Psychological Medicine

cambridge.org/psm

Covariation between spontaneous neural activity in the insula and affective temperaments is related to sleep disturbance in individuals with major depressive disorder

Huawang Wu[1,*], Yingjun Zheng[1,*], Qianqian Zhan[1], Jie Dong[1], Hongjun Peng[1], Jinguo Zhai[2], Jingping Zhao[1], Shenglin She[1] and Chao Wu[3]

[1]The Affiliated Brain Hospital of Guangzhou Medical University (Guangzhou Huiai Hospital), Guangzhou 510370, China; [2]School of Mental Health, Jining Medical University, Jining 272067, China and [3]School of Nursing, Peking University Health Science Center, Beijing 100191, China

Original Article

*These authors contributed equally to this work.

Cite this article: Wu H, Zheng Y, Zhan Q, Dong J, Peng H, Zhai J, Zhao J, She S, Wu C (2021). Covariation between spontaneous neural activity in the insula and affective temperaments is related to sleep disturbance in individuals with major depressive disorder. *Psychological Medicine* **51**, 731–740. https://doi.org/10.1017/S0033291719003647

Received: 29 September 2018
Revised: 13 May 2019
Accepted: 25 November 2019
First published online: 16 December 2019

Key words:
Affective temperaments; fractional amplitude of low-frequency fluctuations; insula; major depressive disorder; partial least squares

Author for correspondence:
Shenglin She, E-mail: shenglinshe@gzhmu.edu.cn; Chao Wu, E-mail: chelseawu@pku.edu.cn

Abstract

Background. Affective temperaments have been considered antecedents of major depressive disorder (MDD). However, little is known about how the covariation between alterations in brain activity and distinct affective temperaments work collaboratively to contribute to MDD. Here, we focus on the insular cortex, a critical hub for the integration of subjective feelings, emotions, and motivations, to examine the neural correlates of affective temperaments and their relationship to depressive symptom dimensions.

Methods. Twenty-nine medication-free patients with MDD and 58 healthy controls underwent magnetic resonance imaging scanning and completed the Temperament Evaluation of Memphis, Pisa, Paris and San Diego (TEMPS). Patients also received assessments of the Hamilton Depression Rating Scale (HDRS). We used multivariate analyses of partial least squares regression and partial correlation analyses to explore the associations among the insular activity, affective temperaments, and depressive symptom dimensions.

Results. A profile (linear combination) of increased fractional amplitude of low-frequency fluctuations (fALFF) of the anterior insular subregions (left dorsal agranular–dysgranular insula and right ventral agranuar insula) was positively associated with an affective-temperament (depressive, irritable, anxious, and less hyperthymic) profile. The covariation between the insula-fALFF profile and the affective-temperament profile was significantly correlated with the sleep disturbance dimension (especially the middle and late insomnia scores) in the medication-free MDD patients.

Conclusions. The resting-state spontaneous activity of the anterior insula and affective temperaments collaboratively contribute to sleep disturbances in medication-free MDD patients. The approach used in this study provides a practical way to explore the relationship of multivariate measures in investigating the etiology of mental disorders.

Introduction

Affective temperament describes the behavior, attitudes, and emotional orientation of a person based on the interaction of genetic and environmental factors. Akiskal proposed five major affective temperament dimensions: depressive temperament is characterized by sensitivity to suffering, self-denying, and striving to devote themselves to others and confirm to social norms; hyperthymic temperament shows upbeat and excessively energetic traits; cyclothymic temperament tends to shift between intense high and low emotions; anxious temperament shows exaggerated worries while irritable temperament can be expressed as anger, frustration, and skeptical traits (Akiskal & Akiskal, 2005*a*, 2005*b*; Akiskal, Akiskal, Haykal, Manning, & Connor, 2005; Rovai et al., 2013). The affective temperaments have been considered as subsyndromal (trait-related) manifestations and commonly the antecedents of affective disorders (Kesebir, Gundogar, Kucuksubasi, & Tatlidil Yaylaci, 2013; Rihmer, Akiskal, Rihmer, & Akiskal, 2010; Serafini et al., 2011; Solmi et al., 2016). For example, depressive and anxious temperaments are linked to a lack of antidepressant response in patients with mood disorder (De Aguiar Ferreira, Vasconcelos, Neves, & Correa, 2014) and composite affective temperament score (cyclothymic + depressive + irritable − hyperthymic) are high-risk factors for suicidal acts or ideation (Pompili et al., 2018; Rihmer et al., 2009; Tondo, Vázquez, Sani, Pinna, & Baldessarini, 2018; Vazquez, Gonda, Lolich, Tondo, & Baldessarini, 2018).

It has been implicated that prefrontal cortex and limbic structures are associated with fundamental dimensions of affective temperaments (Hatano et al., 2019; Whittle, Allen, Lubman, & Yucel, 2006). At present, in major depressive disorder (MDD) patients, neural correlates of

CAMBRIDGE UNIVERSITY PRESS

the affective temperaments and their relationship with depressive symptom dimensions are yet unclear. A possible neural key correlate might be the insula, a brain hub that anatomically has connections with the prefrontal cortex, the limbic system, and the thalamus (Namkung, Kim, & Sawa, 2017). Multiple lines of evidence support the role of the insula in not only subjective feeling states (Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004; Namkung et al., 2017) and cognitive control (Diener et al., 2012; Uddin, 2014), but also certain temperament constructs (Michalska et al., 2018). The insula has also been considered as a pivot for internal and external information exchange and disruptions of the interoceptive–exteroceptive integration have been suggested as core symptoms of depression (Harshaw, 2015).

Previous neuroimaging studies have indicated that the insula is related to the key symptoms in MDD (Manoliu et al., 2013; Serafini et al., 2017; Sprengelmeyer et al., 2011). For example, altered functional connectivity (FC) of the insular cortex has been associated with the severity of depressive symptoms (Manoliu et al., 2013; Wang et al., 2018) and altered blood oxygenation level-dependent (BOLD) signal in the insula has been found to be correlated with a higher level of hopelessness (Serafini et al., 2017; Wiebking et al., 2014). It should be noted that different parts of the insular cortex exhibit variable cytoarchitecture features, connectivity, input–output relations, and therefore different divisions of labor (Craig, 2009, 2010; Namkung et al., 2017; Tian & Zalesky, 2018). The posterior regions (granular region: an isocortical region with six differentiated layers) receive thalamocortical inputs and have a role in somatosensory and motor integration; whereas the anterior regions (agranular region: an isocortical region with a relatively undifferentiated layer II and layer III, and lack of layer IV that contains stellate granule cells), which have reciprocal connections to limbic regions (e.g. the anterior cingulate cortex, the amygdala, and the ventral striatum) and the dorsolateral and ventromedial prefrontal cortex (for a review see Namkung et al., 2017), have been implicated in the integration of interoception (Craig, 2009), subjective feelings (Craig, 2009; Damasio, 2003; Kesebir et al., 2013), and motivations (Naqvi & Bechara, 2009). For example, the mid-posterior insula is activated when subjects receive painful stimulation and plays a greater role in regulating homeostatic states (Craig, 2009, 2010; Menon & Uddin, 2010; Uddin, Nomi, Hebert-Seropian, Ghaziri, & Boucher, 2017). The anterior insula plays a role in the salience network, which is important for monitoring the saliency of external inputs and internal brain events. Thus far, whether different insular subregions contribute to specific depressive symptom dimensions is not clear.

Because of its key anatomical position, compared with the amygdala, which is responsible for impulsive and automatic emotional processing, the insula tends to introduce subjective feelings state into cognition and motivation (Bechara, 2005; Dolan, 2002; Namkung et al., 2017). It seems that the insula or its FC could be more related to the affective temperaments, which identify the stable biological or inherited 'core' of an individual's emotional response tendencies and personality of integration of emotional and cognitional activity (Jabbi et al., 2012; Rihmer et al., 2010, 2013). Previous studies have revealed that a combination of certain affective temperaments is related to suicide risk in MDD (Pompili et al., 2013, 2014, 2018). Moreover, insula hyperactivity has been found to be associated with anxiety-related temperamental traits (Liu, Taber-Thomas, Fu, & Perez-Edgar, 2018*b*; Stein, Simmons, Feinstein, & Paulus, 2007). Thus, the insula may affect certain affective temperaments, and the dysregulated insula-temperament covariation, which represents an alteration in a synergistic effect of emotion and personality, might contribute to certain emotional–cognitive integration-related depressive symptoms in MDD patients. Thus far, whether different insular subregions contribute to specific affective temperaments, leading to specific depressive symptoms, has not been investigated. Answering to the question may be of value in exploring the etiology of MDD and provide a target for neurostimulation therapy of depression.

In this study, we aimed to examine whether spontaneous activity or FC of the insula is related to certain affective temperaments and which insular subregion contributes to the association. Also, we explored whether the covariation of insula-temperament is impaired in patients with MDD and related to depressive symptoms. It has been suggested that there is a multidimensional-to-multidimensional relationship between psychopathological subtypes, clinical manifestation subtypes, and biological data (Kang, Bowman, Mayberg, & Liu, 2016; Moser et al., 2018; Perlis et al., 1997). Unlike previous studies focusing on variations of univariate analyses wherein either a single variable (subscale score) is treated as computationally independent or a global approach that treats the set of variables as a whole (total score of a scale or questionnaire) is used, we adopted a multivariate method, a partial least square regression (PLSR), to identify associations between a set of predictor variables (the resting-state activity of insular subregions) and a set of response variables (the dimensions of affective temperaments). The PLS method is especially appropriate when the predictor variables are highly interdependent or multicollinear (Abdi, 2010; Krishnan, Williams, Mcintosh, & Abdi, 2011). We hypothesized that some distinctive affective-temperament profiles would be associated with certain patterns of insular activity in both medication-free MDD patients and healthy controls. Then, in the MDD patients, we examined whether the altered covariation between the insula-activity profile and the affective-temperament profile was associated with depressive symptoms, and if so, which symptom dimension it would contribute to. To avoid the effect of pharmacological treatments and comorbidity on the current research, we only included patients who had no comorbidity and took no medication within 3 months prior to participating in this study.

Materials and methods

Participant

Twenty-nine patients with MDD (mean age = 26.7 ± 6.0), diagnosed according to the Structured Clinical Interview for DSM-IV (SCID-DSM-IV) Patient Edition, were recruited from the Guangzhou Huiai Hospital, Guangzhou, China. Each of the patients had a score of at least 21 on the 24-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960). None of them had any other comorbid psychiatric or neurological diseases. Twenty-five of the MDD patients never received antidepressant treatment (i.e. medication-naïve) and the rest four MDD patients took no medication within 3 months prior to participating in this study (i.e. medication-free).

Fifty-eight healthy control participants (mean age = 27.9 ± 5.9) were screened with the SCID-DSM-IV Non-Patient Edition to confirm the lifetime absence of Axis I illnesses. The healthy control participants had no history of psychiatric illness in any two lines of first- to third-degree biological relatives.

All participants gave their written informed consent to participate in this study. The procedures of this study were approved by

https://doi.org/10.1017/S0033291719003647 Published online by Cambridge University Press

the Independent Ethics Committee (IEC) of the Guangzhou Huiai Hospital. The investigation was carried out in accordance with the World Medical Association (2013) Declaration of Helsiniki-Ethical Principles for medical research involving human subjects.

Measures

Hamilton Depression Rating Scale

A 24-item HDRS was used to rate the severity of patients' depression by probing seven factors of depressive symptoms: anxiety/somatization (physical and somatic anxiety, general and gastrointestinal somatic symptoms, hypochondriasis and insight); weight loss; cognitive disturbance (guilt, suicide, agitation, depersonalization and derealization, paranoid symptoms, and obsessional symptoms); diurnal variation; retardation (depressed mood, work and interests, slowness of thought, speech and activity, apathy, and loss of libido); sleep disturbance (difficulty in falling asleep, waking during the night, and waking in early hours of the morning and unable to fall asleep again); and hopelessness (helplessness, hopelessness, and worthlessness) (Hamilton, 1960; Rabkin & Klein, 1987). The Chinese version 24-item HDRS has been widely used and has demonstrated adequate validity in studies of clinical and non-clinical populations in China (Leung, Wing, Kwong, Lo, & Shum, 1999; Shi et al., 2008; Tu et al., 2018).

Temperament Evaluation of Memphis, Pisa, Paris, and San Diego

The Temperament Evaluation of Memphis, Pisa, Paris, and San Diego (TEMPS-A) is a 110-item 'yes' or 'no' answer self-rated questionnaire that assesses the five temperaments on five scales: depressive, cyclothymic, hyperthymic, irritable, and anxious temperaments (Akiskal & Akiskal, 2005*a*, 2005*b*; Akiskal et al., 2005). The TEMPS have been widely used in populations of different countries and has shown adequate internal consistency (Akiskal & Akiskal, 2005*b*; Akiskal et al., 2005; Elias et al., 2017; Kawamura et al., 2010; Lin et al., 2013; Pompili et al., 2018).

Magnetic resonance imaging data acquisition

Magnetic resonance imaging (MRI) was conducted at the Department of Radiology, Guangzhou Huiai Hospital, China, on a 3.0-Tesla MRI system (Achieva X-series Scanner; Philips, Medical Systems, Best, The Netherlands) with an eight-channel SENSE head coil. The resting-state functional MRI (rs-fMRI) was acquired using a gradient-echo echo-planar-imaging sequence (TR = 2000 ms, TE = 30 ms, flip angle = 90°, matrix = 64 × 64, field of view = 220 mm × 220 mm, number of slices = 33, slice thickness = 4 mm with interslice gap = 0.6 mm, voxel size = 3.4 mm × 3.4 mm × 4.6 mm). The rs-fMRI scanning lasted 8 min and consisted of 240 time points. Participants were instructed to relax and to remain still with their eyes closed during the rs-fMRI scanning. A high-resolution structural image was acquired with a three-dimensional T1-weighted turbo field-echo sequence (TR/TE = 8.2/3.7 ms, flip angle = 7°, acquisition matrix = 256 × 256, slice thickness = 1 mm, voxel size = 1 mm × 1 mm × 1 mm, and number of slices = 188). Earplugs were used to attenuate scanner noise and a foam pillow and extendable padded head clamps were used to restrain the head motions of participants. No participant was excluded due to excessive head motion (1.5 mm in translation or 1.5° in rotation) during rs-fMRI scanning.

MRI data preprocessing

The rs-fMRI data were processed and analyzed using the SPM 12 software (http://www.fil.ion.ucl.ac.uk/spm/) with FC toolbox v17 (CONN, www.nitrc.projects/conn) (Whitfield-Gabrieli & Nieto-Castanon, 2012). The preprocessing pipeline included participant motion estimation and correction, structural segmentation and normalization [resampling to a voxel size of 2 mm × 2 mm × 2 mm in the standard Montreal Neurological Institute (MNI) space], ART-based functional outlier detection and scrubbing, and functional spatial smoothing with an 8 mm Gaussian kernel. Before the first-level analysis, a denoising step (linear regression and bandpass filtering) was conducted to remove possible confounds including the BOLD signal from the white matter and cerebrospinal fluid, realignment parameters (six motion parameters and six first-order temporal derivatives), and scrubbing parameters (maximum interscan movement and identified invalid scans; the framewise-displacement values for all subjects were below 0.3) (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012).

Fractional amplitude of low-frequency fluctuations (fALFF) in subregions of the insula

The fALFF, which is defined as the fractional sum of the amplitudes within the low-frequency range (0.01–0.08 Hz) divided by the sum of amplitude across the entire frequency range (0–0.25 Hz) (Zou et al., 2008), was calculated first for each GM voxel. The participant-level voxel-wise fALFF maps were then further standardized by subtracting the mean whole-brain voxel-wise fALFF from the participant-level voxel-wise fALFF and dividing it by the standard deviation. The fALFF has been considered as an index for detecting spontaneous brain activities (Zou et al., 2008; Zuo et al., 2010).

The regions of interest for the insular subregions were obtained directly from the Human Brainnetome Atlas (http://atlas.brainnetome.org/bnatlas.html). In this atlas, the insula was first extracted from the Desikan–Killiany (DK) atlas, and the connectional architecture was then mapped with probabilistic tractography using diffusion MRI. The insula was symmetrically divided into six subregions in each hemisphere with anatomical connectivity patterns by calculating the similarity/dissimilarity between the connectivity architecture (Fan et al., 2016). The insular subregions (Fig. 1) contain the hypergranular insula (L1 and R1), the ventral agranular insula (L2 and R2), the dorsal agranular insula (L3 and R3), the ventral dysgranular and granular insula (L4 and R4), the dorsal granular insula (L5 and R5), and the dorsal dysgranular insula (L6 and R6) (Fan et al., 2016; Wang et al., 2018). The fALFF values were extracted from each of the 12 insular subregions using the Marsbar toolbox (http://marsbar.sourceforge.net/).

FC of the insular subregions

We used a Global Brain Connectivity (GBC) method to characterize each insular subregion's full range FC with voxel-wise resolution (Cole, Yarkoni, Repovs, Anticevic, & Braver, 2012; Wang et al., 2016). The GBC of a voxel was generally defined as the averaged FC of the voxel to the rest of the voxels in the whole brain or within a predefined mask (Wang et al., 2016). First, the GBC of each insular subregion was computed as the averaged FC (Pearson correlation) of each insular-subregion voxel to all the voxels in a whole brain gray matter mask (the bilateral insula

https://doi.org/10.1017/S0033291719003647 Published online by Cambridge University Press

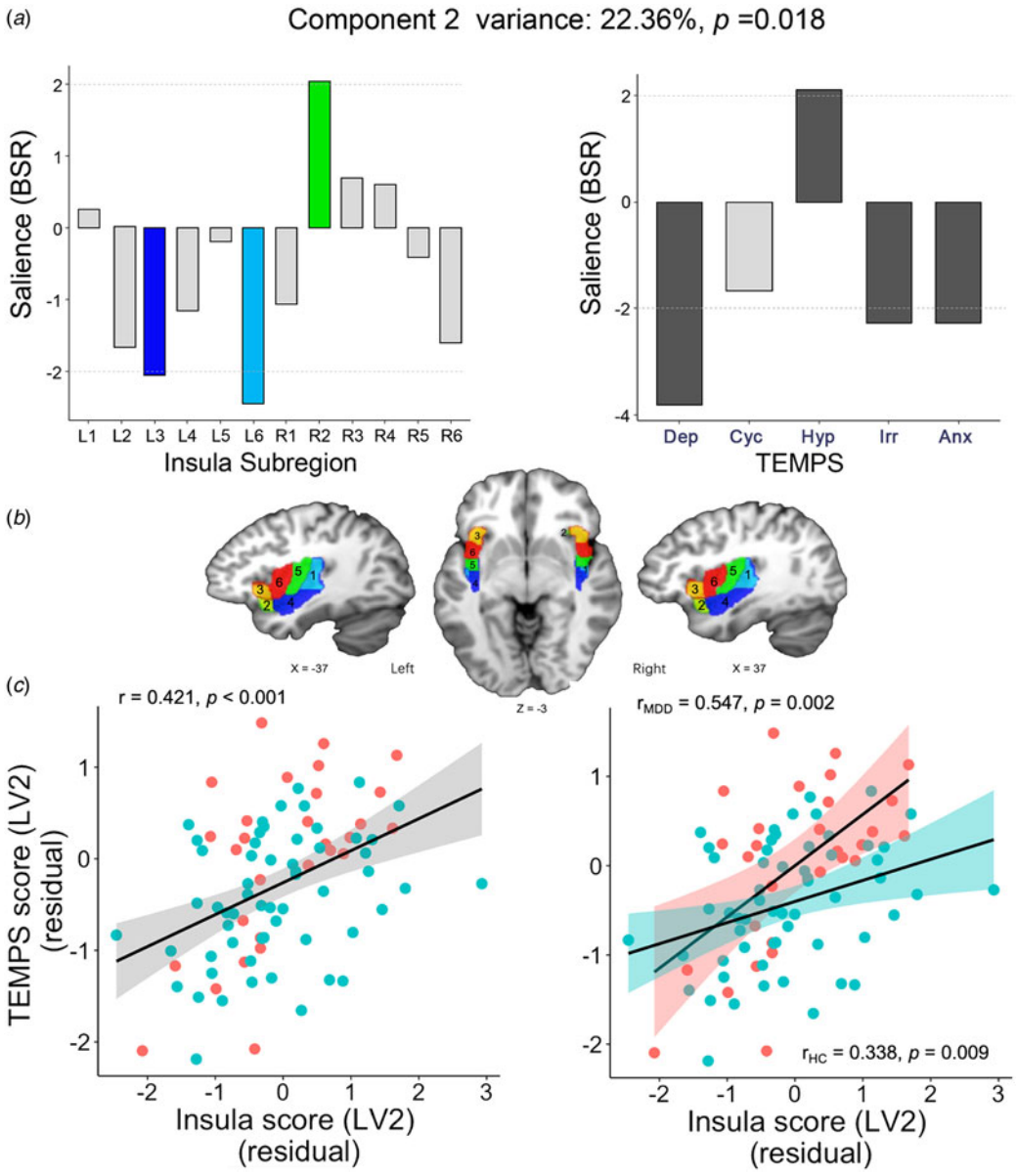

Fig. 1. PLS association analyses in all the participants. (*a*) The second pair of latent variables (LVs) from the partial least squares regression (PLSR) analysis between spontaneous neural activity in the insular subregions measured by the fALFF and affective temperaments measured by the TEMPS (Dep: depressive; Cyc: cyclothymic; Hyp: hyperthymic; Irr: irritable and Anx: anxious). *Left:* Bootstrap ratios (BSR) of saliences (weights) for spontaneous neural activity in each insular subregion; *right:* Bootstrap ratios (BSR) of saliences for each subscale of TEMPS. (*b*) Illustration of the insular subregions from the Human Brainnectome Atlas (http://atlas.brainnetome.org/bnatlas.html). L1 and R1: hypergranular insula; L2 and R2: the ventral agranular insula; L3 and R3: the dorsal agranular insula; L4 and R4: the ventral dysgranular and granular insula; L5 and R5: the dorsal granular insula; L6 and R6: the dorsal dysgranular insula. L, left; R, right. (*c*) The associations between the insular-fALFF profile and the TEMPS profile were significant for not only all the participants (left panel), but also for either the major depressive disorder (MDD) patients or the healthy control participants (right panel).

was excluded from the mask). Then, participant-level GBC maps were transformed to *z*-score maps using Fisher's *z*-transformation to yield normally distributed values. Finally, the GBC values of each participant were entered into a two-sample *t* test for group comparison and a PLS regression for exploring the association with the TEMPS dimensions.

Statistical analysis

Statistical analyses were performed using R 3.4.0. With univariate analyses, we tested differences in scale scores, insula-subregional fALFF values, and insula-subregional GBC values between groups. With partial least squares regression analyses, we investigated patterns of associations between insular-subregional spontaneous activity or GBC and affective temperaments. With partial correlation analyses, we investigate the association of insula-temperament covariation with the depressive symptoms.

The PLS analyses were conducted using the R Package PLS (https://cran.r-project.org/web/packages/pls/index.html) and Morpho (https://cran.r-project.org/web/packages/Morpho/index.html). PLS combines a principal component analysis-style dimensionality reduction with linear regression and finds the

https://doi.org/10.1017/S0033291719003647 Published online by Cambridge University Press

components from predictor variables (e.g. fALFF values or GBC values in each insular subregion: matrix X) that have maximum covariance with the response variables (e.g. the TEMPS subscale scores of participants: matrix Y). The PLS components are ranked by covariance between predictor and response variables, and the first few PLS components (PLS1, PLS2, PLS3, etc.) provide the optimal low-dimensional representation of the covariance between the higher dimensional data matrices (Abdi, 2010; Krishnan et al., 2011; Vertes et al., 2016). To express the saliences relative to the X measures and the Y measures, the original matrices X and Y are projected onto their respective saliences. This creates pairs of latent variables (LVs) – which are linear combinations of the original variables – that are called X and Y scores. A pair of LVs (weighted scores) reflects a relationship between the predictor and the response variable. The significance of the covariance of the components was tested by comparing it with the distribution of covariance arising from random permutation tests (1000 times). The weight (salience) of each variable indicates and ranks the contribution of this variable to a PLS component. A bootstrap procedure (5000 times) was used to test the reliability (bootstrap ratio > 2) of each variable in the significant PLS component (Krishnan et al., 2011; McIntosh & Lobaugh, 2004).

To identify the association between the resting-state activity of the insular cortex and the affective temperaments, a 12 (six insular subregions in each hemisphere) by 87 (number of all participants) matrix of the insula-activity measures and a 5 (TEMPS subscales) by 87 matrix of the affective-temperament measures were entered into a PLSR analysis. Then, the PLS-insula scores and the PLS-TEMPS scores from the insula-TEMPS PLSR were obtained for each participant. Finally, partial correlations were conducted to examine whether the insula-temperament covariation (the mean score averaged across the PLS-insula scores and the PLS-TEMPS scores) contributed to the depressive symptom dimensions.

Results

Comparisons in scale scores, fALFF, and FC between MDD participants and healthy controls

There was no significant difference in age, sex, or educational level between the MDD group and the healthy control group. Compared to the healthy controls, the MDD patients had higher TEMPS depressive scores, higher TEMPS cyclothymic scores, higher TEMPS irritable scores, higher TEMPS anxious scores, and lower TEMPS hyperthymic scores (all p values <0.002) (Table 1).

The fALFF value in the L1 subregion (left hypergranular insula), the L5 subregion (left dorsal granular insula), the R2 subregion (right ventral agranular insula), and the R4 subregions (right ventral dysgranular and granular insula) were lower in the MDD patients than those in the healthy controls (uncorrected p values were 0.030, 0.030, 0.006, and 0.040; two-tailed). All the p values did not survive correction for multiple comparisons with the Benjamini–Hochberg standard false discovery rate (FDR) method (see online Supplementary Fig. S1).

The GBC value of the L3 subregion (left dorsal agranular insula) was higher in the MDD patients than that in the healthy controls (uncorrected $p = 0.038$), and the group difference in the GBC value of the R1 subregion (right hypergranular insula) was marginally significant (uncorrected $p = 0.055$). Both the p values did not survive correction for multiple comparisons with the Benjamini–Hochberg standard FDR method (see online Supplementary Fig. S2).

Multivariate associations between insular fALFF/GBC values and TEMPS scores

The PLSR between the fALFF values in the insular subregions and the TEMPS subscale scores yielded five sets of LVs capturing the insula-temperament associations, ordered by the size of the explained variance (73.80, 22.36, 3.26, 0.33, and 0.25%). Each component (LV pair) was the linear combination of the weighted insula-fALFF scores that most strongly predicted weighted affective-temperament scores. Only the second PLS component (PLS2) was significant (permutation test, $p = 0.018$) (Fig. 1*a*). The bootstrapping test showed that the fALFF value in the L3 subregion, L6 subregion, and R2 subregion of the insula reliably contributed to the PLS2-insula LV (Fig. 1*b*); the depressive temperament (T1), hyperthymic temperament (T3), irritable temperament (T4), and anxious temperament (T5) reliably contributed to the PLS2-temperament LV. Thus, for all the participants, the insula-TEMPS PLS2 identified a profile of spontaneous activity of the left anterior dorsal insula and right anterior ventral insula that positively predicted the depressive, anxious, and irritable temperaments and negatively predicted the hyperthymic temperament (age, sex, and educational years were adjusted) (left panel of Fig. 1*c*). Moreover, the association was also significant for either the MDD patients (age, sex, educational years, age of onset, and illness duration were controlled as nuisance covariates in the MDD group) or the healthy participants (age, sex, and educational years were controlled in the healthy group) (right panel of Fig. 1*c*), supporting our hypothesis that there may be a common pattern of insular-TEMPS covariation between MDD patients and healthy controls.

The PLSR between the GBC values in the insular subregions and the TEMPS subscale scores yielded five sets of LVs representing the insula-temperament associations, ordered by the size of the explained variance (77.46, 16.27, 5.32, 0.86, and 0.09%). However, none of the PLS components was significant (permutation test, all p values >0.05).

Compared with the healthy participants, the MDD patients showed higher PLS2 insula-fALFF scores, significantly higher PLS2 affective-temperament scores, and significantly enhanced covariation between the insula-fALFF profile and the TEMPS profile (Fig. 2).

To examine whether the pair of the insular LV and the TEMPS LV was related to depressive symptoms, in the MDD patients, with the covariates (including age, sex, educational years, age of onset, and illness duration) controlled, we correlated the PLS2 insula-fALFF scores, the affective-temperament (TEMPS) scores, and their covariation index scores with each depressive symptom dimension. The results showed that only the sleep disturbance dimension was significantly correlated with the PLS2-insula scores and the insula-temperament covariation (Fig. 3; FDR-corrected p values <0.05).

Further inspection of each item of the sleep-disturbance dimension showed that both the middle-insomnia (waking during the night) score and the late-insomnia (waking in the early hours of the morning and unable to fall asleep again) score, but not the early-insomnia (difficulty in falling asleep) score, were associated with both the insula profile and the affective-temperament profile (Fig. 4; all FDR-corrected p values <0.05). Moreover, both the middle-insomnia score ($r = 0.521$,

https://doi.org/10.1017/S0033291719003647 Published online by Cambridge University Press

Table 1. Demographic and clinical characteristics in patients with major depressive disorder and healthy controls

Characteristic	MDD ($n = 29$)	HC ($n = 58$)	t/χ^{2a}	p	Cohen's d (95% CI)
Age (years ± S.D.)	26.7 (6.0)	27.9 (5.7)	−0.9	0.398	−0.2 (−0.8 to 0.3)
Male% (n)	41.4 (12)	41.4 (24)	0	1	0
Education (years ± S.D.)	13.8 (3.5)	13.6 (2.4)	0.4	0.671	0.1 (−0.4 to 0.7)
Ill duration (years ± S.D.)	2.1 (2.5)	NA	NA	NA	NA
Age of onset	24.7 (6.5)	NA	NA	NA	NA
TEMPS					
Depressive	13.1 (3.3)	6.1 (2.4)	11.2	<0.001	3.0 (2.2–3.8)
Cyclothymic	13.8 (5.0)	3.2 (3.4)	11.6	<0.001	3.1 (2.3–3.9)
Hyperthymic	6.6 (5.2)	11.0 (4.7)	−3.9	<0.001	−1.1 (−1.6 to −0.5)
Irritable	8.7 (4.1)	1.8 (2.1)	10.4	<0.001	2.8 (2.1–3.6)
Anxious	16.2 (4.5)	3.4 (3.1)	15.5	<0.001	4.2 (3.2–5.2)
HDRS total	31.5 (7.4)		NA	NA	NA
Anxiety/somatization	6.3 (2.5)		NA	NA	NA
Weighting loss	0.6 (0.6)		NA	NA	NA
Cognitive disturbance	5.2 (3.1)		NA	NA	NA
Diurnal variation	0.8 (0.6)		NA	NA	NA
Retardation	8.1 (1.9)		NA	NA	NA
Sleep disturbance	3.5 (1.8)		NA	NA	NA
Hopelessness	6.3 (2.1)		NA	NA	NA

S.D., standard deviation; CI, confidence interval; HC, healthy controls; MDD, major depressive disorder; TEMPS, Temperament Evaluation of Memphis; HDRS, Hamilton Depression Rating Scale. The HDRS was not rated for healthy controls.
[a]Analyses were conducted between the two patient groups by t tests for normally distributed variables and χ^2-tests for categorical variables (two-tailed).

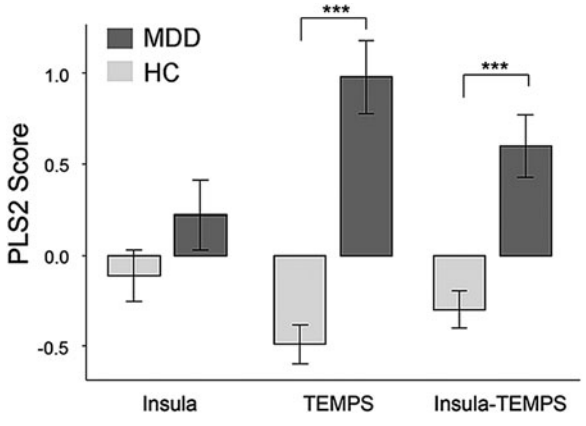

Fig. 2. Comparisons in the PLS2 insula-fALFF scores, PLS2 TEMPS scores, and PLS2 Insula-TEMPS covariation scores between the MDD patients and the healthy participants. ***$p < 0.001$ (two-tailed).

$p = 0.009$, FDR-corrected $p = 0.028$) and the late-insomnia score ($r = 0.527$, $p = 0.008$, FDR-corrected $p = 0.029$), but not the early-insomnia score ($r = 0.387$, $p = 0.062$, FDR-corrected $p = 0.070$), contributed to the positive correlation between the sleep-disturbance symptoms and the insula-temperament covariation (Fig. 4). Thus, for the medication-free MDD patients, the higher the covariation between the spontaneous insular activity and the affective temperaments, the more severe the sleep disturbances (especially for middle insomnia and late insomnia) were.

Discussion

This study, for the first time, investigated the relationship between the resting-state activity of the insular subregions and the affective temperaments. Additionally, in a sample of medication-free MDD patients, we explored whether the insula-temperament covariation was associated with the depressive symptom dimensions. Using a multivariate analysis technique, we identified a profile of spontaneous activity of the insular cortex (left anterior dorsal agranular–dysgranular insula and right anterior ventral agranular insula) that was associated with an affective-temperament (depressive, irritable, anxious, and less hyperthymic) profile. In the MDD patients, the insula-temperament covariation was significantly correlated with sleep disturbance symptoms, especially middle insomnia and late insomnia.

As far as we know, this is the first study investigating the relationship between insular subregional activity and distinct affective-temperament dimensions. In both the MDD patients and healthy control participants, we found that a profile of spontaneous activity of the anterior insular cortex (increased fALFF in the left dorsal agranular–dysgranular insula and decreased fALFF in the right ventral agranular insula) was associated with a profile of affective temperaments (more depressive, irritable, anxious, and less hyperthymic), indicating a role of the anterior insular subregions in the affective temperament profile, a composite cognitive-affective personality characterized by being pessimistic, sensitive to suffering, self-denying, easy to exaggerate anxiety, skeptical and anger/frustration traits. Moreover, the negative results of PLSR analysis between the insular-subregional GBC

https://doi.org/10.1017/S0033291719003647 Published online by Cambridge University Press

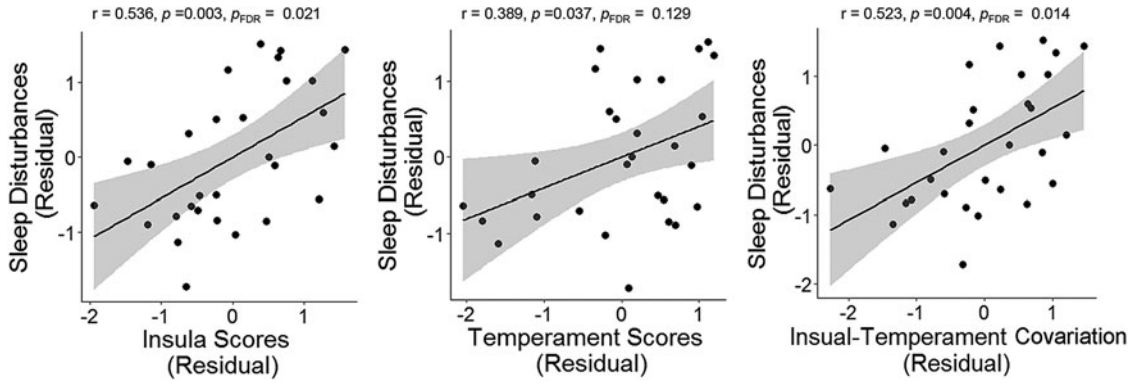

Fig. 3. Associations of the sleep disturbance scores with the PLS2 insula-fALFF scores, PLS2 TEMPS scores, and PLS2 insula-TEMPS covariation scores in MDD patients. Age, sex, educational years, age of depression onset, and illness duration were controlled as covariates.

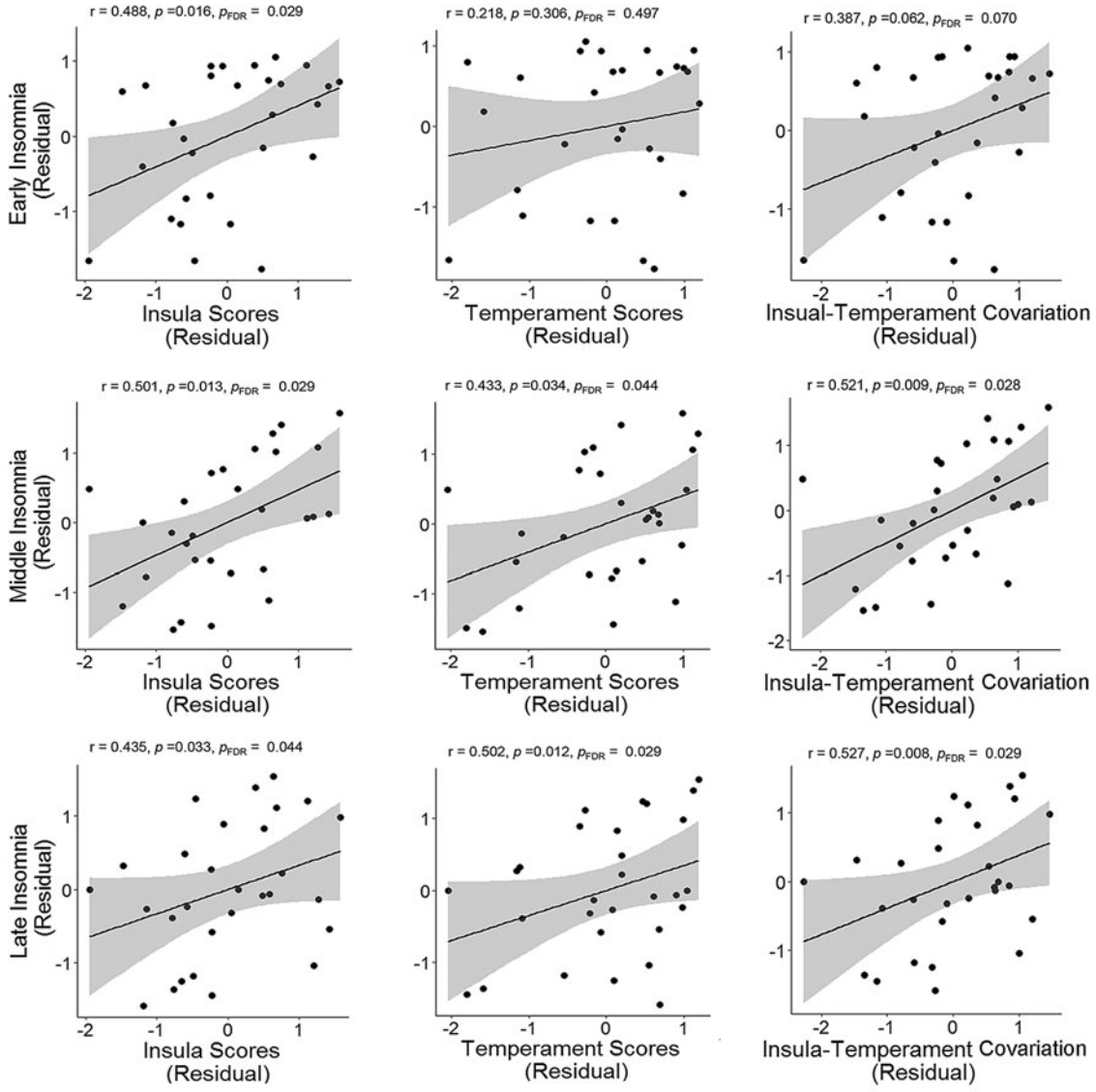

Fig. 4. Associations of the early insomnia, middle insomnia, and late insomnia scores with the PLS2 insula-fALFF scores, PLS2 TEMPS scores, and PLS2 insula-TEMPS covariation scores in MDD patients. Age, sex, educational years, age of depression onset, and illness duration were controlled as covariates.

https://doi.org/10.1017/S0033291719003647 Published online by Cambridge University Press

values and the affective temperament dimensions seem to suggest that it is the functional covariation within the anterior insular subregions, not the insular-subregional GBC, contributes to a specific affective temperament profile. The anterior dorsal insula is considered as a cognitive-control-related region and the anterior ventral insula is considered as a socio-emotion-related region (Kurth et al., 2010). Greater diversity within the anterior insula has been associated with higher scores on measures of positive effect, self-efficacy, emotion recognition, and motor dexterity (Tian & Zalesky, 2018). Moreover, it has been implicated that objective sensory information that is passed from the posterior insula is re-presented in the anterior insula by integration of the primary emotional, cognitive, and motivational information from the limbic system and prefrontal cortex, forming the subjective or interoceptive feeling states (Craig, 2010; Namkung et al., 2017). Thus, it seems that the anterior insula underlies the subjective emotional response that is manifested in emotional personality or affective temperaments. Note that PLS2 of the insular fALFF-temperament association explained only 22.3% of the total variance, suggesting that there may be other unknown neural activities that modulate affective temperaments in addition to the spontaneous activity in the insular subregions.

The role of the insula in depression has been highlighted in several meta-analyses and reviews (Harshaw, 2015; Menon & Uddin, 2010; Otte et al., 2016; Su et al., 2014). Some research reported increased ALFF or fALFF in the insular cortex of patients with MDD (Liu et al., 2015; Yu et al., 2017) but others reported reduced fALFF in the insula (Fitzgerald, Laird, Maller, & Daskalakis, 2008; Liu et al., 2017; Su et al., 2014). The inconsistent results may be explained by several factors including whether patients were comorbid with other disorders, medicated, suffered from a first episode, or were chronic patients (Su et al., 2014). In contrast to previous studies focusing on variations of the univariate analysis, our study extends previous findings by identifying an increased covariation between the spontaneous neural activity of three anterior insular subregions and four affective temperament dimensions in the medication-free MDD patients, and the association was related to only the sleep disturbance symptom dimension when the potential covariates (including age, sex, educational years, illness duration, and the age of onset) were controlled, suggesting that the insula-temperament covariation, which represents a biological-personality interaction, may have a synergistic effect on sleep disturbances in MDD patients.

Human sleep neuroimaging studies support the role for the insular cortex in pathologic sleep associated with both depression and insomnia (Cheng, Rolls, Ruan, & Feng, 2018; Liu et al., 2018*a*; Perico et al., 2005; Wang et al., 2019). For example, it has been found that increased ALFF in the right anterior insula is related to sleep disturbance scores in MDD patients with insomnia complaints (Liu et al., 2018*a*). In primary insomnia people, more negative sleep-onset discrepancy has been associated with the higher relative regional cerebral metabolic rate for glucose in the right anterior insula during non-rapid eye movement sleep (Kay et al., 2017). The results of this study are consistent with the conclusion that the anterior insula is involved in sleep disturbances in MDD patients. Furthermore, the above-mentioned insula-temperament covariation made contributions to the middle and late insomnia in the MDD patients, suggesting that the left anterior dorsal insula and right anterior ventral insula might be involved in regulating the sleep–wake circle, which is more likely through dysregulation of physiological reactivity covering the sympathetic and parasympathetic action (Chouchou et al., 2019; Park et al., 2016). The sympathetic–parasympathetic control spatially distributes from the posterior to anterior insula and regulates cardiovascular and neural activity during sleep and waking (Chouchou et al., 2019; Park et al., 2016). Moreover, an objective–subjective signal processing also distributes spatially from the posterior to anterior insula, which regulates integration of interoceptive–exteroceptive information (Craig, 2009, 2010; Namkung et al., 2017). Thus, alterations in the profile of anterior insular activity might cause disturbances in the sleep–wake circle via the dysregulation of sympathetic–parasympathetic related emotional–cognitive components, which contributes to neural vulnerability to sleep loss in the medication-free MDD patients. The insula-temperament covariation identified in this study may be a potential biopsychological marker underlying the middle and late insomnia in MDD.

In summary, our study indicated that covariation between spontaneous activity in the anterior insula (especially the left dorsal insula and the right ventral insula) and the composite depressive–irritable–anxious temperament (characterized by being pessimistic and self-denying, skeptical and easy to get angry, and prone to exaggerate worries) is associated with sleep disturbance symptoms (middle and late insomnia) in medication-free patients with MDDs. Because of the cross-sectional design, we cannot confirm whether the alterations in insular activity are neurodevelopmental or a consequence of depression onset. This study shed light on the etiology of depression and provides a practical method for exploring the relationship among multivariate measures in mental disorders.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291719003647

Acknowledgments. This work was supported by the National Natural Science Foundation of China (81601168), the Medical Scientific Research Foundation of Guangdong Province of China (B2018063), the Guangdong Natural Science Foundation of China (2015A030313800), and the Shandong Provincial Natural Science Foundation, China (ZR2017LH036).

Author contributions. HW, SS, and CW designed the study and wrote the first draft of the manuscript. CW carried out data analyses. HW, YZ, QZ, SS, JD, HP, JGZ, and JPZ conducted data collection. All authors contributed to and approved the final version of the manuscript.

Conflict of interest. All authors declare no conflicts of interest.

References

Abdi, H. (2010). Partial least squares regression and projection on latent structure regression (PLS regression). *Wiley Interdisciplinary Reviews Computational Statistics*, *2*, 97–106.

Akiskal, H. S., & Akiskal, K. K. (2005a). TEMPS: Temperament Evaluation of Memphis, Pisa, Paris and San Diego. *Journal of Affective Disorder*, *85*, 1–2.

Akiskal, H. S., Akiskal, K. K., Haykal, R. F., Manning, J. S., & Connor, P. D. (2005). TEMPS-A: Progress towards validation of a self-rated clinical version of the temperament evaluation of the Memphis, Pisa, Paris, and San Diego autoquestionnaire. *Journal of Affective Disorders*, *85*, 3–16.

Akiskal, K. K., & Akiskal, H. S. (2005b). The theoretical underpinnings of affective temperaments: Implications for evolutionary foundations of bipolar disorder and human nature. *Journal of Affective Disorders*, *85*, 231–239.

Bechara, A. (2005). Decision making, impulse control and loss of willpower to resist drugs: A neurocognitive perspective. *Nature Neuroscience*, *8*, 1458–1463.

Cheng, W., Rolls, E. T., Ruan, H., & Feng, J. (2018). Functional connectivities in the brain that mediate the association between depressive problems and sleep quality. *JAMA Psychiatry*, *75*, 1052–1061.

Chouchou, F., Mauguiere, F., Vallayer, O., Catenoix, H., Isnard, J., Montavont, A., … Mazzola, L. (2019). How the insula speaks to the heart: Cardiac

responses to insular stimulation in humans. *Human Brain Mapping*, *40*, 2611–2622.
Cole, M. W., Yarkoni, T., Repovs, G., Anticevic, A., & Braver, T. S. (2012). Global connectivity of prefrontal cortex predicts cognitive control and intelligence. *The Journal of Neuroscience*, *32*, 8988–8999.
Craig, A. D. (2009). How do you feel – now? The anterior insula and human awareness. *Nature Reviews: Neuroscience*, *10*, 59–70.
Craig, A. D. (2010). The sentient self. *Brain Structure & Function*, *214*, 563–577.
Critchley, H. D., Wiens, S., Rotshtein, P., Ohman, A., & Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nature Neuroscience*, *7*, 189–195.
Damasio, A. (2003). Feelings of emotion and the self. *Annals of the New York Academy of Sciences*, *1001*, 253–261.
De Aguiar Ferreira, A., Vasconcelos, A. G., Neves, F. S., & Correa, H. (2014). Affective temperaments and antidepressant response in the clinical management of mood disorders. *Journal of Affective Disorders*, *155*, 138–141.
Diener, C., Kuehner, C., Brusniak, W., Ubl, B., Wessa, M., & Flor, H. (2012). A meta-analysis of neurofunctional imaging studies of emotion and cognition in major depression. *Neuroimage*, *61*, 677–685.
Dolan, R. J. (2002). Emotion, cognition, and behavior. *Science*, *298*, 1191–1194.
Elias, L. R., Kohler, C. A., Stubbs, B., Maciel, B. R., Cavalcante, L. M., Vale, A. M., … Carvalho, A. F. (2017). Measuring affective temperaments: A systematic review of validation studies of the Temperament Evaluation in Memphis Pisa and San Diego (TEMPS) instruments. *Journal of Affective Disorders*, *212*, 25–37.
Fan, L., Li, H., Zhuo, J., Zhang, Y., Wang, J., Chen, L., … Jiang, T. (2016). The human brainnetome atlas: A new brain atlas based on Connectional Architecture. *Cerebral Cortex*, *26*, 3508–3526.
Fitzgerald, P. B., Laird, A. R., Maller, J., & Daskalakis, Z. J. (2008). A meta-analytic study of changes in brain activation in depression. *Human Brain Mapping*, *29*, 683–695.
Hamilton, M. (1960). A rating scale for depression. *Journal of Neurology Neurosurgery & Psychiatry*, *23*, 56.
Harshaw, C. (2015). Interoceptive dysfunction: Toward an integrated framework for understanding somatic and affective disturbance in depression. *Psychological Bulletin*, *141*, 311–363.
Hatano, K., Terao, T., Hayashi, T., Hirakawa, H., Makino, M., Mizokami, Y., … Shimomura, T. (2019). Affective temperaments are associated with the white matter microstructure in healthy participants. *Bipolar Disorder*, *21*, 539–546.
Jabbi, M., Kippenhan, J. S., Kohn, P., Marenco, S., Mervis, C. B., Morris, C. A., … Berman, K. F. (2012). The Williams syndrome chromosome 7q11.23 hemideletion confers hypersocial, anxious personality coupled with altered insula structure and function. *Proceedings of the National Academy of Sciences of the USA*, *109*, E860–E866.
Kang, J., Bowman, F. D., Mayberg, H., & Liu, H. (2016). A depression network of functionally connected regions discovered via multi-attribute canonical correlation graphs. *NeuroImage*, *141*, 431–441.
Kawamura, Y., Akiyama, T., Shimada, T., Minato, T., Umekage, T., Noda, Y., … Akiskal, H. S. (2010). Six-year stability of affective temperaments as measured by TEMPS-A. *Psychopathology*, *43*, 240–247.
Kay, D. B., Karim, H. T., Soehner, A. M., Hasler, B. P., James, J. A., Germain, A., … Buysse, D. J. (2017). Subjective-objective sleep discrepancy is associated with alterations in regional glucose metabolism in patients with insomnia and good sleeper controls. *Sleep*, *40*(11), zsx155.
Kesebir, S., Gundogar, D., Kucuksubasi, Y., & Tatlidil Yaylaci, E. (2013). The relation between affective temperament and resilience in depression: A controlled study. *Journal of Affective Disorders*, *148*, 352–356.
Krishnan, A., Williams, L. J., Mcintosh, A. R., & Abdi, H. (2011). Partial least squares (PLS) methods for neuroimaging: A tutorial and review. *Neuroimage*, *56*, 455–475.
Kurth, F., Eickhoff, S. B., Schleicher, A., Hoemke, L., Zilles, K., & Amunts, K. (2010). Cytoarchitecture and probabilistic maps of the human posterior insular cortex. *Cerebral Cortex*, *20*, 1448–1461.
Leung, C. M., Wing, Y. K., Kwong, P. K., Lo, A., & Shum, K. (1999). Validation of the Chinese-Cantonese version of the hospital anxiety and depression scale and comparison with the Hamilton Rating Scale of Depression. *Acta Psychiatrica Scandinavica*, *100*, 456–461.
Lin, K., Xu, G., Miao, G., Ning, Y., Ouyang, H., Chen, X., … Akiskal, H. S. (2013). Psychometric properties of the Chinese (Mandarin) TEMPS-A: A population study of 985 non-clinical subjects in China. *Journal of Affective Disorders*, *147*, 29–33.
Liu, C. H., Guo, J., Lu, S. L., Tang, L. R., Fan, J., Wang, C. Y., … Liu, C. Z. (2018a). Increased salience network activity in patients with insomnia complaints in major depressive disorder. *Frontiers in Psychiatry*, *9*, 93.
Liu, C. H., Ma, X., Song, L. P., Fan, J., Wang, W. D., Lv, X. Y., … Wang, C. Y. (2015). Abnormal spontaneous neural activity in the anterior insular and anterior cingulate cortices in anxious depression. *Behavioural Brain Research*, *281*, 339–347.
Liu, C. H., Ma, X., Yuan, Z., Song, L. P., Jing, B., Lu, H. Y., … Wang, C. Y. (2017). Decreased resting-state activity in the precuneus is associated with depressive episodes in recurrent depression. *Journal of Clinical Psychiatry*, *78*, e372–e382.
Liu, P., Taber-Thomas, B. C., Fu, X., & Perez-Edgar, K. E. (2018b). Biobehavioral markers of attention bias modification in temperamental risk for anxiety: A randomized control trial. *Journal of American Academy of Child & Adolescent Psychiatry*, *57*, 103–110.
Manoliu, A., Meng, C., Brandl, F., Doll, A., Tahmasian, M., Scherr, M., … Sorg, C. (2013). Insular dysfunction within the salience network is associated with severity of symptoms and aberrant inter-network connectivity in major depressive disorder. *Frontiers in Human Neuroscience*, *7*, 930.
McIntosh, A. R., & Lobaugh, N. J. (2004). Partial least squares analysis of neuroimaging data: Applications and advances. *Neuroimage 23*(Suppl 1), S250–S263.
Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: A network model of insula function. *Brain Structure & Function*, *214*, 655–667.
Michalska, K. J., Feldman, J. S., Ivie, E. J., Shechner, T., Sequeira, S., Averbeck, B., … Pine, D. S. (2018). Early-childhood social reticence predicts SCR-BOLD coupling during fear extinction recall in preadolescent youth. *Developmental Cognitive Neuroscience*, *36*, 100605.
Moser, D. A., Doucet, G. E., Lee, W. H., Rasgon, A., Krinsky, H., Leibu, E., … Frangou, S. (2018). Multivariate associations among behavioral, clinical, and multimodal imaging phenotypes in patients with psychosis. *JAMA psychiatry*, *75*, 386–395.
Namkung, H., Kim, S. H., & Sawa, A. (2017). The Insula: An underestimated brain area in clinical neuroscience, psychiatry, and neurology. *Trends in Neurosciences*, *40*, 200–207.
Naqvi, N. H., & Bechara, A. (2009). The hidden island of addiction: The insula. *Trends in Neurosciences*, *32*, 56–67.
Otte, C., Gold, S. M., Penninx, B. W., Pariante, C. M., Etkin, A., Fava, M., … Schatzberg, A. F. (2016). Major depressive disorder. *Nature Reviews Disease Primers*, *2*, 16065.
Park, B., Palomares, J. A., Woo, M. A., Kang, D. W., Macey, P. M., Yan-Go, F. L., … Kumar, R. (2016). Aberrant insular functional network integrity in patients with obstructive sleep apnea. *Sleep*, *39*, 989–1000.
Perico, C. A., Skaf, C. R., Yamada, A., Duran, F., Buchpiguel, C. A., Castro, C. C., … Busatto, G. F. (2005). Relationship between regional cerebral blood flow and separate symptom clusters of major depression: A single photon emission computed tomography study using statistical parametric mapping. *Neuroscience Letters*, *384*, 265–270.
Perlis, M. L., Giles, D. E., Buysse, D. J., Thase, M. E., Tu, X., & Kupfer, D. J. (1997). Which depressive symptoms are related to which sleep electroencephalographic variables? *Biological Psychiatry*, *42*, 904–913.
Pompili, M., Baldessarini, R. J., Innamorati, M., Vazquez, G. H., Rihmer, Z., Gonda, X., … Girardi, P. (2018). Temperaments in psychotic and major affective disorders. *Journal of Affective Disorders*, *225*, 195–200.
Pompili, M., Innamorati, M., Gonda, X., Erbuto, D., Forte, A., Ricci, F., … Girardi, P. (2014). Characterization of patients with mood disorders for their prevalent temperament and level of hopelessness. *Journal of Affective Disorders*, *166*, 285–291.
Pompili, M., Innamorati, M., Gonda, X., Serafini, G., Sarno, S., Erbuto, D., … Girardi, P. (2013). Affective temperaments and hopelessness as predictors of health and social functioning in mood disorder patients: A prospective follow-up study. *Journal of Affective Disorders*, *150*, 216–222.

Power, J. D., Barnes, K. A., Snyder, A. Z., Schlaggar, B. L., & Petersen, S. E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage*, *59*, 2142–2154.

Rabkin, J. G., & Klein, D. F. (1987). The clinical measurement of depressive disorders. In A. J. Marsella, R. M. A. Hirschfeld, & M. M. Katz (Eds.), *The measurement of depression* (pp. 30–83). New York, NY, USA: Guilford Press.

Rihmer, Z., Akiskal, K. K., Rihmer, A., & Akiskal, H. S. (2010). Current research on affective temperaments. *Current Opinion in Psychiatry*, *23*, 12–18.

Rihmer, Z., Gonda, X., Torzsa, P., Kalabay, L., Akiskal, H. S., & Eory, A. (2013). Affective temperament, history of suicide attempt and family history of suicide in general practice patients. *Journal of Affective Disorders*, *149*, 350–354.

Rihmer, A., Rozsa, S., Rihmer, Z., Gonda, X., Akiskal, K. K., & Akiskal, H. S. (2009). Affective temperaments, as measured by TEMPS-A, among non-violent suicide attempters. *Journal of Affective Disorders*, *116*, 18–22.

Rovai, L., Maremmani, A. G., Rugani, F., Bacciardi, S., Pacini, M., Dell'Osso, L., … Maremmani, I. (2013). Do Akiskal & Mallya's affective temperaments belong to the domain of pathology or to that of normality? *European Review for Medical and Pharmacological Sciences*, *17*, 2065–2079.

Serafini, G., Pompili, M., Innamorati, M., Fusar-Poli, P., Akiskal, H. S., Rihmer, Z., … Tatarelli, R. (2011). Affective temperamental profiles are associated with white matter hyperintensity and suicidal risk in patients with mood disorders. *Journal of Affective Disorders*, *129*, 47–55.

Serafini, G., Pompili, M., Romano, A., Erbuto, D., Lamis, D. A., Moraschi, M., … Bozzao, A. (2017). Neural correlates in patients with major affective disorders: An fMRI study. *CNS & Neurological Disorders Drug Targets*, *16*, 907–914.

Shi, M., Hu, J., Dong, X., Gao, Y., An, G., Liu, W., … Sun, X. (2008). Association of unipolar depression with gene polymorphisms in the serotonergic pathways in Han Chinese. *Acta Neuropsychiatrica*, *20*, 139–144.

Solmi, M., Zaninotto, L., Toffanin, T., Veronese, N., Lin, K., Stubbs, B., … Correll, C. U. (2016). A comparative meta-analysis of TEMPS scores across mood disorder patients, their first-degree relatives, healthy controls, and other psychiatric disorders. *Journal of Affective Disorders*, *196*, 32–46.

Sprengelmeyer, R., Steele, J. D., Mwangi, B., Kumar, P., Christmas, D., Milders, M., & Matthews, K. (2011). The insular cortex and the neuroanatomy of major depression. *Journal of Affective Disorders*, *133*, 120–127.

Stein, M. B., Simmons, A. N., Feinstein, J. S., & Paulus, M. P. (2007). Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *American Journal of Psychiatry*, *164*, 318–327.

Su, L., Cai, Y., Xu, Y., Dutt, A., Shi, S., & Bramon, E. (2014). Cerebral metabolism in major depressive disorder: A voxel-based meta-analysis of positron emission tomography studies. *BMC Psychiatry*, *14*, 321.

Tian, Y., & Zalesky, A. (2018). Characterizing the functional connectivity diversity of the insula cortex: Subregions, diversity curves and behavior. *NeuroImage*, *183*, 716–733.

Tondo, L., Vázquez, G. H., Sani, G., Pinna, M., & Baldessarini, R. J. (2018). Association of suicidal risk with ratings of affective temperaments. *Journal of Affective Disorders*, *229*, 322–327.

Tu, Y., Fang, J., Cao, J., Wang, Z., Park, J., Jorgenson, K., … Kong, J. (2018). A distinct biomarker of continuous transcutaneous vagus nerve stimulation treatment in major depressive disorder. *Brain Stimulation*, *11*, 501–508.

Uddin, L. Q. (2014). Salience processing and insular cortical function and dysfunction. *Nature Reviews Neuroscience*, *16*, 55.

Uddin, L. Q., Nomi, J. S., Hebert-Seropian, B., Ghaziri, J., & Boucher, O. (2017). Structure and function of the human Insula. *Journal of Clinical Neurophysiology*, *34*, 300–306.

Vazquez, G. H., Gonda, X., Lolich, M., Tondo, L., & Baldessarini, R. J. (2018). Suicidal risk and affective temperaments, evaluated with the TEMPS-A scale: A systematic review. *Harvard Review of Psychiatry*, *26*, 8–18.

Vertes, P. E., Rittman, T., Whitaker, K. J., Romero-Garcia, R., Vasa, F., Kitzbichler, M. G., … Bullmore, E. T. (2016). Gene transcription profiles associated with inter-modular hubs and connection distance in human functional magnetic resonance imaging networks. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *371*, 1–13.

Wang, C., Wu, H., Chen, F., Xu, J., Li, H., Li, H., & Wang, J. (2018). Disrupted functional connectivity patterns of the insula subregions in drug-free major depressive disorder. *Journal of Affective Disorder*, *234*, 297–304.

Wang, X., Zhen, Z., Song, Y., Huang, L., Kong, X., & Liu, J. (2016). The hierarchical structure of the face network revealed by its functional connectivity pattern. *The Journal of Neuroscience*, *36*, 890–900.

Wang, Y. Z., Han, Y., Zhao, J. J., Du, Y., Zhou, Y., Liu, Y., … Li, L. (2019). Brain activity in patients with deficiency versus excess patterns of major depression: A task fMRI study. *Complementary Therapies in Medicine*, *42*, 292–297.

Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). Conn: A functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connectivity*, *2*, 125–141.

Whittle, S., Allen, N. B., Lubman, D. I., & Yucel, M. (2006). The neurobiological basis of temperament: Towards a better understanding of psychopathology. *Neuroscience and Biobehavioral Reviews*, *30*, 511–525.

Wiebking, C., Duncan, N. W., Tiret, B., Hayes, D. J., Marjanska, M., Doyon, J., … Northoff, G. (2014). GABA in the insula – a predictor of the neural response to interoceptive awareness. *Neuroimage*, *86*, 10–18.

World Medical Association (2013). WMA Declaration of Helsiniki-Ethical Principles for Medical Research Involving Human Subjects. https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/.

Yu, H. L., Liu, W. B., Wang, T., Huang, P. Y., Jie, L. Y., Sun, J. Z., … Zhang, M. M. (2017). Difference in resting-state fractional amplitude of low-frequency fluctuation between bipolar depression and unipolar depression patients. *European Review for Medical and Pharmacological Sciences*, *21*, 1541–1550.

Zou, Q. H., Zhu, C. Z., Yang, Y., Zuo, X. N., Long, X. Y., Cao, Q. J., … Zang, Y. F. (2008). An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: Fractional ALFF. *Journal of Neuroscience Methods*, *172*, 137–141.

Zuo, X. N., Di, M. A., Kelly, C., Shehzad, Z. E., Gee, D. G., Klein, D. F. … Milham, M. P. (2010). The oscillating brain: Complex and reliable. *Neuroimage*, *49*, 1432.

https://doi.org/10.1017/S0033291719003647 Published online by Cambridge University Press