**Trauma-Associated Anterior Cingulate Connectivity During Reward Learning Predicts Affective and Anxiety States in Young Adults**

**Supplementary Material**

**Methods**

*Participant Exclusion Criteria*

Exclusion criteria included any history of serious medical/physical conditions: neurological disorder (past stroke, seizures, dementia), history of brain tumor/brain surgery, progressive endocrine disorder (Cushing’s, Lupus), heart disorder (past history of heart attacks, arteriosclerosis) or other major systemic medical conditions (kidney disease, multiple sclerosis, cerebral palsy, blindness, serious physical disability) or chronic/acute condition including any managed by medication (chronic back problem, recent surgery); taking medication for an excluded medical condition; a visual disturbance (<20/40 Snellen visual acuity) when corrected by glasses; presence of metallic foreign objects in body, such as aneurysm clips or pacemakers, or a questionable history of metallic fragments; positive pregnancy test for female individuals, or self-reporting of pregnancy; claustrophobia; a Mini-Mental State Examination score <24; a premorbid IQ estimate <85 (as determined by the National Adult Reading Test); presence of an alcohol, tobacco, or substance use disorder in the prior 3 months; current treatment with psychotropic medication for >2 weeks; previous psychotropic medication treatment in the past 6 months. For typically-developing individuals, personal history of any psychiatric disorder or psychotropic medication use were also exclusion criteria. Twelve individuals were excluded due to incomplete data, two individuals were excluded due to excessive motion (>5mm), one participant was excluded due to excessive task performance errors (20, other participants <12), and six participants were excluded due to excessive signal loss (>30%).

*Monetary Reward fMRI Task*

In addition to RPE, reward expectancy (RE) and outcome expectancy (OE) were calculated during the reward anticipation period in each trial. RE was defined as the expected value of the arrow: +$0.50 for the possible win condition (50% chance of winning $1), –$0.375 for the possible loss condition (50% chance of losing $0.75), +$0.125 for the mixed condition (50% chance of winning $1; 50% chance of losing $0.75), and zero for the neutral condition. In contrast, OE represented the range of unsigned values of possible outcomes where the greatest value is for the mixed trials ($1−$0.75 = 1.75) and lowest for neutral trials (zero). Possible win ($1−$0 = 1) and possible loss (0−$0.75 = 0.75) trial values were in between.

*fMRI Acquisition Parameters*

Functional neuroimaging data were collected at the University of Pittsburgh using a 3.0 Tesla Siemens Trio 2 MRI scanner. Blood-oxygenation-level-dependent (BOLD) images were acquired with a multi-band gradient echo EPI sequence (18 slices, three-factor multiband; 2.3 mm isotropic voxels; TR=1500ms, TE=30ms; field of view=220 × 220 mm; matrix 96 × 96; flip angle 55°, bandwidth 1860 Hz Px–1). Structural 3D axial MPRAGE images (TR=1500ms, TE=3.19ms; flip angle 8° FOV=256 × 256 mm; 1 mm isotropic voxels; 176 continuous slices) and fieldmaps (2.3 mm isotropic voxels; TR=500 ms, TE1=4.92 ms, TE2=7.38 ms; FOV=220 × 220 mm; flip angle 45°, bandwidth 1302 Hz Px–1) were acquired in the same session. Fieldmaps were not available for 11 participants (6 healthy, 5 distressed).

*Determination of Cluster Threshold*

Recent concern has been raised about the use of less stringent cluster determining thresholds (CDT) in neuroimaging analyses leading to high false positive rates ((Eklund *et al.*, 2016, Woo *et al.*, 2014). The seminal paper by Eklund et al. examines the false positive rate in three independent adult samples (with sample sizes of 20-40 subjects) across image analysis platforms using four different functional MRI tasks, finding that the false positive rate is affected most by the CDT, smoothing kernel, task type, and second-level model. Specifically, less stringent CDTs (e.g. *p*<0.005, *p*<0.01), smaller smoothing kernels, fixed-duration event-related designs, and task activation second-level models had higher false positive rates. In more complex second-level models (e.g. two-sample t-tests, regressions) from a randomized event-related design with 20 subjects, Eklund et al. found that false positive rates ranged between 5-10% with a CDT of *p*<0.001 (see(Eklund *et al.*, 2016), supplementary figure 8). Notably, this false positive range decreased with a larger sample size of 40 subjects to 3-8% (see supplementary figure 8). It was also determined that voxelwise inference was less prone to false positives compared with clusterwise inference. In the present study, we utilized SPM and a 6mm smoothing kernel to analyze data from a randomized event-related design in a regression analysis in a much larger sample correcting for age, gender, race, IQ, and distress – critical demographic variables particularly important to include in young adults that were not included in the aforementioned papers. With the addition of more subjects and stringent control for critical demographic variables, we expect that the voxelwise threshold of *punc*<0.001 will sufficiently control for false positives.

**Results**

*Analyses by Trauma Type*

While analyzing data across trauma subscales was considered, the majority of individuals exposed to trauma had experienced more than one type of trauma and 74% of trauma-exposed individuals had experienced two or more traumas. Further, the only subscale for which there was a robust sample size was general disaster trauma (n=73); however, these individuals comprised 91% of the total trauma-exposed group. Thus, subscale-specific analyses were not performed, given the heterogeneity among the subscales and small sample sizes.

**Supplementary Table 1.** Sample Characteristics by Gender

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Gender** | *p*-value |
|  |  | **Female (N=78)** | **Male (N=33)** |
|  |  | N | Mean±SD | N | Mean±SD |
| Distress | Not Distressed | 39 | n/a | 22 | n/a | 0.107 |
| Distressed | 39 | 11 |
| Race | White | 57 | n/a | 20 | n/a | 0.619 |
| Black or African American | 7 | 4 |
| Asian | 12 | 8 |
| More than one race | 2 | 1 |
| Age |  | n/a | 21.58±1.85 | n/a | 22.10±2.16 | 0.230 |
| IQ |  | n/a | 107.33±7.22 | n/a | 110.14±7.00 | 0.061 |
| Affective Symptoms | Anxiety (HAMA) | n/a | 7.24±7.96 | n/a | 2.52±4.64 | <0.001 |
| Depression (HRSD) | n/a | 8.69±9.00 | n/a | 3.58±6.15 | 0.001 |
| Anhedonic Depression (MASQ-AD) | n/a | 2.90±0.79 | n/a | 2.52±0.72 | 0.019 |
| Anxious Arousal (MASQ-AA) | n/a | 1.45±0.62 | n/a | 1.19±0.29 | 0.003 |
|  | Anhedonia (SHAPS) | n/a | 22.51±8.00 | n/a | 22.45±6.16 | 0.967 |
| Trauma | Events (THQ) | n/a | 2.23±2.04 | n/a | 1.42±1.52 | 0.044 |
| HAMA, Hamilton Anxiety Rating Scale; HRSD, Hamilton Depression Rating Scale; MASQ-AA, Mood and Anxiety Symptom Questionnaire – Anxious Arousal; MASQ-AD, Mood and Anxiety Symptom Questionnaire – Anhedonic Depression; SHAPS, Snaith-Hamilton Pleasure Scale; THQ, Trauma History Questionnaire |

**Supplementary Table 2.** Multiple linear regression analyses by gender.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Anxiety** **(HAMA)** | **Depression** **(HRSD)** | **Anhedonic Depression** **(MASQ-AD)** | **Anxious Arousal** **(MASQ-AA)** | **Anhedonia** **(SHAPS)** |
|  *Females Only* | β | t | *p* | β | t | *p* | β | t | *p* | β | t | *p* | β | t | *p* |
|  Left Frontopolar Cortex | -0.22 | -0.43 | 0.67 | -0.09 | -0.15 | 0.88 | 0.00 | -0.09 | 0.93 | -0.08 | -1.92 | 0.06† | 0.11 | 0.22 | 0.82 |
|  Left Fusiform Gyrus | -0.66 | -1.13 | 0.26 | -0.56 | -0.83 | 0.41 | -0.06 | -1.10 | 0.27 | -0.07 | -1.65 | 0.10 | -0.13 | -0.22 | 0.82 |
|  Left Superior Temporal Gyrus | 0.12 | 0.30 | 0.77 | -0.01 | -0.03 | 0.98 | -0.01 | -0.21 | 0.83 | -0.01 | -0.28 | 0.78 | 0.22 | 0.59 | 0.56 |
|  Right Lingual Gyrus | 0.61 | 1.40 | 0.17 | 0.57 | 1.15 | 0.25 | 0.08 | 1.93 | 0.06† | 0.04 | 1.34 | 0.18 | 0.73 | 1.73 | 0.09† |
|  Right Frontopolar Cortex | -0.29 | -0.97 | 0.34 | -0.23 | -0.67 | 0.51 | 0.00 | -0.15 | 0.88 | -0.01 | -0.59 | 0.56 | -0.56 | -1.93 | 0.06† |
|  Right Inferior Parietal Lobule | **-0.77** | **-1.96** | **0.05** | -0.72 | -1.59 | 0.12 | -0.06 | -1.47 | 0.15 | -0.05 | -1.74 | 0.09† | **-0.84** | **-2.23** | **0.03** |
|  Right Insula | **1.41** | **2.18** | **0.03** | **1.49** | **2.00** | **0.05** | **0.14** | **2.20** | **0.03** | 0.07 | 1.34 | 0.18 | **1.40** | **2.24** | **0.03** |
|  Right Supramarginal Gyrus | 0.19 | 0.43 | 0.67 | 0.07 | 0.13 | 0.89 | 0.02 | 0.46 | 0.65 | **0.07** | **1.97** | **0.05** | 0.01 | 0.02 | 0.98 |
|  Right Middle Temporal Gyrus | 0.09 | 0.25 | 0.80 | 0.09 | 0.20 | 0.84 | 0.00 | -0.06 | 0.96 | 0.02 | 0.60 | 0.55 | -0.05 | -0.14 | 0.89 |
| *Males Only* | β | t | *p* | β | t | *p* | β | t | *p* | β | t | *p* | β | t | *p* |
|  Left Frontopolar Cortex | -0.15 | -0.17 | 0.87 | -0.20 | -0.18 | 0.86 | 0.17 | 1.27 | 0.22 | -0.01 | -0.20 | 0.85 | -0.06 | -0.05 | 0.96 |
|  Left Fusiform Gyrus | 0.22 | 0.28 | 0.78 | 0.51 | 0.51 | 0.61 | 0.11 | 0.91 | 0.37 | 0.04 | 0.79 | 0.44 | 0.95 | 0.94 | 0.36 |
|  Left Superior Temporal Gyrus | -0.06 | -0.16 | 0.88 | -0.26 | -0.54 | 0.59 | -0.04 | -0.70 | 0.49 | -0.01 | -0.51 | 0.61 | -0.44 | -0.92 | 0.37 |
|  Right Lingual Gyrus | 0.05 | 0.06 | 0.95 | 0.03 | 0.03 | 0.97 | -0.12 | -1.07 | 0.29 | -0.02 | -0.41 | 0.69 | -0.35 | -0.36 | 0.73 |
|  Right Frontopolar Cortex | 0.02 | 0.08 | 0.94 | -0.05 | -0.13 | 0.90 | 0.02 | 0.46 | 0.65 | 0.01 | 0.54 | 0.60 | 0.28 | 0.69 | 0.50 |
|  Right Inferior Parietal Lobule | 0.11 | 0.25 | 0.80 | 0.38 | 0.65 | 0.52 | 0.01 | 0.15 | 0.88 | -0.04 | -1.38 | 0.18 | -0.23 | -0.40 | 0.69 |
|  Right Insula | -0.04 | -0.05 | 0.96 | -0.01 | -0.01 | 0.99 | 0.05 | 0.42 | 0.68 | 0.02 | 0.38 | 0.71 | -0.03 | -0.03 | 0.98 |
|  Right Supramarginal Gyrus | -0.29 | -0.74 | 0.47 | -0.44 | -0.90 | 0.38 | -0.06 | -0.98 | 0.34 | -0.01 | -0.58 | 0.57 | -0.14 | -0.28 | 0.78 |
|  Right Middle Temporal Gyrus | 0.04 | 0.12 | 0.90 | 0.17 | 0.44 | 0.66 | 0.02 | 0.45 | 0.66 | 0.03 | 1.92 | 0.07† | 0.34 | 0.89 | 0.39 |
| *Gender\*Region* | F | *p* |  | F | *p* |  | F | *p* |  | F | *p* |  | F | *p* |  |
|  Left Frontopolar Cortex | 0.54 | 0.59 |  | 0.35 | 0.71 |  | 1.60 | 0.21 |  | 2.51 | 0.09 |  | 0.12 | 0.89 |  |
|  Left Fusiform Gyrus | 0.79 | 0.46 |  | 0.58 | 0.56 |  | 1.12 | 0.33 |  | 1.81 | 0.17 |  | 0.32 | 0.72 |  |
|  Left Superior Temporal Gyrus | 0.02 | 0.98 |  | 0.`0 | 0.91 |  | 0.29 | 0.75 |  | 0.29 | 0.75 |  | 0.57 | 0.57 |  |
|  Right Lingual Gyrus | 1.44 | 0.24 |  | 1.04 | 0.36 |  | 2.93 | 0.06† |  | 1.40 | 0.25 |  | 1.56 | 0.22 |  |
|  Right Frontopolar Cortex | 0.73 | 0.49 |  | 0.34 | 0.71 |  | 0.22 | 0.80 |  | 0.41 | 0.67 |  | 2.05 | 0.14 |  |
|  Right Inferior Parietal Lobule | 2.71 | 0.07† |  | 1.79 | 0.17 |  | 1.20 | 0.31 |  | 2.69 | 0.07† |  | 2.53 | 0.09† |  |
|  Right Insula | **3.29** | **0.04** |  | 2.75 | 0.07† |  | 2.78 | 0.07 |  | 1.44 | 0.24 |  | 2.55 | 0.08† |  |
|  Right Supramarginal Gyrus | 1.41 | 0.25 |  | 1.50 | 0.23 |  | 1.16 | 0.32 |  | **3.02** | **0.05** |  | <0.01 | 0.99 |  |
|  Right Middle Temporal Gyrus | 0.20 | 0.82 |  | 0.34 | 0.71 |  | 0.19 | 0.83 |  | 1.16 | 0.32 |  | 0.24 | 0.79 |  |
| HAMA, Hamilton Anxiety Rating Scale; HRSD, Hamilton Depression Rating Scale; MASQ-AA, Mood and Anxiety Symptom Questionnaire – Anxious Arousal; MASQ-AD, Mood and Anxiety Symptom Questionnaire – Anhedonic Depression; SHAPS, Snaith-Hamilton Pleasure ScaleSignificant connectivity effects on affective and anxiety symptoms are denoted in **bold**.†trend-level significance (*p*<0.10) |

Supplementary Figure 1. Reward paradigm. (a) Trial design demonstrating choice (4s), anticipation (2-6s), feedback (0.5s), and outcome (0.5s) phases. (b) Visual representation of potential outcomes with each of the four stimuli (win, mixed, neutral, and loss). (c) Calculation of RPE across trial types.

Supplementary Figure 2. Effect of gender on trauma-associated vACC connectivity relationships with anxiety symptoms (HAMA)

Supplementary Figure 3. Effect of gender on trauma-associated vACC connectivity relationships with depressive symptoms (HRSD)

Supplementary Figure 4. Effect of gender on trauma-associated vACC connectivity relationships with anhedonic depression (MASQ-AD)

Supplementary Figure 5. Effect of gender on trauma-associated vACC connectivity relationships with anxious arousal (MASQ-AA)

Supplementary Figure 6. Effect of gender on trauma-associated vACC connectivity relationships with anhedonia (SHAPS)

**References**

**Eklund, A., Nichols, T. E. & Knutsson, H.** (2016). Cluster failure: why fMRI inferences for spatial extent have inflated false-positive rates. *Proceedings of the National Academy of Sciences* **113**(28), 7900-7905.

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