Hemispheric divergence of interoceptive processing across psychiatric disorders

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Abstract

Interactions between top-down attention and bottom-up visceral inputs are assumed to produce conscious perceptions of interoceptive states, and while each process has been independently associated with aberrant interoceptive symptomatology in psychiatric disorders, the neural substrates of this interface are unknown. We conducted a preregistered functional neuroimaging study of 46 individuals with anxiety, depression, and/or eating disorders (ADE) and 46 propensity-matched healthy comparisons (HC), comparing their neural activity across two interoceptive tasks differentially recruiting top-down or bottom-up processing within the same scan session. During an interoceptive attention task, top-down attention was voluntarily directed towards cardiorespiratory or visual signals, whereas during an interoceptive perturbation task, intravenous infusions of isoproterenol (a peripherally-acting beta-adrenergic receptor agonist) were administered in a double-blinded and placebo-controlled fashion to drive bottom-up cardiorespiratory sensations. Across both tasks, neural activation converged upon the insular cortex, localizing within the granular and ventral dysgranular subregions bilaterally. However, contrasting hemispheric differences emerged, with the ADE group exhibiting (relative to HCs) an asymmetric pattern of overlap in the left insula, with increased or decreased proportions of co-activated voxels within the left or right dysgranular insula, respectively. The ADE group also showed less agranular anterior insula activation during periods of bodily uncertainty (i.e., when anticipating possible isoproterenol-induced changes that never arrived). Finally, post-task changes in insula functional connectivity were associated with anxiety and depression severity. These findings confirm the dysgranular mid-insula as a key cortical interface where attention and prediction meet real-time bodily inputs, especially during heightened awareness of interoceptive states. Further, the dysgranular mid-insula may indeed be a “locus of disruption” for psychiatric disorders.

Introduction

Maintaining the body in a physiologically optimal state is a primary function of the mammalian nervous system. The neurovisceral architecture accomplishing such feats depends on the hierarchical central processing of peripheral internal body signals and operates hierarchically and across heterogenous time scales (Smith et al. 2017), automatically returning the internal state to a setpoint (i.e., homeostasis) (Cannon 1939) or shifting the internal state away from a setpoint in anticipation of future deviations (i.e. allostasis) (Sterling 2012). Importantly, these regulatory operations may depend on the unconscious as well as conscious monitoring of interoceptive states. Interoceptive sensations may even dominate awareness during significant physiological disturbances, motivating behaviors that restore bodily states to homeostatic equilibrium (Khalsa and Lapidus 2016) and forming the basis for emotional experience and numerous cognitive processes (Adolfi et al. 2017; Carvalho and Damasio 2021; Critchley and Garfinkel 2017; Uddin et al. 2014). In turn, dysfunctional interoception has been implicated in the pathophysiology of psychiatric (Bonaz et al. 2021; Khalsa et al. 2018) and neurological disorders (Birba et al. 2022; Drane et al. 2020), underscoring the importance of identifying the form and function of interoceptive neurocircuitry in humans, particularly in relation to conscious experience.

Conscious interoceptive perceptions must, to a considerable extent, reflect the organism's current physiological state. This is conveyed to the brain by cascades of peripheral interoceptive inputs, from spinal and vagal afferent nerves relaying signals transduced from interoceptors located throughout the major internal organ systems (e.g., cardiovascular, respiratory, gastrointestinal, urogenital), as well as via direct chemohumoral signaling (Berntson and Khalsa 2021). Ascending interoceptive pathways converge via brainstem and thalamic nuclei onto the insular cortex, a structurally and functionally diverse region suggested to be the integrative hub for interoceptive awareness, emotional feelings, and overall body awareness (Craig 2002, 2009) (for discussions of additional pathways and cortical targets see (Berntson and Khalsa 2021; Khalsa et al. 2009; Mayeli et al. 2023). A principal role for the insular cortex in interoceptive awareness is supported by previous functional neuroimaging studies from our group, which have highlighted the insula as the primary region dynamically tracking pharmacological perturbations of the heart and lungs in both healthy individuals (Hassanpour et al. 2016, 2018), and those with generalized anxiety disorder (Teed et al. 2022). Our studies have identified multiple subregions of the insula in this "bottom-up" processing of afferently relayed cardiorespiratory signals, including the dysgranular mid and granular posterior sectors, in general agreement with existing theoretical (Barrett and Simmons 2015; Fermin, Friston, and Yamawaki 2022) and cytoarchitectonic (Evrard 2019) arguments regarding their function.

While cortical sensitivity to ascending signals is presumptively required for conscious detection of internal physiological shifts in humans, accumulating evidence also suggests that subjective perceptions (in any sensory modality) are not simply veridical reflections of these ascending inputs, faithfully transmitted and reproduced in a dormant sensory cortex (Clark 2013; Corbetta, Patel, and Shulman 2008; Mechelli et al. 2004; Pace-Schott et al. 2019). Instead, intrinsic "top-down" processes also appear to modulate the activity of sensory cortices (including the insula), potentially altering the resulting percept in the process. For example, internal models of the body that anticipate the sensory consequences of descending regulation can adjust the precision-weighting of prediction error signals in a context-specific manner, increasing or decreasing the

sensory processing of interoceptive inputs (Clark 2016; Petzschner et al. 2021; Pezzulo, Barca, and Friston 2015; Seth and Friston 2016). Extending beyond these model-based influences are goal-directed activities such as the attentional spotlight effect, whereby focusing voluntary attention towards certain environmental signals not only alters their conscious experience but selectively enhances neural activity in the responsive area of cortex (Brefczynski and DeYoe 1999). With respect to interoception, such goal-directed shifts in attention towards endogenous visceral signals have consistently identified activation of the mid insular cortex in healthy individuals (Avery et al. 2017; Simmons et al. 2013), and revealed abnormal mid insula activity associated with symptoms in those with eating disorders (Kerr et al. 2016), depression (Avery et al. 2014; Burrows et al. 2022), and substance use disorders (Stewart et al. 2020).

Conscious perceptions of interoceptive signals are thus underpinned by reciprocal interactions between bottom-up, ascending interoceptive afferents and the coupled, top-down descending efferent systems that enact adaptive regulation and attentional control (Berntson and Khalsa 2021). Based on the available evidence, the insula is uniquely positioned to serve as a cortical interface between these two streams for the processing of interoceptive signals. The highly interconnected nature of the mammalian insula with other cortical (including prefrontal (Mufson and Mesulam 1982), somatosensory (Augustine 1996), and cingulate (Mufson and Mesulam 1982) cortices) and subcortical (amygdala, thalamus (Gehrlach et al. 2020), and brainstem nuclei (Gogolla 2017)) areas facilitates the ability for salient bottom-up activity to recruit conscious attention mechanisms and/or give rise to perceptions and feelings that can adaptively drive behavior and promote homeostatic regulation (Evrard 2019). Furthermore, disrupted processing in this region may be characteristic of individuals with certain psychiatric disorders. In a previous human functional magnetic resonance imaging (fMRI) meta-analysis, abnormal activation of the left dorsal mid-insula differentiated healthy individuals from groups of individuals with anorexia nervosa, anxiety disorders, depression, bipolar disorder, or schizophrenia, across tasks involving the bottom-up modulation of pain, dyspnea, or hunger or the top-down manipulation of interoceptive attention (Nord, Lawson, and Dalgleish 2021). This insular "locus of disruption" was inferred as a possible transdiagnostic and domain-general neuromarker of psychopathology, and the finding advanced the field by pointing toward the mid-insula as an important area for interoceptive dysfunction and a putative target for clinical intervention (Paulus and Khalsa 2021). Despite the theoretical and empirical convergence related to bottom-up and top-down processing in the insula , no study to date has directly investigated both interoceptive processing streams together in the same sample.

Here, we conducted a pre-registered conjunction analysis of two established neuroimaging tasks preferentially manipulating the assessment of top-down and bottom-up interoception (Adamic et al. 2021). Our hypotheses, based on a voxelwise whole-brain approach, were threefold: 1) broad regions of the dysgranular mid insula would be co-activated when a) engaging top-down interoceptive attention under physiological resting conditions and b) during bottom-up perturbation of peripheral interoceptive signals via the adrenergic drug isoproterenol, 2) the agranular anterior insula would be preferentially selective to manipulations of interoceptive uncertainty, such as those occurring during saline infusions, and 3) relative to healthy individuals, interoceptive processing in the insula would show lateralized differences across a transdiagnostic group of individuals with anxiety, depression, and/or eating disorders. To extend the functional

interpretation of convergence, we used the convergent dysgranular insula regions identified in the initial analysis as seeds in an exploratory whole-brain resting state functional connectivity (FC) analysis. Finally, we isolated divergent voxels that were selective to the engagement of top-down expectancies about the body, focusing on temporal windows of the ISO task when the anticipation of potential interoceptive change was maximal.

Methods

Participants

Seventy individuals with primary diagnoses of anxiety, depression, and/or eating disorders (ADE) and 57 healthy comparisons (HC) were included in the study. Diagnoses were based on DSM-5 criteria using the Mini-International Neuropsychiatric Interview (Sheehan et al. 1998). Comorbid anxiety and depression were allowed, but those with panic disorder were excluded to reduce the likelihood of isoproterenol-induced panic anxiety (Pohl et al. 1988). We further required ADE individuals with a primary diagnosis of anxiety to have scores greater than seven for the GAD-7 (Spitzer et al. 2006) and greater than 10 for the Overall Anxiety Severity and Impairment Scale (OASIS) (Campbell-Sills et al. 2009) indicative of clinically significant anxiety levels. Participants with eating disorders were required to meet lifetime DSM-5 criteria for anorexia nervosa but were required to be weight restored as defined by a minimum body mass index of 18.5 or greater for at least 1 month to control for the well-described state effects of acute starvation (Wierenga et al. 2015). We employed several self-report scales of measure symptom severity across the sample due to our interest in transdiagnostic markers of pathology. Psychotropic medications were allowed as long as there was no change in dosage during the four weeks preceding the MRI. Finally, a medical evaluation including a 12-lead electrocardiogram read by a cardiologist ensured all participants were generally healthy, free of cardiorespiratory, endocrine, metabolic, or other illness, as well as free of major neurological disorders or psychiatric disorders besides ADE. Additional exclusion criteria involved anything would render the participant unfit for the MRI environment, such as inability to remove body piercings or implanted electrodes. The study was part of a larger project investigating interoception in anorexia nervosa and generalized anxiety disorder (ClinicalTrials.gov Identifier: NCT02615119). Additional recruitment, screening, and exclusion information can be found in (Teed et al. 2022), who used a subset of our sample for a separate analysis.

All study procedures were approved by the Western Institutional Review Board, and all participants provided written informed consent before participation. All data were collected at the Laureate Institute for Brain Research in Tulsa, OK.

Experimental design and statistical analysis

During a single scanning session, participants completed two fMRI tasks designed to preferentially assess intersecting aspects of cardiorespiratory interoception while measuring blood oxygen level-dependent (BOLD) signals (Figure 1). The Isoproterenol Infusion (ISO) task was always performed before the Visceral Interoceptive Attention (VIA) task, which we considered to preferentially emphasize the bottom-up or top-down processing of interoceptive signals, respectively. The order of these tasks reflected the project's primary emphasis on acquiring the

infusion data. Complete details of these paradigms are described in the following sections. Both tasks were immediately preceded by and followed by eight-minute eyes-open resting state scans. For these resting state scans, participants were instructed to remain as still as possible, to keep their eyes open and fixated on a white cross centered upon a dark screen, and to "clear your mind and do not think about anything in particular." To minimize head motion, a layer of tape was gently placed over the participant's forehead to provide them with tactile feedback regarding head position. Participants were monitored throughout scanning and would be notified via intercom if they were displaying excessive motion. During all fMRI scans, cardiac and respiratory activity was measured simultaneously at 40Hz using a pulse oximeter and thoracic respiratory belt, respectively. Finally, before any resting state or task-based imaging data was collected, participants completed a high-resolution anatomical MRI scan.

Isoproterenol Infusion Task

We used intravenous bolus infusions of the peripherally-acting beta-adrenergic agonist isoproterenol (ISO) or Saline to manipulate afferent interoceptive signals in a bottom-up fashion. Infusions of 2.0 micrograms (mcg), 0.5 mcg, or Saline were administered 60 seconds into each four-minute infusion scan as per Hassanpour et al. (Hassanpour et al. 2018) and Teed et al. (Teed et al. 2022). Infusion delivery was randomized, double-blinded, and repeated twice for each dose for a total of six infusions (*i.e.,* six scans). Throughout the infusion scan, participants focused on sensations from their heart and lungs while providing real-time, continuous intensity ratings using an MRI-compatible dial (Current Designs Inc.) in their dominant hand, the rating of which was visible to them on the screen while scanning. The intensity rating scale ranged from 0 (none or normal) to 10 (most ever). Thus, this task engages top-down attention towards cardiorespiratory sensations while parametrically perturbing the bottom-up stream in the ISO trials, with an absence of such perturbation in Saline trials. Following our lab's previous studies (Hassanpour et al. 2018; Teed et al. 2022) we split the time course of this task into pre-defined epochs of interest, and focused on the Anticipation period (seconds 60 to 80, directly following the infusion but before the ISO-induced rise in heart rate) and the Peak period (seconds 80 to 120, encompassing the onset and peak magnitude of ISO-induced heart rate change), and contrasted these with the Baseline period (initial 45 seconds of the task). Following each scan, participants also retrospectively reported the intensity of their perceived cardiac and respiratory sensations, separately, that they felt during the scan, as well as their anxiety and excitement. To familiarize participants with the task and isoproterenol-induced sensation, they were given single-blind infusions of 1mcg ISO and Saline before the MRI session.

Visceral Interoception Attention Task

We used the Visceral Interoceptive Attention (VIA) task to assess the top-down attentional processing of interoceptive signals at rest (*i.e.,* no infusions were delivered). This minimalistic task, adapted from Simmons et al. (Simmons et al. 2013), involved repeated trials of interoceptive or exteroceptive attention delivered across three six-minute runs, with the order of these runs randomized across participants. During the interoceptive attention trials, the words "HEART", "LUNGS", or "STOMACH" were presented on the screen for eight seconds, during which participants

were instructed to focus their attention on the naturally-occurring sensations experienced from that organ. During the exteroceptive attention control trials, the word "TARGET" was shown on the screen at changing shades of black to grey, and participants were instructed to pay attention to the degree of color change. After half of all trials in each condition participants were asked to indicate via an MRI-compatible dial the intensity of sensations they had perceived from that organ (interoceptive condition) or the intensity of the color change (exteroceptive condition) on a rating scale of 1 to 7.

MRI data acquisition

All scans were conducted in a 3 Tesla General Electric scanner with an eight-channel head coil (GE MR750; GE Healthcare). We acquired anatomical images via T1-weighted, magnetization-prepared, rapid gradient-echo sequences images (MPRAGE). MPRAGE parameters were: 190 axial slices, slice thickness = 0.9mm, TR/TE = 5/2.012 ms, FOV = 240 x 192mm^2, matrix size 256 x 256, flip angle 8 degrees, inversion time 725 ms, SENSE acceleration R =2, with a sampling bandwidth of 31.2. For the task-based and resting state functional scans, T2*-weighted images were acquired via an echoplanar sequence of the following parameters: single-shot gradient echo planar imaging (EPI) sequence covering the whole brain, obtaining 39 axial slices, 2.9mm thick with no gap and a voxel size of 1.875 x 1.875 x 2.9mm^3, with TR/TE = 2000/27ms, FOV = 240 x 240mm^2, flip angle 78 degrees, SENSE acceleration R = 2 with a 96 x 96 matrix.

fMRI task-based analysis

Each task was analyzed separately in AFNI (Cox 1996), following previously-published preprocessing pipelines specific to each task (Simmons et al. 2013; Teed et al. 2022). Preprocessing steps common to both tasks included removal of the first four volumes (ISO) or five volumes (VIA) for steady state tissue magnetization, despiking, slice time correction, spatial smoothing (6mm full width at half maximum Gaussian kernel), co-registration to the first volume, scaling of each voxel, and normalization to Talairach space. In the ISO task, temporal fluctuations of cardiac and respiratory frequencies and their first-order harmonics were additionally removed from the imaging data using RETROICOR, implemented via custom code in MATLAB (MathWorks).

After preprocessing, whole-brain individual-level activation maps were created. For the ISO task, this involved regressing out baseline drift and contrasting average BOLD percent signal change (PSC) within each epoch of interest (Anticipation and Peak, separately) against the average PSC during the Baseline period. For the VIA task, this involved a multiple linear regression model that included terms for baseline drift, six motion parameters, and each interoceptive and exteroceptive attention condition, and contrasting PSC associated with cardiorespiratory attention (*i.e.,* collapsed across "Heart" and "Lung" trials) against that of exteroceptive attention ("Target" control condition). This particular cardiorespiratory contrast was chosen as it best matched the cardiorespiratory perturbation induced by ISO.

After the creation of individual-level maps, we performed quality control checks and excluded any subject from group analysis who had poor quality imaging data according to the previously established approaches in each task. For the ISO task, we excluded any scan from a participant where greater than 20% of all time points had motion over 0.3mm based on the

Euclidean norm of the six motion parameters, and then removed any participant who was missing both runs of any dose. For the VIA task, we censored out all time points that had an outlier fraction greater than 5% or an estimated motion greater than 0.3mm, and excluded any run in which this censoring removed over 20% of volumes. If a participant was missing at two or more VIA runs (out of three), they were removed from the analysis. To avoid an unbalanced conjunction analysis in the following step, participants excluded from one task were also removed from the other task. The groups were then propensity matched based on age, sex, and BMI to equalize the sample sizes (*MatchIt* function in R), resulting in a final sample of participants who had high quality imaging data in *both* tasks.

Using this propensity-matched sample, group-level statistical activation maps were created for each task and group separately, using whole-brain voxel-wise t-tests (*3dttest++* in AFNI) with Equitable Threshold And Clustering corrections for an overall False Positive Rate of 5% (Cox 2019). With these group activation maps, we were thus able to identify voxels significantly active at the group level in each experimental condition.

Convergence maps

To identify voxels co-activated across the ISO and VIA tasks, we restricted our analysis to the specific condition of each task that best exemplifies the interoceptive processes of interest: namely, Heart + Lung interoceptive attention relative to exteroceptive attention in the VIA task, and the Peak period of the 2.0mcg dose relative to the Baseline period in the ISO task. We narrowed our focus to this specific period and dose of the ISO task because it represents, as shown in our previous studies, the peak level of cardiorespiratory perturbation that can be confidently ascertained by subjective report in all individuals. Using the statistically-thresholded group activation maps from each task, we binarized these maps into active and non-active voxels and created convergence maps by joining the binary maps across the specified contrasts of each task. These convergence maps thus identified which voxels were commonly activated, *i.e.*, *convergent*, at the group level during both tasks. Due to our pre-specified focus on cytoarchitectural processing of the insular cortex, we subdivided these convergence maps (after converting to MNI space) into three bilateral insular subregions of interest, defined using the Brainnetome atlas (Fan et al. 2016), as follows: agranular anterior (regions 165 and 167 for the left hemisphere, 166 and 168 for the right) , dysgranular mid (regions 169 and 173 for the left hemisphere, 170 and 174 for the right), and granular posterior (region 163 and 171 for the left hemisphere, 164 and 172 for the right).

We evaluated each convergence map in four ways: 1) the spatial extent of convergence (*i.e.,* number of convergent voxels) within each insular subregion, tested for group differences via chi-square tests, 2) the spatial similarity of patterns of convergence between HC versus ADE groups, separately for each hemisphere, measured via Dice coefficient (using AFNI's *3ddot*), 3) the degree of task overlap within each group and hemisphere separately, quantified via the overlap coefficient (*i.e.,* the proportion of the ISO cluster overlapping with the VIA cluster, as per (Bowring, Maumet, and Nichols 2019), and finally 4) the average PSC across convergent voxels within each cytoarchitectonic subregion, compared across groups and hemispheres using linear mixed effects regression (*lmerTest* library in R). In these linear models, we tested the fixed effects of group and hemisphere on the magnitude of activation within subregion-specific convergent voxels, and

interpreted significant main effects using post-hoc contrasts between the estimated marginal means of each condition (*emmeans* library in R). Additionally, we used linear regression to examine possible associations between PSC in hemisphere- and subregion-specific convergent voxels and the following variables: trait measures of anxiety and depression, the objective degree of peripheral perturbation (*i.e.,* mean change in heart-rate during the Peak period of the 2.0mcg dose), real-time subjective rating of cardiorespiratory intensity (*i.e.,* mean change in dial rating during the Peak period of the 2.0mcg dose), and retrospective ratings of cardiac and respiratory intensity, anxiety, and excitement following the 2.0mcg dose. For these associations, we took a dimensional approach and collapsed across groups, considering all participants on a continuous scale.

fMRI resting state analysis

In this exploratory whole-brain resting state functional connectivity (FC) analysis we aimed to identifying which connections, if any, from these convergent regions to the rest of the brain changed in strength following engagement of interoception and if these patterns of connectivity change differed between groups. Because resting state scans were collected before and after the interoceptive tasks, we could investigate these post- minus pre-interoception effects.

Resting state scans were analyzed in AFNI with a preprocessing pipeline optimized for connectivity analysis. After discarding the first five images to ensure steady-state tissue magnetization, we removed spikes in the signal intensity time course, and removed physiological noise using respiration volume per time (RVT) (Birn et al. 2008) and RETROICOR (Glover, Li, and Ress 2000) regressors that model slow blood oxygenation level fluctuations and time-locked cardiac and respiratory artifacts, respectively. The RVT regressors consisted of the RVT function and four delayed terms at 5, 10, 15, and 20 seconds, and the RETROICOR regressors consisted of four respiratory and four cardiac generated on a slice-wise basis by AFNI's "RetroTS.m" script (Jo et al. 2010). Further preprocessing steps included slice-timing correction, rigid body realignment, warping to MNI space, and smoothing with a 6-mm full-width half-maximum Gaussian kernel. Next, we applied a high-pass filter to remove all non-relevant ultra-slow fluctuations below 0.01 Hz, and then further reduced potential contributions of non-neural sources by regressing out the following nuisance variables: (1) 12 head motion regressors (6 realignment parameters and their derivatives), (2) voxel-wise local white matter regressors, and (3) three principal components of ventricle signals (ANATICOR). Following preprocessing, we excluded from further analysis any subject who had greater than 10% of volumes above our motion cut-off of 0.3mm for the Euclidean norm of the motion derivatives.

From the pre- and post-interoception resting state scans, we extracted the time series from the convergent ROIs within the left and right hemispheres for HC and ADE groups separately, and correlated these respective time series against those of all other voxels in the brain to create an FC map for each resting state period (i.e., pre- and post-interoception). The resulting correlation coefficients were converted to Fisher z-statistics to produce a more normally distributed variable. We then tested for group differences in the effect of time (*i.e.,* pre- versus post-interoception task) on resting state FC, in each hemisphere individually. All resulting clusters of this group*time interaction analysis were corrected for multiple comparisons using the *3dClustSim* function in AFNI, using 10,000 Monte Carlo simulations of whole-brain fMRI data to determine the cluster size at which the false positive probability was below $\alpha<0.05$ with an uncorrected voxel-wise threshold of

367 $p<0.001$, yielding a threshold of 150 contiguous voxels. This approach allowed us to identify regions
368 that changed connectivity with the convergent insula differently for HC vs. ADE groups following
369 top-down and bottom-up interoception. Finally, we conducted a correlational analysis to explore
370 whether individual changes in FC were related to trait measures of anxiety and depression in our
371 transdiagnostic sample, using the Bonferroni method for multiple comparison correction.
372
373 *Divergence maps*

374 To isolate voxels selective to the engagement of top-down expectancies about the body, we
375 focused on specific time windows of the ISO task where expectations of potential interoceptive
376 change were preferentially engaged: namely, the Anticipation period across all doses, and the Peak
377 period of the Saline dose. The Anticipation period covered the 20 second epoch right after the
378 intravenous infusion was delivered but before the onset of any cardiorespiratory perturbation.
379 Such perturbations would ensue in the case of an isoproterenol dose but would not occur following
380 a saline infusion, and thus, the Anticipation period represented a time when no peripheral change
381 was happening, but participants were unsure about whether or not one was coming. This state of
382 uncertainty continued into the Peak period of the Saline dose, where no peripheral change
383 occurred. Based on our previous experiences with the task, we expected that by the end of the Peak
384 window some participants would be update their beliefs about what had occurred after realizing
385 that they had not rated experiencing a cardiorespiratory perturbation. Together, the Anticipation
386 period and the ensuing Peak period of the Saline dose share some similarities with the VIA task in
387 their involvement of top-down interoceptive attention during physiological rest, but critically differ
388 in their added expectancies about possible changes in the state of the body. In an effort to separate
389 the top-down effect of interoceptive expectancy from the general effect of interoceptive attention,
390 we isolated voxels that were preferentially active during these ISO expectancy windows from those
391 that were active during the VIA task.
392 For this aim, we took group-level activation maps from the Anticipation period (averaged
393 across all doses) and the Peak period of the Saline dose, both relative to the Baseline period. Like
394 the convergence map approach described above, we binarized these maps within each period and
395 group separately, transformed them to MNI space, reduced them to the cytoarchitecturally-
396 subdivided insular cortex, and joined them with the binarized activation map for the Heart and
397 Lung relative to Target contrast in the VIA task. Here, we instead focused on the *divergent* voxels,
398 *i.e.,* those that were active only during specific periods of the ISO task and that were not co-active
399 during the VIA task. As per our hypothesis regarding anterior insula involvement in interoceptive
400 expectancy processing, we restricted these "ISO task only" voxels to those within the agranular
401 anterior insula, and evaluated them in two ways: 1) spatial extent of activation (*i.e.,* number of
402 voxels) across groups, time periods, and hemispheres, testing for differences via chi-square tests
403 with Bonferroni correction for multiple comparisons, and 2) average PSC, testing for main effects of
404 group, hemisphere, and time period using linear mixed effects regression and interpreting
405 significant main effects using post-hoc contrasts of estimated marginal means (as previously
406 described). Finally, we also examined possible associations between average PSC in these agranular
407 expectancy-specific regions and trait measures of anxiety using linear regression
408 transdiagnostically across groups.

Physiological and behavioral analysis

To test for group differences in the time courses of heart rate and real-time dial ratings during the ISO task (2.0mcg isoproterenol and Saline infusions), we used cluster-based permutation testing (*permutes* package in R) separately for each dose after averaging across both trials. We considered a significant group difference as any contiguous set of time points (two or more continuous seconds) where permutation testing revealed a statistical difference at $p<0.05$. To test if the magnitude of heart rate change or dial rating, when averaged across each epoch of interest, was associated with dimensional measures of trait anxiety and depression, we used linear regression applied across the entire sample. Finally, to test group differences in retrospective ratings of interoceptive intensity, anxiety, and excitement in the ISO task and interoceptive and exteroceptive intensity in the VIA task, we used two-tailed t-tests separately for each task and condition. This approach reflected our primary interest to investigate group differences in perceptual ratings, rather than performing additional within-group comparisons across modalities or doses.

Code accessibility

Custom AFNI and R scripts are available from the authors upon request.

Results

Of the 127 total participants included in this study, 57 ADE individuals and 46 HC individuals fulfilled criteria for high-quality brain imaging data on both of our tasks. To obtain equal-sized groups for group-level comparison, propensity matching on age, self-reported sex, and body mass index resulted in 46 individuals per group (Table 1). After propensity matching, the two groups were not significantly different regarding age, body mass index, and sex but differed significantly on trait measures of anxiety (OASIS, GAD7, and Anxiety Sensitivity Index (ASI) total and subscales), depression (Patient Health Questionnaire-9 (PHQ-9) (Kroenke, Spitzer, and Williams 2001), and eating disorders (SCOFF) (Kutz et al. 2020).

Convergence analysis

Bilateral patches of the ventral mid-insular cortex were commonly activated during the ISO and the VIA tasks (Figure 2). Quantification of the spatial extent of this convergence in the pre-defined probabilistic agranular, dysgranular and granular subregions revealed that these convergent clusters were localized primarily to the dysgranular insula bilaterally (Figure 3A), but with a hemispheric asymmetry across groups: the ADE group showed a greater number of overlapping voxels within the left dysgranular insula ($\chi^2 = 26.7$, $p<0.001$) whereas the HC group showed a greater number of overlapping voxels within the right dysgranular insula ($\chi^2 = 26.7$, $p<0.001$) (Figure 3B). Correspondingly, the spatial similarity of these convergently activated voxels between the groups was lower in the left dysgranular insula (Dice coefficient of 0.58) than in the right (Dice coefficient of 0.78) (Figure 2). In addition to these group hemispheric differences in

spatial extent, there was a lower amount of overlap between the two tasks in the left dysgranular insula for both groups (left hemisphere overlap coefficients: 0.42 and 0.63 for HC and ADE, respectively; right hemisphere overlap coefficients: 0.83 and 0.78, for HC and ADE, respectively).

Despite these group differences in the spatial extent and spatial similarity of convergence, the magnitude of activation within convergent dysgranular subregions during the Peak of perturbation (ISO 2.0mcg dose) or Heart and Lung attention (VIA task) was not different between groups (F_{90} = 0.64 and 0.05, p = 0.43 and 0.82 for main effect of group in ISO and VIA tasks, respectively). However, during both of these task conditions, activation was greater within the right hemisphere than the left (F_{90} = 6.13 and 5.10, p = 0.015 and 0.027 for main effect of hemisphere during ISO and VIA tasks, respectively; t_{90} = -2.48 and -2.25, p = 0.02 and 0.03 for post-hoc contrasts between the left versus right hemisphere when collapsed across groups) (Figure 4A). This hemispheric effect on activation magnitude was specific to interoceptive processing, as no similar hemispheric difference was seen during the exteroceptive condition of the VIA task (F_{90} =0.68, p=0.41). Furthermore, the magnitude of activation within the right (but not the left) dysgranular convergent region during the peak of cardiorespiratory perturbation was associated with the intensity of real-time cardiorespiratory sensations (*i.e.,* mean dial rating) during this period when examined across the entire sample (Pearson's *r* = 0.12 and 0.21, *p* = 0.25 and 0.046 for the left and right hemispheres, respectively; Figure 4B). Conversely, PSC in either convergent hemisphere during perturbation was not associated with objective measures of physiological arousal (*i.e.,* mean change in heart rate, Pearson's *r* = 0.11 and 0.09, p = 0.31 and 0.40 for left and right hemispheres, respectively), post-scan ratings of cardiac intensity (Pearson's r = 0.16 and 0.20, p = 0.15 and 0.06), post-scan ratings of respiratory intensity (Pearson's *r* = 0.12 and 0.06, p = 0.26 and 0.58 for left and right hemispheres, respectively), post-scan ratings of state anxiety (Pearson's r = 0.14 and 0.08, p = 0.20 and 0.49 for left and right hemispheres, respectively), or trait measures of anxiety sensitivity (*i.e.,* ASI-Total, Pearson's *r* = 0.12 and 0.16, p = 0.25 and 0.13 for left and right hemispheres, respectively).

Finally, pre- and post-task resting state functional connectivity of the convergent regions in the ADE group showed a different pattern of change compared to the HC group: functional connectivity between the right dysgranular convergent cluster and left middle frontal gyrus increased in the ADE group only, while the HC group saw no differences ($p < 0.05$, corrected; Figure 5A and 5B). Across the entire sample the magnitude of functional connectivity change between these two areas was associated with trait anxiety (Pearson's r = 0.43 and 0.36 for OASIS and GAD7, respectively, $p < 0.001$) and trait depression (Pearson's r = 0.38, $p < 0.001$ for PHQ-9) (Figure 5C) but not the magnitude of ISO-induced heart rate (Pearson's r = -0.05, p = 0.66) or dial rating (Pearson's r = 0.01, p = 0.90) at the 2.0μg dose.

Divergence analysis

The Anticipation window of the ISO task engaged large swaths of the bilateral agranular insular cortex that were not activated at any point during the VIA task (Figure 6A). Here, the right hemisphere was activated to a greater spatial extent than the left in both groups (χ^2 = 22.7, $p < 0.001$ for the HC group; χ^2 = 7.3, p = 0.007 for the ADE group), and to a greater spatial extent in the HC than ADE individuals (χ^2 = 7.3, p=0.007 in the right). There was no group difference in the spatial extent of agranular insula activation in the left hemisphere (χ^2 = 2.0, p = 0.15) (Figure 6B).

The spatial extent of activation in the agranular insula during the Peak period of the Saline dose involved significantly more voxels than the Anticipation period, for both groups and hemispheres (χ^2 = 75.3 and 71.4, p < 0.001 for left and right hemispheres in HC; χ^2 = 36.3 and 12.1, p < 0.001 for left and right hemispheres in ADE) (Figure 6B). This expansion occurred to a greater degree in the HC versus ADE group (χ^2 = 17.2 and 70.3 for left and right, corrected p < 0.001). Finally, agranular insula recruitment within the HC group during the Saline Peak period was shifted towards the right hemisphere (*i.e.*, more active voxels in the right than the left, χ^2 = 24.3, p < 0.001), while the ADE group showed no hemispheric difference during this period (χ^2 = 0.10, p = 0.75).

Alongside these differences in spatial extent, the activation magnitude also increased from the Anticipation period to the Saline Peak period, with no differences between groups (F_{270} = 0.25, 7.2, and 28, p = 0.62, 0.008, and < 0.0001 for the main effects of group, hemisphere and epoch, respectively, F_{270} = 0.04–2.31, p = 0.13–0.85 for all two- and three-way interaction effects) (Figure 6C). While this increase in signal intensity from the Anticipation to the Saline Peak period occurred in both hemispheres (t_{270} = 2.7 and 4.8, p = 0.008 and < 0.001 for post-hoc contrasts between epochs in the left and right hemispheres, respectively, when collapsed across groups), the right hemisphere had greater signal intensity than the left during the Saline Peak window (t_{270} = -3.0, p = 0.003), with no differences in activation during the Anticipation period (t_{270} = -0.8, p = 0.41). The magnitude of agranular insula activation was not related to trait measures of anxiety sensitivity in either epoch or hemisphere when examined transdiagnostically across groups (ASI * hemispheric interaction effect: F_{90} = 1.9, p = 0.17 for Anticipation, and F_{90} = 1.7, p = 0.19 for Saline Peak).

Physiological response and perceptual ratings

During the 2.0mcg dose there were large perturbations in heart rate in both groups, and while the ADE appeared to have a slightly greater response on average, this reached statistical significance via permutation testing only during two continuous time points in the Peak period (seconds 98 through 99, t = 1.7 and 1.5, p = 0.044 and 0.045, Figure 7A). However, the ADE group reported significantly higher real-time dial ratings for the latter portion of the Peak period extending into much of the ensuing recovery period (seconds 110 through 142, t = 1.8–2.3, p = 0.008–0.042, Figure 7B). During the Saline dose of the ISO task, both heart rate and perceptual dial ratings remained near baseline levels. While heart rate did not differ between groups (t = -0.53 – 1.64, p = 0.05 – 1.00), the HC group showed small but statistically significant increases in dial ratings compared to those of the ADE group for portions of the Peak period and the ensuing recovery period (t = -1.67 – -2.60, p = 0.005 – 0.048) (Figure 7A and 7B). Although there were ISO-induced differences in continuous measures of cardiorespiratory sensation, there were no group differences in retrospective cardiac or respiratory intensity ratings for the 2.0 mcg infusion (t_{80} = 1.5 and 1.2 for heart and lungs respectively, p = 0.13 and 0.25) or the saline infusion (t_{80} = 0.3 and 1.2, p = 0.75 and 0.22) (Figure 7C). The ADE group reported greater retrospective anxiety than the HC group following infusions (t_{85} = 4.8 and 3.8 for 2.0mcg and Saline, respectively, p < 0.001), with no differences in excitement (t_{85} = 0.2 and 0.9, p = 0.80 and 0.37 for 2.0mcg and Saline, respectively). During the VIA task, the ADE group reported higher cardiac intensity ratings than the HC group during heart-focused attention (t_{89} = 2.0, p = 0.04) but lower exteroceptive intensity ratings during the target condition (t_{90} = 2.7, p < 0.01). There were no group differences in the intensity of reported lung or stomach sensations (t_{89} = 1.6 and 1.4, p = 0.10 and 0.16) (Figure 7D).

Discussion

The current study revealed overlapping patterns of neural activation within the dysgranular mid-insula during top-down and bottom-up interoceptive processing, corroborating previous studies examining each task independently (Hassanpour et al. 2018, Simmons et al. 2013), and supporting the notion of the insula as a key functional interface between goal-directed and sensory-driven interoceptive streams. Importantly, individuals with anxiety, depression, and eating disorders were showed hemispheric differences in the patterns of dysgranular activation, as hypothesized. This hemispheric imbalance was concurrent with a perceptual hypersensitivity often reported in anxiety and/or eating disordered individuals, as well as with changes in right dysgranular insula-to-left middle frontal gyrus functional connectivity following the interoceptive tasks. Finally, activity within the agranular insula was selective to periods of increased interoceptive expectancies, aligning with a prediction-generating role for this region and distinct from the convergent effects seen in the dysgranular mid-insular subregion.

Top-down processing commonly refers to cognitive influences on perception, such as attention, memory, and expectation, that putatively help the brain to make sense of the stimuli it encounters. In contrast, bottom-up processing commonly refers to the stimulus-driven signals that are processed hierarchically as they reach the brain. With respect to interoception, top-down processing can refer to the brain's ability to predict or interpret how the internal body should feel (*i.e.,* the expected interoceptive signals) based on the previous or current environmental context. In turn, bottom-up processing can refer to the actual state of the body in the current moment. For instance, top-down influences may include the anticipation of an upcoming meal leading to feelings of hunger, while bottom-up influences may refer to actual signals of hunger from the body at lunch time, such as hormonal bloodstream signals and stomach contractions. The continuous balance between these processes determines the qualia of conscious perceptions and has been suggested to be key to health and the optimization of internal and subjective states (Sterling 2012). However, as processes, they are rarely studied concomitantly in the manner conducted in this study. The convergence identified between top-down and bottom-up interoceptive processing within the dysgranular mid-insula suggests that this cortical subregion is an area where further detail regarding the reciprocal interactions underpinning interoceptive perceptions should be investigated, particularly in relation to psychiatric disorders involving disturbances of mood, anxiety, or appetite.

Although the current findings emphasize the role of interoceptive processing within dysgranular subregions of the insula, a number of experimental and analytic next steps are worth considering. Due to the broad systems-level of inference allowed by fMRI methodology and the relatively rudimentary block averaging analytic approach employed, additional steps are necessary to establish the cellular and molecular mechanisms underlying the observed dysgranular co-activation. Such approaches would be important for verifying a true intersection of interoceptive neurocircuitry in which connecting top-down attention and prediction circuits directly modulate, or are modulated by, incoming sensory afferences. An alternative outcome could be identifying an overpassing flow of signals across these circuits within the same region. Implementing high resolution approaches to imaging these processes via high-field fMRI on a trial-by-trial basis could inform the laminar architecture underpinning attention-driven modulation of incoming viscerosensory signals as well as allowing for computational modelling of these processes at the level of cortical laminar subfields (Kasper et al. 2022). In an interoceptive application of this analyses employing a respiratory bottom-up perturbation approach, activation of the anterior

insula was associated with the prediction certainty but not prediction error for different inspiratory breathing loads, with parameters distinguishing between high versus low trait anxiety individuals (Harrison et al. 2021). This computational approach could be adapted for cardiorespiratory processing within the dysgranular insula. While typically restricted to high field strengths, such as 7 Tesla, there are emerging reports that laminar imaging can be performed at lower field strengths (Knudsen et al. 2023). Thus, there are a number of currently feasible steps for further extending the present findings.

While we did not set out to test predictive coding or other computational models in the current study, these theories (such as the Embodied Predictive Interoceptive Coding model (Barrett and Simmons 2015) or the Insula Hierarchical Modular Adaptive Interoception Control model (Fermin et al. 2022)) provide useful frameworks for interpreting the observed dysgranular co-activation during each imaging task, and by extension potential sources of interoceptive dysfunction in psychiatric disorders (see (Allen et al. 2022), (Paulus, Feinstein, and Khalsa 2019), and (Petzschner et al. 2021) for additional theoretical discussions of the computational functional neuroanatomy of interoception). Collectively, these theories suggest a model of bidirectional signal flow along the antero-posterior axis of the insula: while the granular posterior subregions may be the first to receive ascending visceral input, the agranular anterior subregions continuously issue predictions regarding the anticipated states of such input (Barrett and Simmons 2015). From this perspective, the dysgranular mid-insula is well-positioned between the agranular and granular poles to integrate the signals from each source. As suggested in the EPIC model, computations within dysgranular layers can link incoming sensations with top-down expectations, effectively translating visceral signals into endogenously meaningful interoceptive prediction error signals, providing the rest of the brain with an internal context for interpreting the current bodily state (Seth and Friston 2016).

Applying computational frameworks for interoception to the present findings may be useful for interpreting the broad agranular insula activation found only during periods of increased bodily expectancies (*i.e.*, "divergence"). This subregion's engagement during the Anticipation window that expanded throughout the Peak period of the Saline dose likely reflects an increase in predictive processing during these conditions, as both groups expected a potential perturbation and were subsequently required to update their predictions upon experiencing a physiologically quiescent state instead. The reduced spatial extent of this activation in the ADE versus HC group could plausibly stem from an overreliance on top-down predictions and perhaps a pruning of spatial activation in the associated regions, similar to the functional alterations of neural activity that have been observed following the acquisition of a motor skill (Wiestler and Diedrichsen 2013). Such 'efficient' neural exchanges might even result from a chronic inability to adjust confidence in bodily input in relation to its ambiguity (*i.e.,* increasing confidence in visceral signals during bodily perturbation), and could occur alongside maladaptive allostatic adjustments described in psychiatric disorders (Barrett, Quigley, and Hamilton 2016; Smith et al. 2020). Computational theories of interoception can also be applied to the observed co-activation within the dysgranular mid-insula (i.e., "convergence"). Dysgranular convergence aligns with the proposed intersectional (as opposed to overpassing) role for this region, and could represent the generation of prediction errors as inflows of isoproterenol-induced viscerosensation are compared to task-specific interoceptive predictions focused by the lens of directed attention. While internally-directed attention alone might selectively enhance cortical representations of the viscera across wide

swaths of posterior to mid-insula, as suggested previously (Avery et al. 2014; Pollatos et al. 2007), these past studies were hampered by the ambiguity of visceral sensation inherent to solely studying interoceptive awareness under physiological resting conditions. By manipulating cardiorespiratory signals during the ISO 2.0mcg dose and eliciting unambiguous perceptual states, the current study effectively spotlights the dysgranular subregion's role in heightened interoceptive awareness and provides a neuroanatomical focus for further examinations of bi-directional interoceptive processing in this important region.

The right-hemispheric increase in mid-insula activity, as well as the hemispheric asymmetries and altered functional connectivity found in the ADE group has several implications for an interoceptive processing role for the dysgranular insula. Previous theories have suggested a right-hemispheric dominance in interoceptive processing in healthy populations (Craig 2009; Schulz 2016), but have pointed towards the right anterior insula as the locus of body awareness (Craig 2009). While we saw a rightward emphasis in the magnitude of insular activation during ISO-induced cardiorespiratory sensations across both groups, activation was located more posteriorly, towards the mid insula (similar to previous isoproterenol fMRI studies (Hassanpour et al. 2016, 2018)), where it was correlated with cardiorespiratory sensation. While this finding supports a role for the mid-insula as an integrative hub for interoceptive awareness, it was in contrast to a lack of clear associations with physiological signals, emotional feelings such as anxiety or excitement or trait measures of ADE severity, despite the evocation of greater state anxiety following perturbation in the ADE individuals. Probing the symptomatic relevance these potential brain-behaviour relationships may call for more refined approaches to the study of interoceptive perturbation. One option might be to evaluate the intersecting influence of several forms of perturbation, by studying both exogenous approaches such as isoproterenol infusion, and endogenous approaches such as mental stressors (i.e., Stroop task (Gianaros et al. 2005)), physical stressors (i.e., Valsalva (Song et al. 2018) or lower body negative pressure (Kimmerly et al. 2005)), or social stressors (i.e., Trier Social Stress task (Allen et al. 2017) within the same individuals.

Despite observing several right hemispheric associations across both groups, the ADE group demonstrated a clear leftward activation asymmetry and distinct activation patterns in the left dysgranular mid-insula across both the ISO and VIA tasks. These left insula abnormalities are compatible with the "locus of disruption" identified by Nord et al. (2021), when conducting an fMRI meta-analysis of existing top-down or bottom-up interoception studies of various psychiatric populations. However, beyond identifying a plausible region of cortex, the precise mechanisms underlying this disruption remain unclear. Assuming an intersectional role involving intermingling of top-down and bottom-up streams, it seems plausible that numerous mechanisms could contribute to the aberrant processing identified in the current study. Such mechanisms include a reduced attentional enhancement of ascending visceral input (Avery et al. 2014; Kerr et al. 2016), and/or an inflexibility in adjusting to the salience of interoceptive signals occurring during perturbation (Smith et al. 2020). Reduced flexibility of neural processing may also explain the singular finding of altered connectivity between the right mid-insula and left middle frontal gyrus following the interoceptive tasks in the ADE group, as this frontal region is likely involved in attentional control during the tasks themselves (Andersson et al. 2009). An inability to return this coupling to baseline following interoceptive engagement (or a shift in the opposite direction than HCs) could suggest a dysregulated attentional switching mechanism, particularly since the degree of connectivity change was associated with trait measures of anxiety and depression severity.

The observed hemispheric divergence in interoceptive processing is worth considering from an affective standpoint in the context of the functional lateralization of emotion theories proposed within the insula. Such theories have attributed the left anterior insula to the energy-enriching behaviors of the parasympathetic nervous system, and the right anterior insula with the energy-expending ones of the sympathetic nervous system (Oppenheimer et al. 1992). The current finding of right-hemispheric dominance for conscious awareness of isoproterenol (an arousal-like, and thus sympathetic-emulating manipulation), as well as the increased signal intensity in the right anterior insula during the expectancy of such manipulation, may be considered aligned with this perspective. Correspondingly, activity within the right insula has been associated with conscious awareness of threat (Critchley, Mathias, and Dolan 2002), and mouse models have shown causal evidence for the right anterior insula in orchestrating stress responses to aversive visceral stimuli (Wu et al. 2020). However, assumptions of autonomic opponency are likely overly simplistic, and it is more appropriate to consider these systems as functionally coupled across spinal and vagal systems instead of anatomically distinct (Jänig 2006; Jänig et al. 2017). In this manner, an admittedly speculative emotional lateralization account of functional coupling might posit that the right anterior insula generates interoceptive predictions of spinal efference and the left anterior insula for those of vagal efference; in turn the respective dysgranular areas would then be specialized towards receiving and computing the prediction errors of these different inputs. However, since there does not appear to be an neuroanatomical lateralization for afferent tracts (*i.e.,* spinal input does not have a preference for the right hemisphere, and vagal not for the left) (Dum, Levinthal, and Strick 2009; Shipley and Sanders 1982), it seems more plausible that the contribution of interoceptive processing to emotion requires a carefully integrated balance of both autonomic systems across both hemispheres. Based on the present data, such a balance appears to be asymmetrically shifted in the setting of psychiatric disorders affecting the anxiety, depression, and disordered eating spectrums, particularly during modulations of interoceptive state.

There are several limitations and strengths of the present approach to consider. First, while the quantification of insula asymmetries was strengthened by the use of previously established task-specific pipelines and justified by cytoarchitectural hypotheses leveraging prominent theories about interoceptive neurocircuitry, several analysis steps differed between the tasks that could impact the observed results, including the use of a block versus event-related design and differences in the regression of physiological noise and motion correction. This pre-specified approach was intentional, allowing us to be confident that the analysis metrics were sound (i.e., that a voxel counted as co-activated would have good task-validity in relation to the analysis methods of previously published ISO and VIA studies). However, it also meant that there was variability within-individuals in how the data was handled for each task. Future approaches involving harmonized pre-processing steps across both tasks may be considered. Second, the present approach to determining 'convergence' was admittedly simplistic in relying on co-activation as a determinant. Applying more sophisticated analysis techniques, such as those amenable to individual-level pattern analyses (as opposed to group activation maps) would provide greater insight into the individual responses of each participant. Such approaches are worth considering as they are a core component of personalized evaluations applying functional neuroimaging to diagnostic validation or treatment selection (Goldstein-Piekarski et al. 2022). Third, our sample was predominantly female, reflecting the recruitment of disorders that disproportionately affect women. Fourth, while

our conceptual approach to the top-down/bottom-up construct may mask the complexity of the brain (Rauss and Pourtois 2013) and the process of attention (Hommel et al. 2019), the integration of this concept with several behaviorally relevant tasks in the same individuals offers a useful and informative heuristic in its application. Finally, while our results focus on the insula's role in cardiorespiratory awareness, it's important to note that the insula isn't the only brain area involved in sensing internal body states (Berntson & Khalsa, 2021). Future research should explore how the insula interacts with other brain regions to inform interoception.

Conclusion

This study confirms the pivotal role of the dysgranular insula in the processing of top-down and bottom-up interoceptive inputs, and validates the dysgranular mid-insula as a possible locus of interoceptive disruption in psychiatric disorders.

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Figures and Tables

Table 1. Demographic information for transdiagnostic anxiety, depression, and/or eating disorders (ADE) group and propensity matched healthy comparison (HC) group. MDD = major depressive disorder; GAD = generalized anxiety disorder; AN = anorexia nervosa; BMI = body mass index; PHQ-9 = depression module of the Patient Health Questionnaire; OASIS = Overall Anxiety Severity and Impairment Scale; ASI = Anxiety Sensitivity Index, with a Total Score, and Physical, Cognitive, and Social subscales; GAD7 = Generalized Anxiety Disorder assessment; SCOFF = "Sick, Control, One, Fat, Food" eating disorder questionnaire.

	HC Mean (SE)	**ADE Mean (SE)**	**t**	**df**	**p-value**
Age	24 (5)	24.61 (6.61)	0.57	80.49	0.57
Sex	41 Female 5 Male	45 Female 1 Male	-	-	-
BMI	23.48 (3.26)	23.34 (3.33)	-0.20	89.97	0.84
PHQ-9	0.78 (1.11)	10.39 (5.98	10.72	48.12	**<0.0001**
OASIS	1.44 (1.82)	9.13 (3.66)	12.76	65.99	**<0.0001**
ASI-Total	7.39 (5.26)	13.98 (8.89)	8.89	57.47	**<0.0001**
ASI-Physical	1.15 (1.59)	5.19 (5.81)	5.81	53.38	**<0.0001**
ASI-Cognitive	0.98 (1.47)	7.41 (6.53)	6.52	49.54	**<0.0001**
ASI-Social	5.26 (4.04)	13.83 (6.08)	7.96	78.28	**<0.0001**
GAD7	0.96 (1.50)	11.28 (5.46)	12.38	51.81	**<0.0001**
SCOFF	0 (0)	1.09 (1.23)	6.01	45	**<0.0001**

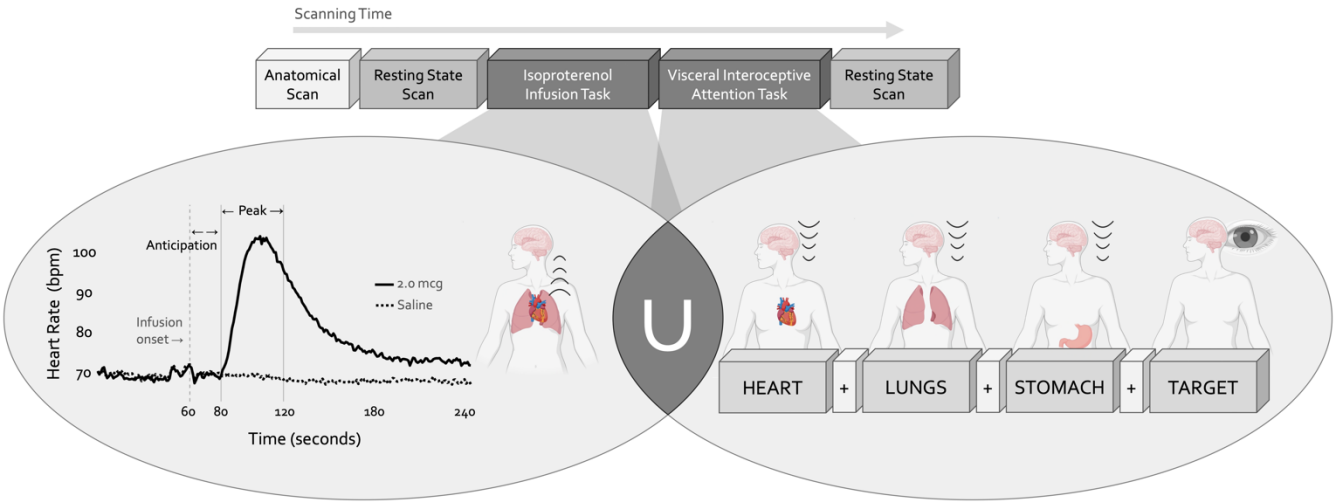

Figure 1. Experimental design. During a single functional neuroimaging scanning session two neuroimaging tasks were used to localize the convergence between bottom-up and top-down interoceptive processing. The Isoproterenol Infusion (ISO) task manipulated interoceptive input in a bottom-up manner, while the Visceral Interoceptive Attention (VIA) task manipulated interoceptive attention in a top-down manner. During the ISO task, participants were asked to attend to their cardiorespiratory sensations while receiving double-blinded infusions of either isoproterenol 2.0 micrograms (mcg, solid line) or 0.5mcg (not pictured), a rapidly acting peripheral beta-adrenergic analogue eliciting transiently increased cardiovascular and respiratory signals in a manner akin to adrenaline, or infusions of saline (dashed line), resulting in no physiological change. During the VIA task, on-screen cues directed participants to shift their attention towards naturally occurring body sensations from a particular internal organ (heart, lungs, or stomach, the interoceptive attention conditions) or the word "TARGET" (the exteroceptive attention condition) that would flash at different intensities. No infusions were given during this task, so the body remained at physiological rest. Both tasks were preceded and followed by a resting state scan.

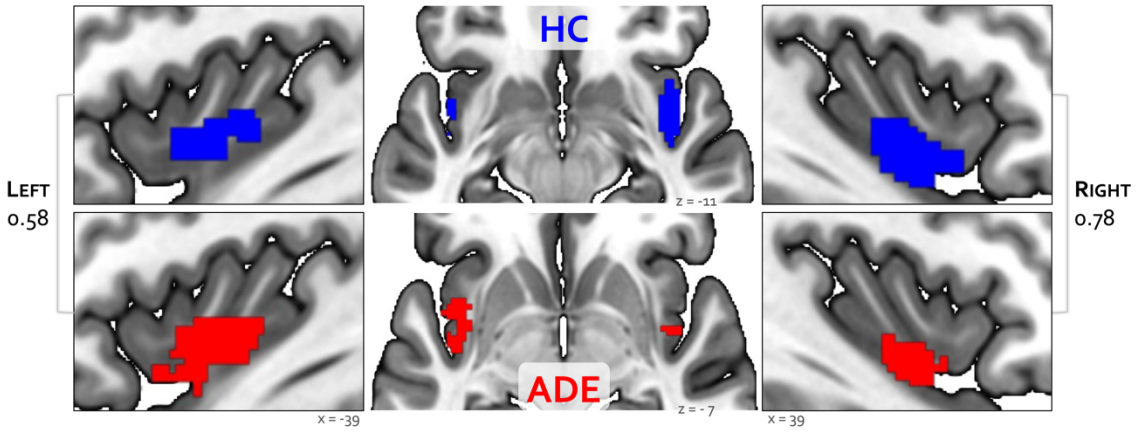

Figure 2. Convergence between bottom-up and top-down interoceptive processing in healthy comparisons (HC, top row) and individuals with anxiety, depression, and/or eating disorders (ADE, bottom row). Each convergence map reflects the only cluster of voxels that were co-activated across the whole brain during the ISO task, involving perturbation of cardiorespiratory signals, and the VIA task, involving goal-directed interoceptive attention towards cardiac and respiratory signals at rest. Numbers on the left and right reflect the Dice similarity coefficient, which are used to quantify the degree of spatial overlap between groups. Relative to HCs, the ADE group showed lower spatial similarity for the left insular cortex (Dice coefficient of 0.58), than the right insular cortex (Dice coefficient of of 0.78).

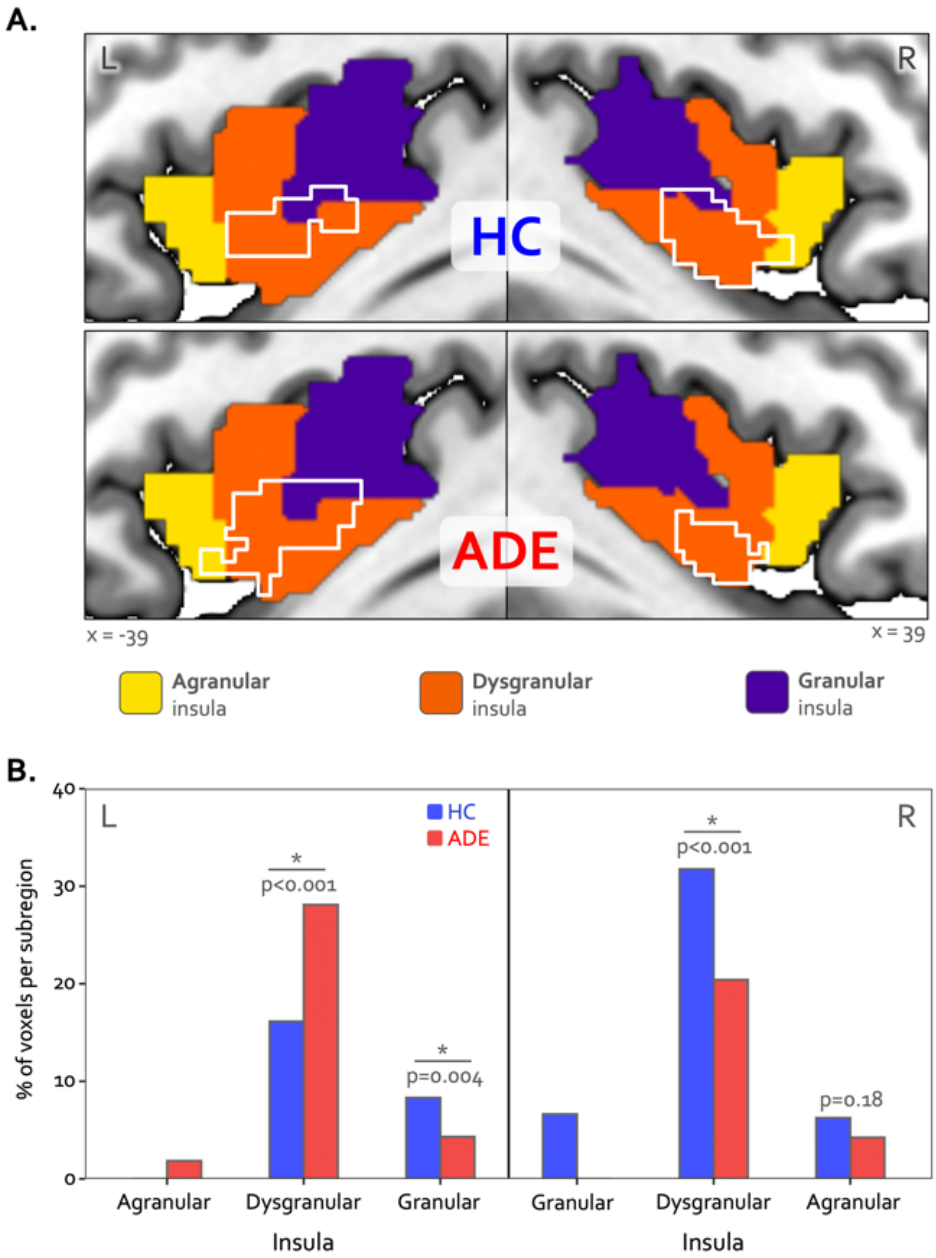

Figure 3. Hemispheric divergence of bottom-up and top-down interoceptive processing across psychiatric disorders. (A) Hemisphere-specific convergence maps (white outlines) overlaid on a tripartite probabilistic cytoarchitectonic division of the insular cortex. (B) Quantification of convergence within each cytoarchitectonic subregion (*i.e.,* number of co-activated voxels in relation to the total number of voxels within that subregion), showing asymmetric proportional voxel co-activation in the left and right dysgranular insula between the groups. *indicates significant group difference for the proportion of co-activated voxels in each subregion via chi-square test. HC: healthy comparison. ADE: anxiety, depression, and/or eating disorder.

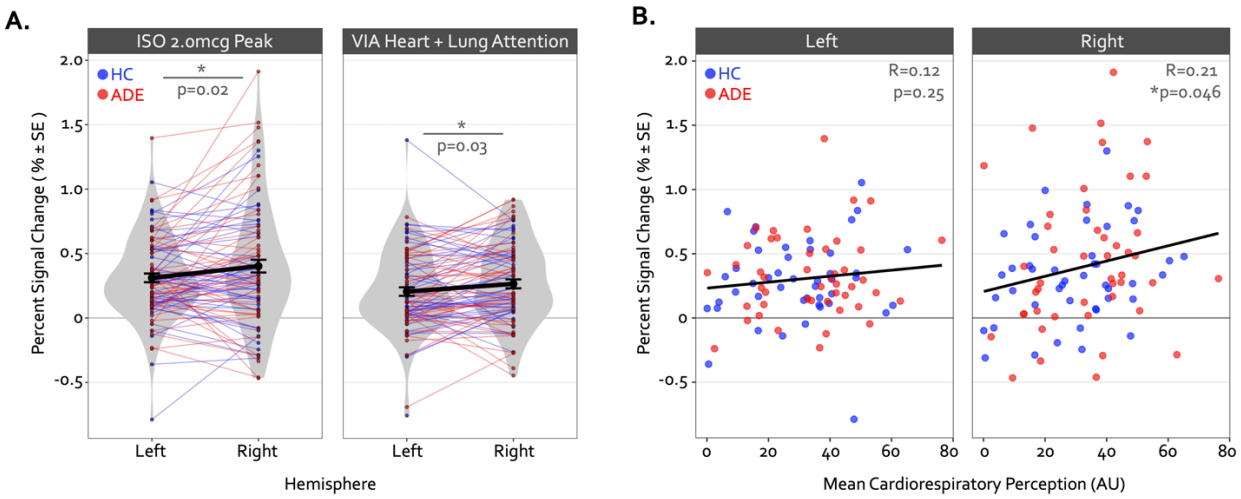

Figure 4. Hemispheric differences in the activation magnitude of the convergent dysgranular insula subregion. (A) Co-activated voxels within the right dysgranular insula subregion exhibited a greater degree of percent signal change during the Peak period of the 2.0mcg dose in the ISO task (left sub panel) and during the Heart and Lung attention condition in the VIA task (right sub panel). As there were no main effects of group, mean percent signal change is collapsed across groups. *indicates $p<0.05$ for the left versus right post-hoc contrast, following a main effect of hemisphere in the linear mixed effects regression. (B) Across both groups the mean percent signal change within the right but not left convergent dysgranular insula subregion was correlated with real-time cardiorespiratory intensity ratings during the Peak period of the 2.0mcg dose in the ISO task. *indicates $p<0.05$ for the Pearson's R correlation coefficient, separately for each hemisphere.

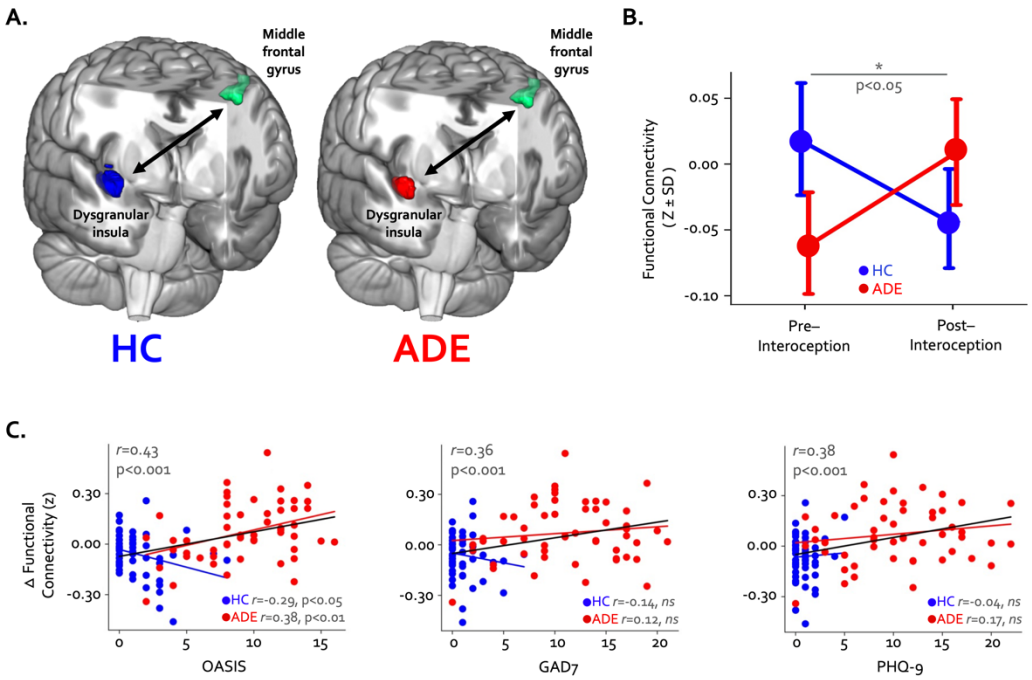

Figure 5. Changes in whole-brain functional connectivity of convergent dysgranular insula subregions following performance of the interoceptive tasks. (A) Functional connectivity between the right convergent dysgranular insula subregion and the left middle frontal gyrus showed opposing effects in the ADE versus HC groups. (B) While functional connectivity between these regions increased from baseline following interoception in the ADE group, it decreased in the HC group, resulting in a significant interaction effect. (C) Across the entire sample changes in functional connectivity were associated with trait measures of anxiety and depression. When examined in each group individually, associations between connectivity change and trait anxiety occurred in opposite directions (left and middle panel).

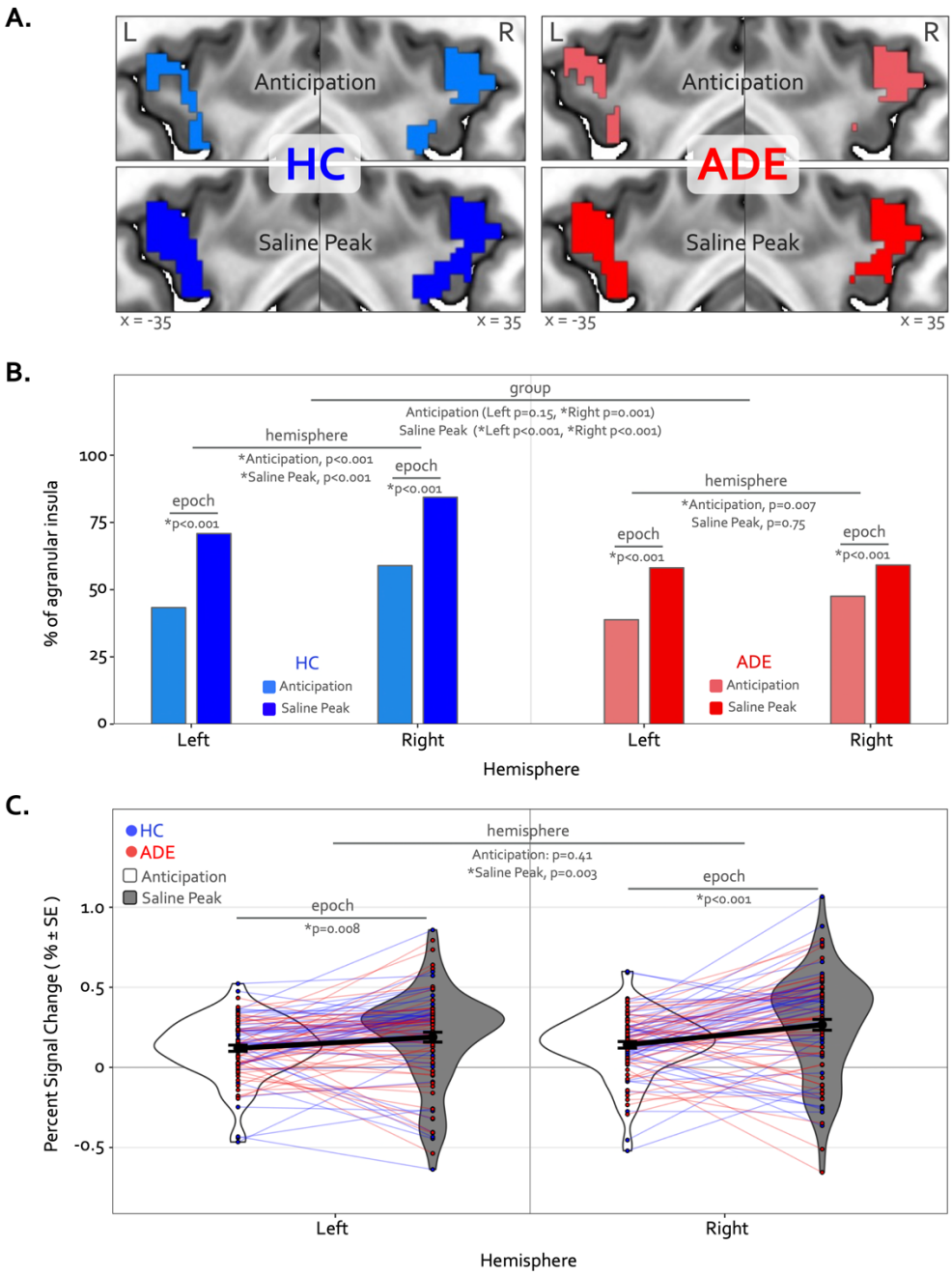

Figure 6. Divergence of top-down processing during interoceptive uncertainty in individuals with anxiety, depression, and/or eating disorders (ADE) and healthy comparisons (HC). (A) Selective activation of the bilateral agranular anterior insula during the Anticipation period (top) and Saline Peak period (bottom) of the ISO task. Both epochs demarcate periods of maximal expectancy about future changes in body state while the body remains at physiological rest (i.e., no ensuring evidence of heart rate or breathing rate increases). These brain areas showed activity only during these periods of the ISO task and were not active during the Heart and Lung attention part of the VIA task, indicated they have specialized roles. (B) Quantifying the spatial extent of this agranular activation (*i.e.,* number of active voxels in relation to total number of voxels in that subregion) revealed that

827 greater right hemisphere activation during the anticipation window occurred for both groups, and
828 more so in the HC than the ADE group. During the peak period of Saline this activation covered
829 more of the bilateral agranular insula in both groups. However, the right hemisphere was activated
830 more than the left in both groups, and the HC group exhibited more active voxels in the right
831 hemisphere than the left, and more than the ADE group in both hemispheres, while the ADE group
832 showed no hemispheric difference during this window. (C) Increased ISO-specific activation of
833 agranular insular during the anticipation period versus the saline peak period. This pattern,
834 occurring for both hemispheres and across both groups, was greater in the right than the left
835 hemisphere.
836

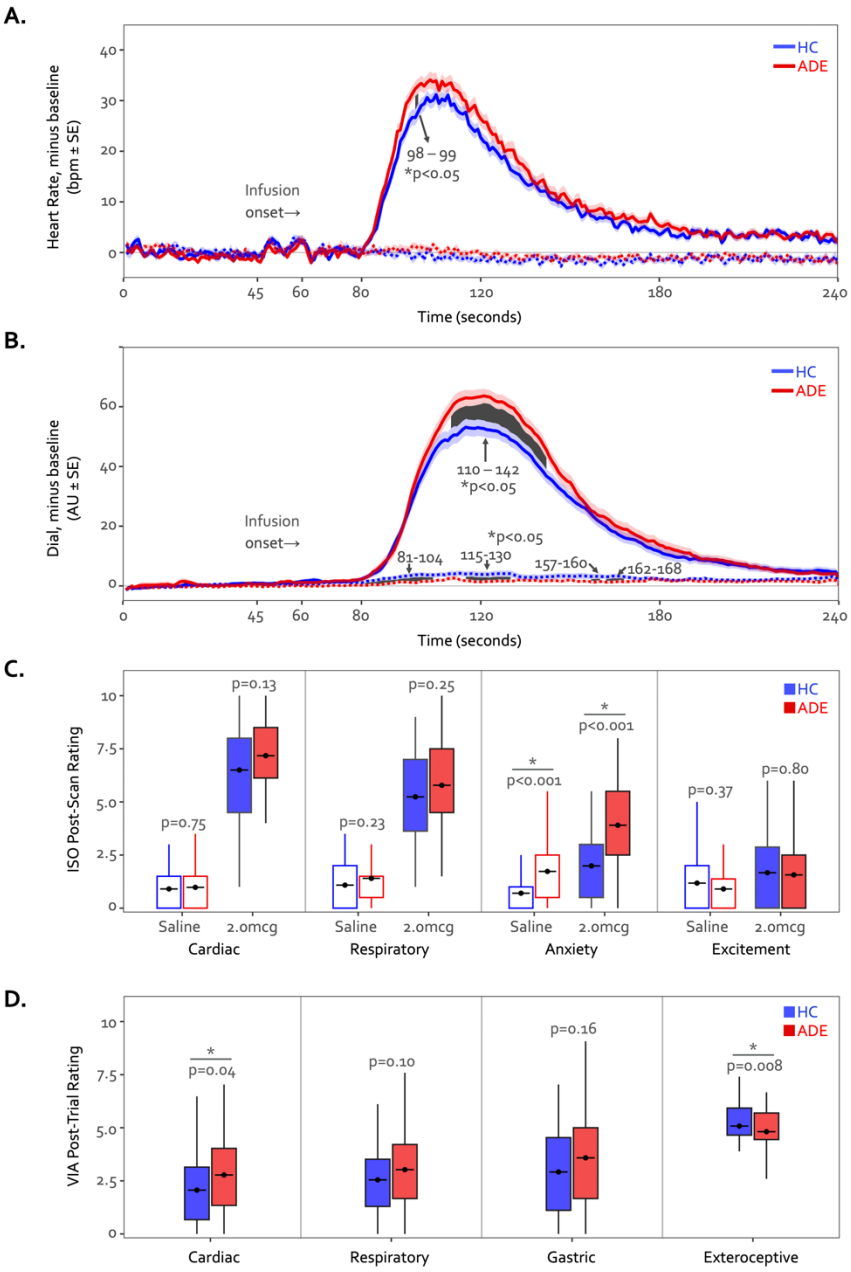

Figure 7. Time courses of heart rate (A) and continuous dial ratings (B) during the 2.0mcg (solid lines) and Saline (dotted lines) doses of the ISO task. Heart rate changes observed during both infusions were generally similar between both groups across the majority of the infusion period. The cluster-based permutation analysis showed a two-second instance of significantly higher heart rate in the ADE group starting at the 98th second of the 2.0mcg dose only during the Peak period, followed by a prolonged increased in dial rating for large portions of the Peak and Recovery periods (seconds 110 through 142, shown via the shaded area). No heart rate differences were observed during the Saline infusion. (C) Retrospective ratings on the ISO task showed no significant group differences in cardiac or respiratory intensity or excitement following saline or the 2.0mcg infusion,

while the ADE group reported increased anxiety following both infusions. (D) Sensation ratings during the VIA task. The cardiac group reported higher cardiac intensity ratings in the ADE group but lower exteroceptive ratings in the target condition, and no group difference in respiratory or gastric sensation ratings. *indicates $p<0.05$ for two-sample t-test between groups, separately for each condition. Where appropriate, both the p-value and Cohen's d are shown.

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