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ORIGINAL ARTICLE

Salience network connectivity in the insula is associated with individual differences in interoceptive accuracy

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Abstract The insula and the anterior cingulate cortex are core brain regions that anchor the salience network, one of several large-scale intrinsic functional connectivity networks that have been derived consistently using resting-state functional magnetic resonance imaging (fMRI). While several studies have shown that the insula and anterior cingulate cortex play important roles in interoceptive awareness, no study to date has examined the association between intrinsic salience network connectivity and interoceptive awareness. In this study, we sought to test this idea in 26 healthy young participants who underwent a resting-state fMRI scan and a heartbeat counting task outside the scanner in the same session. Greater salience network connectivity in the posterior insula (but not the anterior cingulate cortex) using independent component analysis correlated with higher accuracy in the heartbeat counting task. Furthermore, using seed-based approach, greater interoceptive accuracy was associated with greater intrinsic connectivity of all insular functional subdivisions to salience network regions, including the anterior insula, orbitofrontal cortex, ventral striatum and midbrain. These associations remained after correcting for voxel-wise grey matter volumes. The findings underscore the critical role of insular salience network intrinsic connectivity in interoceptive awareness and pave the way for future investigations into how salience network dysconnectivity affects interoceptive awareness in brain disorders.

Keywords Salience network · Interoceptive awareness · Insula · Intrinsic functional connectivity

Introduction

Intrinsic connectivity network (ICN) functional magnetic resonance imaging (fMRI) provides a novel tool to map temporally synchronous, spatially distributed, spontaneous low frequency (<0.08 Hz) blood-oxygen-level-dependent (BOLD) signal fluctuations at rest, or, more accurately, in task-free settings (Biswal et al. 1995; Fox and Raichle 2007). To date, ICN fMRI has been used to predict individual differences in human behaviour and cognition (Di Martino et al. 2009; Hampson et al. 2006; Reineberg et al. 2015; Seeley et al. 2007) and detect symptom-specific connectivity changes in disease (e.g. Greicius et al. 2004; Sheline and Raichle 2013; Zhou et al. 2010). Of the multiple large-scale ICNs consistently discovered across sites, the salience network, consisting of the insula, anterior cingulate cortex (ACC) and subcortical regions such as the ventral striatum, amygdala, ventral tegmental area, and midbrain, serves as the 'dynamic switch', biasing activation of other task-positive or task-negative networks when a salient external event is detected (Menon and Uddin 2010; Seeley et al. 2007). Intrinsic connectivity within the salience network regions, referred to as salience network

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connectivity, corresponds closely to task-related co-activations (Menon 2015) and shows disruptions in a range of brain disorders including schizophrenia (Palaniyappan et al. 2013), autism spectrum disorders (Uddin et al. 2013), and frontotemporal dementia (Zhou et al. 2010). Of the salience network regions, the most prominent structures are the anterior insula and anterior cingulate cortex (ACC), which consistently show coactivation in response to both internal and external salience (Craig 2009). The anterior insula, a major hub of the salience network, is proposed to integrate external (such as sights and sounds) and internal (such as feelings and emotions) stimuli in order to, together with the ACC, guide an individual's actions and decisions (Craig 2009). These structures are thus commonly implicated in interoceptive awareness—the awareness of feelings such as hunger, thirst, and body temperature, which arises from changes in internal body states and visceral organs (Craig 2003).

To probe individual differences in interoceptive accuracy, heartbeat detection tasks are usually employed. One such task is the heartbeat counting task, which involves participants counting their own heartbeats for a certain period of time while their heart rate is recorded using an electrocardiogram (e.g. Schandry 1981). Another commonly utilised task is the heartbeat discrimination task, in which participants report the onset of heartbeats, either through tapping, or determining the synchrony between their heartbeats and external stimuli such as tones (e.g. Brener and Kluvitse 1988; Whitehead et al. 1977). Using these tasks, several studies have demonstrated that significant differences in interoceptive awareness exist between different clinical populations. Patients with depressive, somatoform, and personality disorders, for example, show lower degrees of interoceptive awareness (Mussgay et al. 1999) while patients with panic disorders show the opposite relation (Ehlers et al. 1988). Even in healthy populations, substantial differences in interoceptive awareness have been found (Cameron 2001; Wiens and Palmer 2001).

Additionally, these tasks have been employed in brain imaging studies to support the role of the insula and ACC in interoceptive awareness. Critchley et al. (2004) reported enhanced blood-oxygen-level-dependent (BOLD) responses in the insular, somatomotor and cingulate cortices while participants performed a heartbeat discrimination task during an fMRI scan. Further, neural activity in the right anterior insular/opercular cortex correlated with participants' accuracy in the task (Critchley et al. 2004). Similarly, Pollatos et al. (2005) recorded electroencephalogram (EEG) during a heartbeat perception task and found a positive association between accuracy of heartbeat perception and dipole strength at the ACC and the right insula. Other measures of interoceptive awareness, such as gastrointestinal sensations (Van Oudenhove et al. 2004) and breath monitoring (Farb et al. 2013), have also implicated the ACC and insula as important salience network regions for interoceptive awareness.

However, to our knowledge, no study to date has looked directly at whether intrinsic salience network connectivity is associated with individual differences in behavioural measures of interoceptive awareness. This is despite the fact that, as aforementioned, individual differences in behavioural measures of interoceptive awareness are correlated with neural activity in certain salience network structures (Critchley et al. 2004; Pollatos et al. 2005). Therefore, the present study sought to fill this gap by examining the relationship between intrinsic salience network connectivity and individual differences in interoceptive awareness in a group of healthy young participants. We employed the heartbeat counting task to probe inter-individual differences in interoceptive accuracy, given that the task is more reflective of internal monitoring processes than the heartbeat discrimination task, which additionally requires processing of exteroceptive stimuli (Garfinkel et al. 2015; Schulz et al. 2013). We hypothesized that better performance on the heartbeat counting task will be correlated with greater functional connectivity of the salience network, particularly in the anterior insula and the ACC which forms the core of the network.

Methods

Participants

We studied 26 healthy young adults [12 females, 1 left-handed, mean age 26.1 (SD = 5.6) years]. All participants provided informed consent in accordance with the guidelines of the Institutional Review Board for the National University of Singapore.

Image acquisition

The participants underwent one neuroimaging session on a 3T Siemens Tim Trio system (Siemens, Erlangen, Germany). The neuroimaging consisted of a high-resolution T1-weighted image of the entire brain in 192 sagittal slices (magnetization prepared rapid gradient echo sequence with repetition time = 2300 ms, echo time = 2.98 ms, inversion time = 900 ms, flip angle = 90°, field of view = 256×256 mm^2, voxel size = 1.0 mm isotropic) and an 8-min resting-state fMRI scan (T2*-weighted echo planar sequence with repetition time = 2000 ms, echo time = 30 ms, flip angle = 90°, field of view = 192×192 mm^2, voxel size = 3.0 mm isotropic, slice thickness = 3 mm, 36 axial slices, interleaved collection) in which participants were instructed to remain awake and fixate on a white cross in the centre of a black screen.

Body awareness questionnaire

After the scan, subjective measures of bodily awareness were obtained from all participants using the Body Awareness Questionnaire (BAQ) (Shields et al. 1989). Each item on the 18-item questionnaire was rated on a 7-point rating scale ranging from not true at all (1) to very true (7).

Reliability of the BAQ was determined by calculating Cronbach's alpha, using the 'psy' package in R (Falissard 2012; R Core Team 2015). The 18-item questionnaire had high reliability, $\alpha = 0.86$.

Heartbeat counting task

All participants completed the heartbeat counting task immediately after the scan and questionnaire on the same day. Following previous work (Meissner and Wittmann 2011; Pollatos et al. 2005; Schandry 1981), participants were instructed to attend to their own heartbeats and count them silently over four heartbeat counting intervals (25, 35, 45 and 60 s). Each counting interval was presented three times, giving rise to a total of 12 trials. The start and end of each counting interval were indicated by a change in colour of the cross on the screen (i.e. from white cross to green cross). At the end of each interval, participants were prompted to indicate the number of heartbeats counted, following which a 30 s rest period was given before the start of the next counting interval. Participants were also instructed to refrain from closing their eyes and using any other physical strategies to aid the counting of heartbeats.

Heartbeats of participants were continuously monitored and recorded throughout the heartbeat counting task using a Comet-PLUS 57-Channel Digital PSG Record & Review Portable System and an AS40-PLUS Amplifier System, with the TWin PSG software for acquisition of data (GRASS Products, Natus Neurology Incorporated, Middleton, WI, USA). The electrocardiogram (ECG) signal was sampled at a rate of 500 Hz. Gold electrodes were attached to the participants prior to the heartbeat counting task, and participants were given a 5-min break to stabilize their heart rate before the task commenced.

Heartbeat counting scores were calculated using the formula from Pollatos et al. (2007). Each subject's accuracy in the perception of their heartbeats was quantified by first taking the absolute difference between the actual number of heartbeats recorded by the electrocardiogram and the number of heartbeats counted by the subject, divided by the actual number of heartbeats. This value was then subtracted from 1 and then averaged across all trials to yield a heartbeat counting score such that the higher the score, the better the performance on the heartbeat counting task. The mathematical formula is thus:

$$\frac{1}{12}\Sigma\left(1-\frac{|\text{recorded heartbeats} - \text{counted heartbeats}|}{\text{recorded heartbeats}}\right).$$

Image preprocessing

The task-free fMRI data were preprocessed following our previous work (Ng et al. 2016; Wang et al. 2016) using the FMRIB Software Library (FSL) (Jenkinson et al. 2012) and the Analysis of Functional NeuroImages software (Cox 1996). The preprocessing included the following steps: (1) discarding the first five volumes to allow for magnetic field stabilization; (2) correcting for motion; (3) spatial smoothing and grand mean scaling; and (4) co-registering the structural MRI image using Boundary-Based Registration (Greve and Fischl 2009) and then registering the fMRI image to the Montreal Neurological Institute (MNI) 152 standard space using a nonlinear registration tool (FNIRT). For the seed-based analyses, further preprocessing steps were performed, including: (1) time series despiking, bandpass temporal filtering and removal of linear and quadratic trends prior to co-registration; and (2) regressing out global, cerebrospinal fluid (CSF), white matter and six motion signals from the co-registered fMRI images. All subjects passed quality control measures (absolute displacement <3 mm and relative displacement <1 mm).

Subject-level grey matter (GM) volume probability maps were derived from T1-weighted images using voxel-based morphometry (VBM). VBM was performed using VBM8 toolbox (Structural Brain Mapping Group; http://dbm.neuro.uni-jena.de/software/) for Statistical Parametric Mapping (SPM8) (Wellcome Trust Centre for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm/software/spm8/), and included: (1) segmenting individual T1-weighted images into GM, white matter (WM) and CSF using an adaptive Maximum A Posterior technique (Rajapakse et al. 1997), which eliminates the use of tissue priors; (2) registering each GM/WM probability map to an existing DARTEL template derived from 550 healthy subjects of the IXI-database (http://www.brain-development.org) (Ashburner 2007); (3) performing modulation by multiplying voxel values with the non-linear component of the Jacobian determinant to account for individual brain sizes; (4) applying smoothing on the normalized GM maps by an 8 mm full-width at half-maximum (FWHM) Gaussian kernel.

Salience network connectivity

ICN maps for the salience network were obtained from the preprocessed task-free fMRI data using spatial independent component analysis (ICA) with the Group ICA of fMRI toolbox (GIFT) in MATLAB (Medical Image Analysis Lab; http://mialab.mrn.org/software/gift/index.html). Spatial ICA estimates a set of spatially independent components from the combined time courses of whole-brain volumes across all participants via minimization of mutual information among components (Calhoun et al. 2001). Each component consists of a time course and an associated voxel-wise spatial map, where each voxel has a z score representing the correlation between the voxel's time course and the mean time course of the component. To perform spatial ICA, principal component analyses (PCA) were first performed to reduce the dimension of the fMRI data. ICA was then applied to separate the combined subjects' data into a set of group-level spatially independent components. The number of components was set to twenty in accordance with previous studies (Damoiseaux et al. 2006). Individual subject spatial maps and their corresponding time courses were subsequently back-reconstructed from the group-level components and PCA-reduced data using GICA3. Briefly, GICA3 derives the subject-specific spatial maps and time courses by partitioning the inverse of the group PCA matrix while maintaining two desirable properties: (1) the sum of the estimated subject spatial maps is equal to the group-level spatial map; and (2) the PCA-reduced data matches the PCA-reduced product of the estimated subject time courses and spatial maps (Erhardt et al. 2011). Finally, spatial template matching was performed using multiple linear regression followed by visual inspection to identify the best-fit spatial component map corresponding to the salience network (the template was derived from binarized group ICA maps of 15 healthy young adults in previous published work (mean age: 26.5 years, range: 19–40 years, nine females, all right-handed) (Habas et al. 2009) which were thresholded at a z score of 4.0 to visually match the ICNs reported by Damoiseaux et al. (2006). As a control, subject-level spatial maps for the visual network (based on the template by Habas et al. (2009)) were also derived using the same procedure described above.

Statistical analyses

To examine the relationship between the salience network connectivity and measures of body awareness, whole-brain voxel-wise regression analysis of subject-level salience network functional connectivity maps (derived from ICA) against the heartbeat counting scores was performed across all subjects using the Statistical Parametric Mapping (SPM8) toolbox (Wellcome Trust Centre for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Results were thresholded using an uncorrected joint height ($p < 0.05$) and extent threshold ($p < 0.05$), and group-averaged salience network connectivity maps were used as an inclusive mask (thresholded at a false discovery rate (FDR) of $q < 0.05$). To visualize the results, mean connectivity z scores of clusters (representing the mean correlation between the time course of each voxel in the cluster and the mean time course of the salience network component across all voxels in the cluster) that showed significant negative or positive correlations with HBC scores were then extracted using the MarsBar toolbox (Brett et al. 2002) and plotted in a scatterplot against heartbeat counting scores.

To confirm that the association between intrinsic connectivity and heartbeat counting scores was specific to the salience network and not to other networks or confounded by other variables, the following additional analyses were performed: (1) addition of grey matter probability maps as a voxel-wise covariate using the biological parametric mapping (BPM) toolbox (Casanova et al. 2007); (2) whole-brain voxel-wise regression of grey matter volumes against heartbeat counting scores; and (3) whole-brain voxel-wise regression of subject-level visual network connectivity maps against heartbeat counting scores.

The insula has been shown to be functionally differentiated into four separate regions each with distinct functions: social-emotional, sensorimotor, chemical sensory and cognitive (Kurth et al. 2010). To clarify which insular subdivision(s) and their connected brain regions were linked to interoceptive awareness, we further examined the relationship between voxel-wise connectivity maps of each insular subdivision and heartbeat counting scores. We first derived eight insular functional seeds [i.e. two (left and right) × four (insula subdivisions)] using the functional parcellation of the insula based on the meta-analysis of 1768 functional neuroimaging experiments (Kurth et al. 2010; http://anima.fz-juelich.de). After removing overlapping regions between the seeds and reslicing them to match the resolution of the fMRI data, whole-brain voxel-wise seed-based connectivity maps were then obtained for each of the eight seeds. Briefly, this involves for each subject extracting the average fMRI BOLD time series across all voxels within each seed and then computing Pearson's correlations between the seed's time series and every other voxel's time series. The correlation values were converted using Fisher's r-to-z transformation to obtain subject-level z score maps for each seed. Finally, whole-brain voxel-wise regression of each seed-based connectivity map against the heartbeat counting scores was performed to identify brain regions connected to the insular subdivisions that were correlated with heartbeat counting scores. All results were thresholded using an uncorrected joint height ($p < 0.05$) and extent threshold ($p < 0.05$). To ensure that the results were not confounded by grey matter volumes, the seed-based analyses were repeated with voxel-wise grey matter volumes as an additional covariate.

Results

Behavioural results

Subjects yielded total BAQ scores of between 58 and 112 ($M = 84.62$, $SD = 14.91$), and heartbeat counting scores of between 0.377 and 0.934 ($M = 0.687$, $SD = 0.140$). Total BAQ scores were significantly positively correlated with HBC scores [$r(24) = 0.54$, $p = 0.004$], suggesting that accurate perception of one's heartbeat is correlated with one's subjective perception of bodily awareness. This finding is in agreement with the findings by Garfinkel et al. (2015) who found positive associations between the two measures in individuals with high interoceptive accuracy, although we noted as well that some studies have reported no correlations between subjective and objective measures of interoceptive awareness (Critchley et al. 2004).

Association between salience network connectivity and accuracy in heartbeat perception

Within the group-averaged salience network map derived from ICA ($p < 0.05$, FWE-corrected, Fig. 1a), we found a significant positive correlation between heartbeat counting scores and salience network connectivity in the right posterior insula and a trend towards a positive correlation in the left posterior insula (right insula: 48, −10, 2; cluster size of 252 voxels (extent threshold $p < 0.05$), peak t score of 4.94; left insula: −44, −18, 8; cluster size of 167 voxels (extent threshold slightly below the threshold, $p = 0.090$), peak t score of 3.39), but no correlation was found for the anterior insula or the ACC (Fig. 1b, c). No significant negative correlations were detected between heartbeat counting scores and salience network connectivity.

Even after controlling for grey matter volumes in a voxel-wise manner, positive correlations between salience network connectivity in the bilateral posterior insula and heartbeat counting scores remained, although clusters were smaller and no longer significant (right insula: 48, −10, 2; cluster size of 232 voxels, extent threshold $p = 0.068$), peak t score of 4.72; and left insula: −44, −18, 8; cluster size of 164 voxels, extent threshold $p = 0.121$), peak t score of 3.32) (Supplementary Fig. S1). Additionally, we did not find any significant correlations between grey matter volumes and heartbeat counting scores, as well as between visual network connectivity and heartbeat counting scores.

Association between the connectivity of insular subdivisions and accuracy in heartbeat perception

Given the positive association between salience network connectivity in the posterior insula and heartbeat counting scores, we sought to examine further the relationship between insular connectivity and interoceptive accuracy. To this end, we derived seed-based voxel-wise connectivity maps for each insular subdivision [based on the functional parcellation of the insula by Kurth et al. (2010)] and correlated them with heartbeat counting scores. Consistent with our results from the ICA analyses, greater right sensorimotor insular connectivity in salience network-related regions, including the bilateral anterior insula, medial orbitofrontal cortex, ventral striatum and midbrain was associated with higher interoceptive accuracy (Fig. 2; Supplementary Table S1). More interestingly, we found similar results for the other seven insular seeds, with extensive overlap observed across each seed's results (≥four) in these same regions (Supplementary Fig. S2b). In contrast, no negative correlations between interoceptive accuracy and insular functional connectivity were observed in any brain regions within the salience network. Similar results were obtained even after controlling for grey matter volumes in a voxel-wise manner.

Discussion

The present results provide new insights regarding how salience network intrinsic functional connectivity at rest correlated with individual differences in interoceptive awareness. Using the ICA approach, participants who showed greater salience network connectivity in the posterior insula at rest perceive their own bodily responses more readily. Additionally, seed-based intrinsic connectivity of all insular subdivisions to brain regions within or related to the salience network, including the anterior insula, orbitofrontal cortex, ventral striatum and midbrain, showed a positive correlation with heartbeat counting scores, thereby further supporting the role of intrinsic salience network connectivity in interoceptive awareness. Further, these results for both ICA and seed-based analyses remained after controlling for grey matter volumes.

Our study is, to our knowledge, the first to demonstrate that differences in insular functional connectivity in the salience network at rest can be associated with individual differences in interoceptive awareness. According to the proposed model by Zhou and Seeley (2014), the salience network is anchored by the afferent anterior insula, which integrates incoming internal and external information (Craig 2002) and represents "feeling" states, and the efferent anterior cingulate cortex, which regulates and activates responses to salience. Internal interoceptive and visceromotor information to the anterior insula comes from the posterior insula, which is postulated to be the primary interoceptive area in the cortex (Craig 2002) and in turn receives autonomic inputs from the thalamus and the

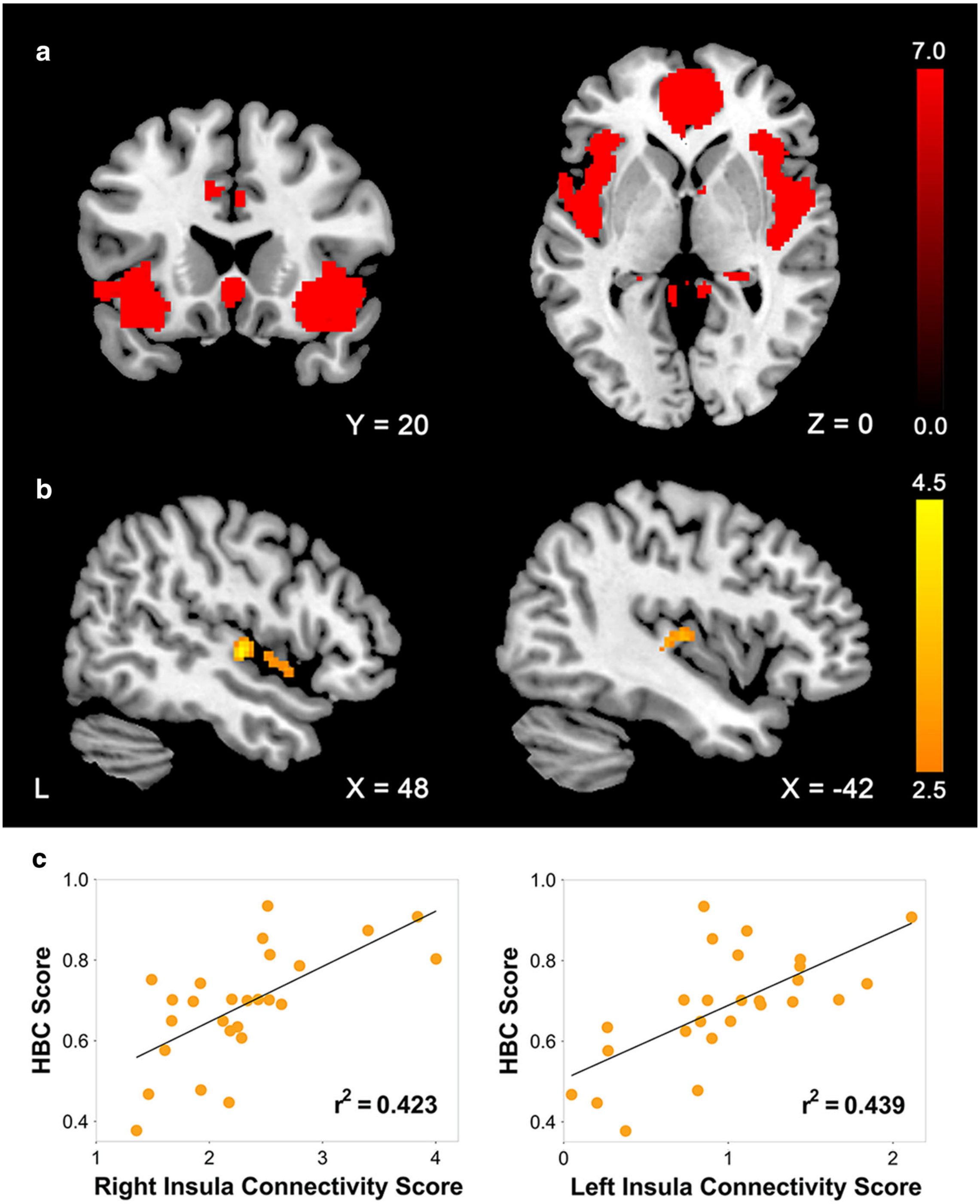

Fig. 1 Salience network connectivity in the insula is positively correlated with accuracy in the heartbeat counting task. **a** Salience network averaged across all participants (height threshold: $p < 0.05$, corrected for family-wise error, extent threshold: 200 voxels, masked with a binary grey matter map (grey matter tissue probabilistic map thresholded at 0.2, SPM8). **b** The posterior insula, but not the ACC, shows increased salience network connectivity with increased accuracy in the heartbeat counting task. **c** Scatterplots further demonstrate the positive relationship between connectivity scores in the posterior insula and heartbeat counting scores. Coefficients of determination (r^2) are given in the scatterplots

midbrain (Critchley and Harrison 2013). External information, on the other hand, is sent to the anterior insula via the closely allied semantic-appraisal network, which comprised the temporal pole, ventral striatum, medial orbitofrontal cortex, and amygdala. These regions show some evidence of strong structural connections to the anterior insula (specifically the orbitofrontal cortex; Wiech et al. 2014) and are postulated to assign hedonic values to

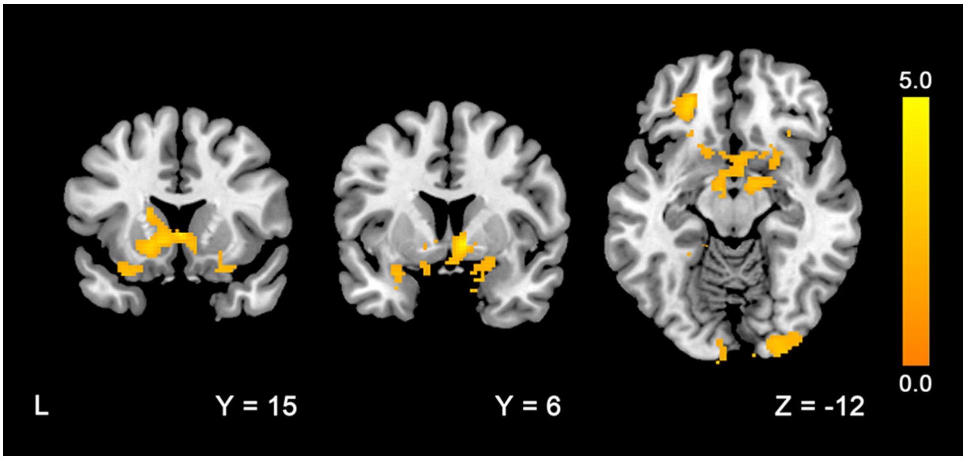

Fig. 2 Increased intrinsic connectivity of the right sensorimotor insular seed to salience network regions is associated with greater accuracy in the heartbeat counting task. We derived the intrinsic functional connectivity to the bilateral sensorimotor insular seeds for each participant (Kurth et al. 2010) (Supplementary Fig. S2a). Across individuals, increased right sensorimotor insular connectivity to salience network regions (*highlighted* in *orange*), including the anterior insula, orbitofrontal cortex, ventral striatum and midbrain, is associated with greater interoceptive accuracy (heartbeat counting scores)

incoming external stimuli through bidirectional interactions with the anterior insula (Guo et al. 2013).

In light of this model, it is thus expected that greater interoceptive awareness would be associated with greater connectivity between these regions that are involved in the integration, processing and representation of internal and external information. For example, greater connectivity between the anterior insula and regions involved in representation of interoceptive states could indicate more information flow about interoceptive states to the integrative centre (anterior insula), while greater connectivity between the anterior insula and regions in the semantic-appraisal network could suggest more feedback from the anterior insula to reduce the salience of incoming external stimuli. In agreement with this, we found increased posterior insular connectivity to the salience network with greater interoceptive accuracy, which supports its role as the main interoceptive cortex. Further, interoceptive awareness was positively associated with insular connectivity (both posterior and anterior insular seeds) to the ventral striatum, orbitofrontal cortex, anterior insula and midbrain, all of which are regions involved in either the representation of internal (midbrain) and external (ventral striatum, orbitofrontal cortex) information or the integration of both types of information (anterior insula).

We hypothesized that the salience network connectivity of the two major hubs of the salience network, the anterior insula and ACC, in particular, would be positively correlated with interoceptive awareness. Unexpectedly, we did not find any associations between interoceptive awareness and intrinsic ACC connectivity, which contradicts past research reporting higher dipole strength at the ACC in good heartbeat perceivers (Pollatos et al. 2005). This lack of association might be related to differences in paradigms used in the current study and those employed in previous studies. In previous studies, researchers used task-based fMRI to examine brain regions that were recruited during interoceptive awareness tasks (Critchley et al. 2004). By comparison, the present study examined ICNs where the task was performed separately from the neuroimaging scan. It has been proposed that changes in bodily states are a two-order process: a first-order context-independent autonomic representation within the insula and somatosensory cortices, and a second-order context- and experience-dependent representation within the cingulate and ventromedial prefrontal cortices (Critchley et al. 2001). The lack of a context-dependent representation of bodily states in this study might thus explain why no change was observed in the salience network connectivity of the ACC.

Interestingly, Kuehn et al. (2016) found reduced task-based functional connectivity between the anterior and posterior insula as well as reduced network centrality in the posterior insula in participants with high interoceptive awareness. By comparison, we found increased connectivity between the insular subdivisions (which included posterior insular seeds) and anterior insula to be associated with greater interoceptive accuracy. The difference in findings might be attributed to differences in task conditions: we examined functional connectivity under task-free conditions while Kuehn et al. (2016) examined functional connectivity during a heartbeat monitoring task. Studies have demonstrated that task-free (intrinsic) and task-based (extrinsic) functional connectivity are weakly correlated and are likely to reflect different facets of connectivity, with intrinsic connectivity reflecting task-independent spontaneous BOLD signal fluctuations and task-based connectivity largely modulated by the specific task (Mennes et al. 2013; Rehme et al. 2013). In agreement with our results, high functioning individuals with autism spectrum disorder, who are impaired in the emotional awareness of self and others, also showed reduced intrinsic connectivity between the anterior and posterior insula compared to controls (Ebisch et al. 2011).

Given that the heartbeat counting task was performed independently of the scan, our findings suggest that intrinsic salience network connectivity, particularly in the posterior insula, may potentially serve as a trait-like marker for predicting interoceptive accuracy in individuals. In addition, our results have potential implications in the understanding of the salience network and its role in disease pathophysiology. By establishing a link between interoceptive awareness and insular connectivity in the salience network in healthy controls, our study provides further clarity into the roles and functions of the salience network, as we have outlined earlier in the discussion. Given that salience network connectivity is affected in several brain disorders, the findings in this study could pave the way for further investigations into how dysfunctional changes in salience network connectivity could relate to interoceptive awareness changes in these patients. Conversely, salience network connectivity changes of patients suffering from disorders with known impairments in interoceptive awareness, such as diabetic neuropathy (Leopold and Schandry 2001), arrhythmias (Ehlers et al. 2000) and somatoform disorders (Mussgay et al. 1999; Schaefer et al. 2012), could be investigated to determine if such connectivity changes underlie impairments in body awareness and perception.

One limitation of the study is that it did not differentiate between endogenous and exogenous sources of interoceptive awareness. Endogenous interoceptive awareness refers to awareness which arises when one explicitly directs attention to internal (visceral) bodily states. On the other hand, exogenous interoceptive awareness refers to awareness arising due to bottom-up physiological signals such as pain, heat, or touch (Craig 2002; Farb et al. 2013). Therefore, a possible avenue for future exploration would be to examine the relation between these different types of interoceptive awareness and salience network connectivity. Additionally, our study only examined the relationship between intrinsic connectivity and interoceptive accuracy. Further studies are needed to examine the relationships between task-free intrinsic connectivity and task-based activation/connectivity in interoceptive awareness. Finally, despite past reports of a positive association between insular/opercular grey matter volumes and interoceptive accuracy (Critchley et al. 2004), we did not find any significant association between whole-brain grey matter volumes and heartbeat counting performance. Future studies utilising larger samples and more complicated analytical models (such as mediation) could be performed to investigate the joint effects of structure and function on interoceptive awareness.

In conclusion, the present study showed a significant positive association between salience network connectivity of the posterior insula and interoceptive awareness. Additionally, increased intrinsic connectivity of the insular functional subdivisions to salience network regions, including the anterior insula, orbitofrontal cortex, ventral striatum and midbrain, was associated with greater interoceptive accuracy. Our findings provide further insight into the functions of the salience network, a key intrinsic connectivity network that is implicated in several brain disorders, and may therefore further advance the understanding of the pathophysiologies underlying brain disorders in which salience network dysconnectivity is implicated.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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