

1           **Neural correlates of cardiac interoceptive accuracy across development:**  
2           **implications for social symptoms in autism spectrum disorders**

3  
4           Michelle D. Failla<sup>1</sup>, Lauren K. Bryant<sup>1</sup>, Brynna H. Heflin<sup>2</sup>, Lisa E. Mash<sup>3</sup>, Kim Schauder<sup>4</sup>,  
5           Samona Davis<sup>1</sup>, Madison B. Gerdes<sup>1</sup>, Amy Weitlauf<sup>5</sup>, Baxter P. Rogers<sup>6</sup>, Carissa J. Cascio<sup>1</sup>

6  
7           **Affiliations**

8           <sup>1</sup>Department of Psychiatry and Behavioral Science, Vanderbilt University Medical Center,  
9           Nashville, TN; <sup>2</sup>Florida International University Doctoral Program in Clinical Science, Miami, FL;  
10           <sup>3</sup>SDSU/UC San Diego Joint Doctoral Program in Clinical Psychology, San Diego, CA; <sup>4</sup>Clinical  
11           and Social Sciences in Psychology, University of Rochester, Rochester, NY; <sup>5</sup>Department of  
12           Pediatrics, Vanderbilt University Medical Center, Nashville, TN; <sup>6</sup>Vanderbilt University Imaging  
13           Institute, Nashville, TN.

14  
15           **Corresponding Author:**

16           Carissa J. Cascio  
17           Vanderbilt University Medical Center

18  
19           **Funding:** This work was supported by National Institutes of Mental Health [1R01MH102272 to  
20           C.J.C, T32-MH18921 supporting M.D.F]; and Autism Science Foundation [2082 to M.D.F].

21  
22           **Acknowledgements**

23           We also acknowledge the support from the Vanderbilt Kennedy Center Treatment and  
24           Research Institute for Autism Spectrum Disorders (NICHD U54HD083211) and Vanderbilt  
25           Institute for Clinical and Translational Research grant support (UL1 TR000445 from

1 NCATS/NIH). We thank Norman Farb for sharing regions of interest in the insula and for his  
2 guidance in their implementation.

3 **Running Title:** Interoception in autism and development

4

1 **Abstract**

2 **Background:** Interoception involves the processing of sensory information relevant to  
3 physiological functioning and is integral to building self-awareness, emotional states, and  
4 modulating social behaviors. With the role of interoception in emotional processing and social  
5 functioning, there is growing interest in characterizing interoception in autism spectrum disorder  
6 (ASD), yet, there are mixed results regarding cardiac interoceptive accuracy in ASD.

7 **Methods:** In this study, we explored the neural basis of cardiac interoception using an fMRI  
8 heartbeat counting task in order to assess neural correlates of primary interoception. We  
9 predicted that interoceptive-specific response in the insula, a “hub” for interoception, would be  
10 related to ASD symptomatology. We investigated the relationship of insula responses during  
11 cardiac interoceptive accuracy and a self/caregiver-reported autism-related symptom scale  
12 (Social Responsiveness Scale-2 (SRS)). Participants included 46 individuals with autism  
13 spectrum disorder (ASD) (age 8-54, mean= 19.43±10.68 years) and 54 individuals with typical  
14 development for comparison (TC, age 8-53, mean= 21.43±10.41 years).

15 **Results:** We found no significant difference in cardiac interoceptive accuracy or neural  
16 response to cardiac interoception in ASD. Several insula subdivisions had a curvilinear  
17 relationship to age, peaking in early adulthood. Interoceptive-specific insula response was  
18 associated with adult self-report SRS scores; this association differed by diagnostic group and  
19 self/other report.

20 **Conclusions:** This work suggests that 1) there is no global deficit in interoception in ASD, but  
21 that integrating interoceptive cues with social information may distinguish individuals with ASD,  
22 and 2) there is a developmental trajectory for interoceptive processing in the insula that may be  
23 relevant for socio-emotional health.

24 **Keywords:** interoception, development, autism, fMRI, insula

25

## 1 **Introduction**

2           The human experience of emotion begins in the body, with sensory cues generated by  
3 autonomic responses to emotionally-salient events, such as a racing heart after a near-miss in  
4 traffic, or the feeling of butterflies in the stomach on a first date. This internally-generated  
5 landscape of sensations is known as interoception, though this term is now often used more  
6 broadly to describe all somatic input that confers information about the physiological state of the  
7 body, whether arising from the viscera or from the skin (1). Higher-order representations of  
8 emotional states are built from these bodily sensations; thus accuracy and interpretation of  
9 interoceptive signals can influence emotional processing and regulation (2, 3). By learning to  
10 associate interoceptive information with external stimuli, we build a sense of self in relation to  
11 others that serves as a foundation for social function(4) .

12           While few experimental studies have investigated interoception across development (for  
13 a review see (5)), the available evidence distinctly indicates a trajectory of interoceptive  
14 perception that changes with development. Maister and colleagues demonstrated that infants as  
15 young as five months can distinguish stimuli presented asynchronously vs. synchronously with  
16 their own cardiac rhythm. This implicit perception of cardiac interoceptive cues was altered by  
17 emotional state, providing support for the idea that interoception is central to the development of  
18 socio-emotional awareness (6). Older children show similar levels of cardiac interoceptive  
19 accuracy to adults in a experimental heartbeat counting paradigm (7, 8), suggesting early  
20 maturation of explicit interoceptive abilities. These studies indicate perception and processing of  
21 interoceptive cues begins early in development, thus, understanding their trajectory will be  
22 important for characterizing the role of interoception in socio-emotional development.

23           Given the role of interoception in emotional processing and social functioning, there is a  
24 growing interest in characterizing interoception in autism spectrum disorder (ASD) (9–11). ASD  
25 is characterized by social communication difficulties, restricted and repetitive behaviors, and  
26 altered sensory perception and reactivity. Thus, altered interoception could be related both to

1 perceptual differences and social-emotional differences in this population. There is mounting  
2 evidence of altered insular connectivity in ASD across development (12, 13). Examining  
3 interoceptive abilities in ASD, specifically across the lifespan, may provide significant insight into  
4 the social communication deficits that are central to ASD (14, 15). Some studies report  
5 diminished cardiac interoceptive accuracy in both school-age children (16) and adults with ASD  
6 (17, 18), but most have found no significant differences (10, 11, 19–21). For a review, see (22)).  
7 While the field may be moving towards an understanding that cardiac interoceptive accuracy is  
8 not systematically/dramatically different in ASD, how these cues are interpreted, and relayed to  
9 the rest of the brain – and how they relate to autism symptoms - remains to be seen.

10         Understanding the role for interoceptive processing in autism related-symptoms is likely  
11 tied to one’s ability to pair interoceptive information with external events. Altered integration of  
12 interoceptive information could have early consequences. Recently, Quattrocki and Friston  
13 proposed that the ability to pair interoceptive signals, like satiety, with external cues, like a  
14 caregiver’s presence, could influence infant social learning and attachment (15). This  
15 relationship between social functioning and interoception is maintained over the lifespan. In  
16 adolescence, heightened social sensitivity increases the influence of social context on  
17 interoceptive functioning (23, 24). In adults, heightened interoceptive accuracy may be a  
18 protective factor against social exclusion (25). Given the role of social functioning in typical  
19 development, and in developmental disorders like autism spectrum disorders (ASD), it is  
20 important to understand interoceptive functioning across development and how it may influence  
21 socio-emotional health at different ages.

22         Examination of neural correlates of interoception provides an opportunity to assess the  
23 connection between perceptual and social-affective brain networks, while avoiding potential  
24 confounds related to self-report and experimental task performance. A meta-analysis of cardiac  
25 interoceptive fMRI studies shows consistent response in the somatomotor cortex, medial frontal  
26 regions including the cingulate, and most prominently, in the bilateral insula (26). The posterior

1 insula contains primary interoceptive cortex (27), while salience and emotional processing  
2 involves the anterior insula (28). Studies have also shown a specific role of the anterior insula in  
3 modulating the influence of interoceptive processing on social behaviors (29, 30). While there  
4 has been limited work specifically addressing developmental changes in interoceptive neural  
5 responses, there is evidence of a non-linear developmental trajectory, with peak neural  
6 responses (24) and structural changes in insular connectivity (31) emerging in  
7 adolescence/early adulthood.

8         In this study, we aim to examine the neural responses underlying unisensory cardiac  
9 interoceptive processing in ASD and to understand how interoceptive processing may change  
10 across development. By investigating how interoception changes with age, we can better  
11 understand its role in both typical and atypical social development. The use of neuroimaging  
12 provides a window into stages of perceptual processing that *precede* interoceptive awareness  
13 and its influence on socio-emotional functioning. Thus, in the current study, we used functional  
14 neuroimaging in combination with a heartbeat counting task (Schandry, 1981). Among several  
15 alternatives, we chose this task for two reasons: 1) its widespread use maximizes the ability to  
16 interpret findings in the context of a large literature on this topic, and 2) its interoceptive  
17 “purity”—compared to other established interoceptive tasks like the heartbeat discrimination  
18 task that requires comparison of external stimuli to cardiac rhythms, the Schandry task  
19 demands focus on interoceptive signals alone. This feature allows for the establishment of  
20 neural correlates of interoception with minimal exteroceptive influences.

21         Given the mixed results regarding cardiac interoceptive accuracy in ASD (22), we  
22 explored the neural basis of interoception, and the relationship between neural responses and  
23 age in a cross-sectional sample of individuals with both typical development and ASD, spanning  
24 middle childhood through middle adulthood. We first focused on the insula as the proposed  
25 “hub” of interoceptive processing (26–28), and then further expanded our investigation into a  
26 whole-brain search, incorporating age and diagnostic status in our model. Given the links

1 between interoception, insula response, and socio-emotional behavior, we predicted that  
 2 interoceptive-specific response in the insula would be related to ASD symptomatology.

3

4 **Methods**

5 *Participants*

6 The Vanderbilt University Medical Center Institutional Review Board approved this study.  
 7 Informed consent and/or assent was obtained for every participant. In total, 48 individuals with  
 8 ASD and 60 individuals in a typically developing comparison group (TC) were recruited for this  
 9 study. Data from 8 participants were not included in the analyzed dataset: 5 participants were  
 10 excluded for excessive motion (see motion exclusion criteria in Methods, 1 ASD, 4 TC, ages 8-  
 11 14), and 3 participants had significant acquisition errors during the scan (1 ASD, 2 TC, ages 8-  
 12 30). The final analyzed sample included 46 individuals with ASD (age 8 to 54, mean=  
 13 19.43±10.68 years) and 54 individuals in a typically developing comparison group (TC, age 8 to  
 14 53, mean= 21.43±10.41 years). A summary of demographic information is provided in **Table 1**.

15

16 **Table 1. Participant demographic and motion characteristics.**

	<b>Autism Group n=46</b>	<b>Typical Comparison Group n=54</b>	<b>p-value</b>
<b>Demographics</b>			
<b>Male, # (%)</b>	32 (69.6)	35 (63.6)	0.677
<b>Age (years), mean (SD)</b>	20.84±10.11	19.85±11.14	0.644
<b>Full Scale IQ</b>	103.93±15.25	109.07±13.28	0.080
<b>Scanner Motion</b>			
<b>Max Rotation</b>			
<b>X</b>	0.021±0.032	0.014±0.023	0.015
<b>Y</b>	0.005±0.005	0.005±0.005	0.087
<b>Z</b>	0.004±0.006	0.004±0.005	0.274
<b>Max Translation</b>			
<b>X</b>	0.196±0.261	0.180±0.197	0.049
<b>Y</b>	0.413±0.507	0.342±0.444	0.081
<b>Z</b>	0.743±0.872	0.571±0.701	0.002
<b>% dropped volumes</b>	2.657±2.515	2.371±2.275	0.329

17

1 All participants had IQ>70, as measured by Wechsler Abbreviated Scales of  
2 Intelligence—Second Edition (WASI-II; Wechsler, 2011). ASD diagnosis was confirmed by  
3 research-reliable administration of the Autism Diagnostic Observation Schedule— Second  
4 Edition (ADOS-2; (32)), and the judgment of a licensed clinical psychologist based on DSM-5  
5 criteria. TC individuals were excluded if they had a history of any psychiatric or learning disorder  
6 or had a first-degree relative with ASD. All participants had normal or corrected-to-normal vision.  
7 Exclusion criteria for both groups included genetic conditions, neurological disorders, significant  
8 head injuries, MRI contraindications, and a number of psychotropic medications (including  
9 selective serotonin reuptake inhibitors, serotonin and noradrenergic reuptake inhibitors,  
10 noradrenergic and specific serotonergic antidepressants, norepinephrine-dopamine reuptake  
11 inhibitors, tricyclics, atypical antipsychotics, mood stabilizers and/or anticonvulsants, anti-  
12 hypertensives, and anti-anxiety medications; see **Supplemental Table 1** for complete list).  
13 Stimulant medications were permitted, but suspended for at least 24 hours prior to the scan.  
14 The Social Responsiveness Scale (SRS) (33), a 65-question survey assessing traits relevant for  
15 ASD, was administered. For children, a parent or caregiver completed the parent-report SRS  
16 version. For adults, either a parent or spouse reported (relative/other report version of SRS,  
17 n=9) or participants completed the self-report version. Some individuals did not complete the  
18 SRS (total analyzed sample, children: ASD=17, TC=15; adults: ASD=19, TD=16).

### 19 *Heartbeat Counting Task*

20 During the functional scan, participants completed a modified cardiac interoceptive  
21 accuracy task (7) adapted from (10, 11). The task was presented using e-Prime 2.0 and  
22 consisted of 4 runs, with 6-8 randomized blocks of counting heartbeats or a visual stimulus.  
23 Instructions were as follows: “*You are going to see either a heart or a star. Each stimulus will be*  
24 *preceded by a fixation cross. Please stare at the cross until the picture appears. When a heart*  
25 *appears, focus on counting the number of times your heart beats. When a star appears, focus*  
26 *on counting the number of times the star appears.*” During the heartbeat condition, a heart



1 shape was static on the screen and individuals were instructed to count their own heartbeat  
2 while lying still in the scanner. During the visual condition, a low-contrast star shape flashed at a  
3 rate similar to an average resting heart rate. The flashing visual counting stimulus was designed  
4 to be difficult to detect visually. Following each block, participants reported their count using  
5 hand signals to a researcher in the scanning room. There was a 15 second rest between each  
6 block. This task was practiced outside of the scanner to ensure task comprehension and  
7 participants were continually reminded that they were not to physically feel any part of their body  
8 for a pulse.

9         During the entire scan, the participant's heart rate was recorded using the peripheral  
10 pulse oximeter associated with the 3.0 Tesla Phillips Achieva MRI scanner (sampling rate of  
11 0.002 seconds). Accuracy on the heartbeat task was calculated by comparing the participant  
12 counts to the physiological heart rates from the scanner. Accuracy was calculated on each trial,  
13 or block during each scanner run as the absolute value of ((actual heartbeats from scanner  
14 output - reported heartbeats) / actual heartbeats). Overall accuracy was based on the average  
15 for all completed blocks of heartbeat counting. Accuracy on the visual counting task was  
16 calculated as follows: absolute value of ((actual flashes in e-prime output – reported flashes) /  
17 actual flashes in e-prime output). Overall accuracy was based on the average for all completed  
18 blocks of visual counting. Some visual (n=9) and heartbeat (n=18) counting data were missing  
19 due to technical issues during data transfer from the scanner. Physiological recording data were  
20 cleaned prior to analysis; recordings of fewer than 20 beats per 30 seconds were rejected as  
21 unlikely to reflect accurate heartrates. Similarly, participant-report counts that were below 5  
22 were rejected as inconsistent with task understanding. Counts were not used if the participant  
23 indicated they were falling asleep or unable to sustain attention for a particular block. For these  
24 reasons, 5 participants' accuracy data were not included in the final analysis. The final sample  
25 of accuracy data included for heartbeat accuracy: ASD, n=28, TC, n=41 and for visual accuracy:  
26 ASD, n=29, TC, n=44.

## 1 *Image Acquisition*

2 Image data was collected using a 3.0 Tesla Phillips Achieva MRI scanner with a 32-  
3 channel SENSE head coil. A high-resolution T1-weighted anatomical image was acquired  
4 (1mm<sup>3</sup> resolution, TR=8 msec, TE= 3.7 msec, acquisition matrix=256x256) for registration of  
5 functional images. Functional images were acquired with a whole-brain T2\*-weighted EPI  
6 sequence (axial slices, voxel size=2.5x2.5x3mm, TR=2 s, TE=25 msec, FOV= 96x96, flip  
7 angle=90°).

## 8 *Image Preprocessing and Analysis*

9 For processing anatomical images, we used a large-scale neuroimaging data platform  
10 (34). A multi-atlas segmentation algorithm (35) with a set of manually labeled atlases  
11 (Neuromorphometrics, Inc., Somerville, MA, USA) was applied to the T1-weighted image in  
12 order to obtain a gray matter image. All gray matter regions were summed to make a total gray  
13 matter image, and then the gray matter image was nonlinearly normalized to a MNI-152 gray  
14 matter probabilistic template (OASIS project (<http://www.oasis-brains.org>), provided by  
15 Neuromorphometrics, Inc. (<http://www.neuromorphometrics.com>)). The registration algorithm  
16 minimized the sum of squared differences between gray matter image and template (SPM12  
17 “Old Normalise”, (36, 37)) and modeled spatial nonlinearity with a discrete cosine basis of 25  
18 mm lower cutoff. Using parameters resulting from the anatomical normalization step, each  
19 subject’s coregistered functional images were then warped to MNI space.

20 FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version  
21 6.00, part of FSL (FMRIB's Software Library, [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)). Motion correction was  
22 conducted with MCFLIRT (38); brain extraction with BET (39), a high-pass filter (300s), grand-  
23 mean intensity normalization of the entire 4D dataset by a single multiplicative factor and spatial  
24 smoothing (FWHM=5mm). Time-series statistical analysis was conducted with prewhitening  
25 using FILM with local autocorrelation correction (40).

1 Each block was modeled as a single contrast of stimulus – baseline (rest of 15s),  
2 convolved with a double-gamma hemodynamic response function with temporal filtering added  
3 to the GLM model. Standard motion parameters were included in the general linear model  
4 (GLM), with the addition of DVARS (D, temporal derivatives of time courses; VARS, variance of  
5 root mean squares of head motion across voxels) and framewise displacement metrics for head  
6 motion (41) to the GLM as confound explanatory variables (EV) to remove effects of outlier  
7 volumes from the parameter estimates of interest. Individual runs were rejected based on peak  
8 motion > 5mm or if DVARS/FD metrics flagged more than 10% of volumes as outliers. In the  
9 analyzed dataset, there were no differences in the number of outlier volumes by diagnostic  
10 group. There was a significant difference in motion by diagnostic group such that ASD had  
11 higher max translation in x ( $p=0.049$ ) and z ( $p=0.002$ ) and rotation around x (pitch,  $p=0.015$ ).

12 Second-level analyses combined runs for each subject using a fixed-effects model, by  
13 forcing random effects variance to zero in FLAME (FMRIB's Local Analysis of Mixed Effects)  
14 (42–44).

#### 15 *Region of Interest Analysis*

16 Regions of interest (ROI) in this study included sub-divisions of the bilateral insula as  
17 defined cyto-architecturally (45), as previous work has suggested different interoceptive  
18 functions per laminar architecture (46). The Farb et al. insular ROIs include 16 subdivisions on  
19 right (8) and left (8) insular cortex. Percent signal change was extracted using featquery in FSL  
20 for each of the 16 insular ROIs and then averaged into 3 ROIs on each insular cortex: anterior  
21 (3 most anterior ROIs), mid (2 middle ROIs), and posterior (3 most posterior ROIs). Figure 1  
22 shows a depiction of these bilateral insular subdivisions.

#### 23 *Whole-brain Group Analysis*

24 Group analyses were carried out using FLAME (FMRIB's Local Analysis of Mixed  
25 Effects) stage 1 model (42–44). Given the developmental age range in this sample, age was  
26 considered a covariate in the GLM, as both a linear and nonlinear (quadratic) association. All

1 group maps were made using a cluster threshold of  $z = 2.3$  and a  $p$ -value = 0.05 (family-wise  
2 error corrected based on clusters defined by Gaussian random field theory (47)). Additionally,  
3 group contrasts of ASD>TD and TD>ASD were examined. Nonparametric permutation testing of  
4 group results was conducted using FSL's randomize (48). Using the Threshold-Free Cluster  
5 Enhancement method, 5000 iterations of each statistics map were generated, results are  
6 reported at the  $p < 0.05$  level.

7

### 8 *Statistical Analysis*

9 Demographic and survey data for this study was collected and managed with REDCap  
10 electronic data capture tools (49). Data processing and statistical analyses were conducted  
11 using Python (v.2.7) and R (v.3.3.2). Multiple comparison corrections were calculated using the  
12 Holm method (50).

13 To examine diagnostic group differences in demographic variables, motion parameters,  
14 and percent signal change in insula subdivisions, we used parametric tests (t-tests), non-  
15 parametric tests (Mann-Whitney), or chi-square tests, where appropriate. To investigate  
16 associations between age and percent signal change in insula subdivisions, we first investigated  
17 nonlinear, quadratic fit regression models and then calculated spearman's rho for those insula  
18 subdivisions where a nonlinear, quadratic fit was not significant.

19 To address missing data in interoceptive accuracy data and SRS scores, we used  
20 multiple imputation by chained equations (51, 52); data were imputed to 5 datasets, with up to  
21 40 iterations, using predictive mean matching and estimates from all reported tests were pooled.  
22 To investigate relationships between heartbeat counting accuracy, age, and IQ, we calculated  
23 spearman's rho. To compare group means in heartbeat counting accuracy, we calculated  
24 student's t-tests.

25 To investigate effects of neural responses in the insula, diagnostic group, and age on  
26 social symptomology, we employed linear regression models with SRS total T-scores as the

1 dependent variable. Age, diagnostic group, and percent signal change from the left posterior  
2 insula were entered into the model, as well as a diagnostic group by insula variable. Models  
3 were calculated separately in self- and other-reporter versions of the SRS.

4

## 5 **Results**

### 6 *Cardiac interoceptive accuracy*

7 Heartbeat counting accuracy did not differ by group (ASD: 71.50 ± 26.59%, TC: 75.71 ±  
8 20.09%,  $t=-0.936$ ,  $df=51.54$ ,  $p=0.354$ ), and reached accuracy levels consistent with our previous  
9 results outside of the scanner (10). Age was not significantly associated with heartbeat accuracy  
10 in the total sample ( $r=0.114$ , 95% CI (-0.140-0.355),  $p=0.379$ ); additionally, there was no  
11 significant interaction between age and diagnostic group on heartbeat accuracy (age\*diagnosis:  
12  $\beta=-0.021$ ,  $p=0.957$ ). Heartbeat counting accuracy did not differ by gender (Female: 74.09 ±  
13 24.42%, Male: 73.62 ± 22.09%,  $t=0.102$ ,  $df=40.56$ ,  $p=0.919$ ). IQ was significantly correlated with  
14 heartbeat accuracy across both groups ( $r=0.243$ , 95% CI (0.024-0.439),  $p=0.030$ ). Using linear  
15 regression, we investigated the possibility of an interaction between IQ and diagnostic group on  
16 heartbeat accuracy, correcting for age ( $r^2=0.122$ ). IQ was still associated with heartbeat  
17 accuracy ( $\beta=0.502$ ,  $p=0.014$ ); age ( $\beta=0.226$ ,  $p=0.283$ ), diagnostic group ( $\beta=39.52$ ,  $p=0.180$ ),  
18 and diagnostic group\*IQ ( $\beta=-0.351$ ,  $p=0.205$ ) were not significant predictors of heartbeat  
19 accuracy.

20 In the control condition, mean visual counting accuracy was over 92% for both groups  
21 with no significant group differences (ASD: 92.61 ± 16.47%, TC: 93.60 ± 13.90%,  $t=-0.329$ ,  
22  $df=43.80$ ,  $p=0.744$ ). Visual counting accuracy was correlated with heartbeat counting accuracy  
23 ( $r=0.286$ ,  $p=0.026$ ), however, when visual counting accuracy and IQ were in the same linear  
24 regression model, IQ was still associated with heartbeat counting accuracy ( $\beta=0.314$ ,  $p=0.046$ )  
25 while visual counting accuracy was not ( $\beta=0.121$ ,  $p=0.552$ ,  $r^2=0.083$ ).

### 26 *Insula response during interoception*

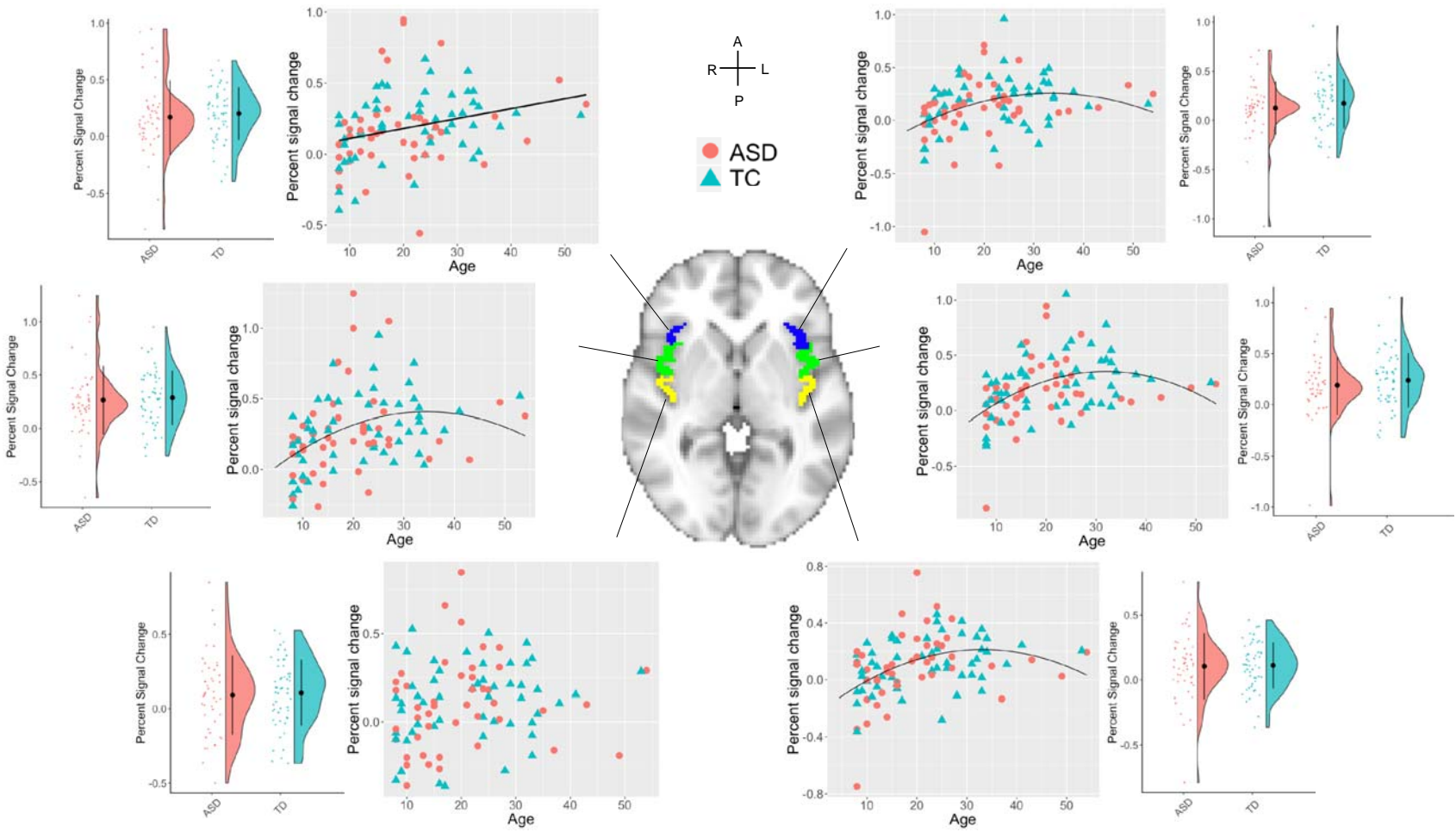
1 We examined percent signal change in subdivisions of the insula as it related to  
2 diagnosis and age. For each subdivision of the insula, mean percent signal change was not  
3 significantly different by diagnostic group, for anterior, left:  $ASD=0.124\pm0.325$ ,  $TD=0.171\pm0.233$ ,  
4  $W=1086$ ,  $p=0.353$ , and right:  $ASD=0.169\pm0.326$ ,  $TD=0.202\pm0.233$ ,  $W=1052$ ,  $p=0.243$ ; for  
5 middle, left:  $ASD=0.190\pm0.291$ ,  $TD=0.238\pm0.265$ ,  $W=1068$ ,  $p=0.291$ , and right:  
6  $ASD=0.265\pm0.321$ ,  $TD=0.287\pm0.253$ ,  $W=1099$ ,  $p=0.402$ ; and posterior, left:  $ASD=0.105\pm0.254$ ,  
7  $TD=0.111\pm0.177$ ,  $W=1211$ ,  $p=0.958$ , and right:  $ASD=0.091\pm0.266$ ,  $TD=0.107\pm0.222$ ,  $W=1139$ ,  
8  $p=0.577$  (**Figure 1**).

9 For age, we explored both linear and nonlinear, quadratic models as previous studies  
10 have demonstrated both linear and nonlinear developmental trajectories in the brain (53).  
11 **Figure 1** shows the inverted curvilinear relationship between age and percent signal change in  
12 4 of the 6 insular subdivisions. Of the remaining 2 subdivisions, the right anterior insula had a  
13 better fit with a linear model, whereas the right posterior insula did not show a significant  
14 relationship with age in either linear or nonlinear models. **Table 2** shows the best-fit model  
15 parameters for each ROI and age.

16

17

18



1  
2  
3

Figure 1

1 **Figure 1. Percent signal change in subdivisions of the bilateral insula (heartbeat**  
 2 **counting-visual counting) is not different by diagnostic group but is related to age.**  
 3 Regions of interest are shown in the middle on the MNI template brain. Insula regions are  
 4 subdivided into 3 regions on each side, blue: anterior, green: mid, yellow: posterior insula.  
 5 **There were no differences in percent signal change in any of the insula subregions by**  
 6 **diagnostic group:** for anterior, left: ASD=0.124±0.325, TD=0.171±0.233, W=1086, p=0.353,  
 7 and right: ASD=0.169±0.326, TD=0.202±0.233, W=1052, p=0.243; for middle, left:  
 8 ASD=0.190±0.291, TD=0.238±0.265, W=1068, p=0.291, and right: ASD=0.265±0.321,  
 9 TD=0.287±0.253, W=1099, p=0.402; and posterior, left: ASD=0.105±0.254, TD=0.111±0.177,  
 10 W=1211, p=0.958, and right: ASD=0.091±0.266, TD=0.107±0.222, W=1139, p=0.577.  
 11 **For relationships with age,** model fit and parameters for each insula subdivision can be found  
 12 in **Table 2.**

13  
 14  
 15 **Table 2. Linear and nonlinear models of age by percent signal change in insular regions.**

Insula Region	Overall Model Fit (p-value)	R <sup>2</sup>	Variable	β	Standard Error	t	p-value	
<b>Left</b>								
<i>Anterior</i>	Quadratic	0.0013*	0.1302	Age	0.02847	0.00899	3.167	<i>0.0021</i>
				Age <sup>2</sup>	-0.00042	0.00017	-2.504	<i>0.0140</i>
<i>Mid</i>	Quadratic	0.00004*	0.1915	Age	0.03846	0.00911	4.222	<i>&lt;0.0001</i>
				Age <sup>2</sup>	-0.00060	0.00017	-3.491	<i>0.0007</i>
<i>Posterior</i>	Quadratic	0.00006*	0.1838	Age	0.02736	0.00696	3.931	<i>0.0002</i>
				Age <sup>2</sup>	-0.00041	0.00013	-3.136	<i>0.0023</i>
<b>Right</b>								
<i>Anterior</i>	Linear	0.0045*	0.0809	Age	0.00699	0.00241	2.907	<i>0.0045</i>
<i>Mid</i>	Quadratic	0.0005*	0.1449	Age	0.03033	0.00939	3.232	<i>0.0017</i>
				Age <sup>2</sup>	-0.00044	0.00018	-2.479	<i>0.0150</i>
<i>Posterior</i>	Linear	0.1047	0.0272	Age	0.00367	0.00224	1.638	0.1050

Note: italicized p-value denotes p<0.05, \*denotes p-values survive Holm multiple comparison correction

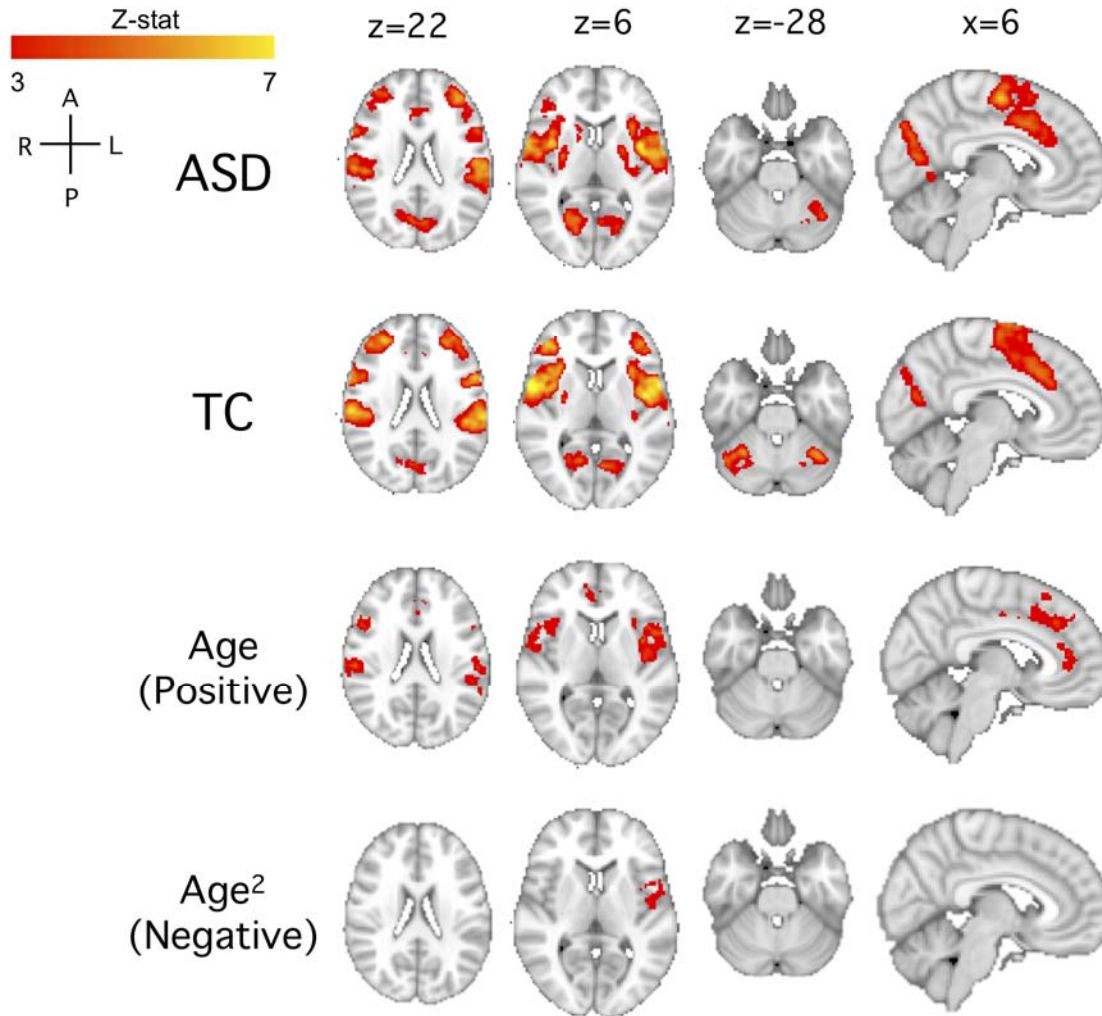
16  
 17 There were no significant relationships between percent signal change in insula  
 18 subregions and heartbeat counting accuracy ([anterior: left, r=-0.020,p=0.865; right, r=0.132,



1 p=0.268]; [mid: left, r=0.046, p=0.692; right, r=0.105, p=0.372]; [posterior: left, r=0.079, p=0.484,  
2 right: r=0.006, p=0.958]).

3 *Interoception-specific whole brain responses*

4 For the ASD and TC groups, interoception-specific BOLD response was observed  
5 bilaterally in the insula, medial frontal gyrus, anterior cingulate, primary and secondary  
6 somatosensory regions, visual cortex, and cerebellum (**Figure 2**). We also investigated group  
7 differences: there were no significant clusters where ASD>TC or TC>ASD. There were several  
8 responses associated with age, both with a positive linear association (bilateral anterior, mid,  
9 and posterior insula, secondary somatosensory, anterior cingulate) as well as with a negative  
10 quadratic association (left insula). Following permutation testing, both groups retained clusters  
11 in the bilateral insula, while the ASD group also showed response in the visual cortex and  
12 cerebellum. In the permutation testing, positive linear associations with age expanded to include  
13 the majority of the interoceptive network (**Supplemental Figure 1**). There were no clusters  
14 related to quadratic, nonlinear age effects.



1

2 **Figure 2. Whole brain heartbeat counting-visual counting in the total sample.** Group maps

3 are shown for the autism spectrum disorders (ASD), typical comparison group (TC), and linear

4 and nonlinear age associations. Group maps were calculated with  $z > 3$  and  $p < 0.01$ . We also

5 tested group comparison of  $ASD > TC$  and  $TC > ASD$  (thresholded at  $z > 2.3$  and  $p < 0.05$ ), but there

6 were no significant clusters. There were no negative linear age associations and no positive

7 nonlinear age<sup>2</sup> associations. See **Supplemental Figure 1** for permutation testing results.

8

9 *Associations of interoceptive response and autism-related behaviors*

10 We investigated the relationship between insular response during our interoception task

11 and ASD-related symptomology, using SRS total T-score as the dependent variable in separate

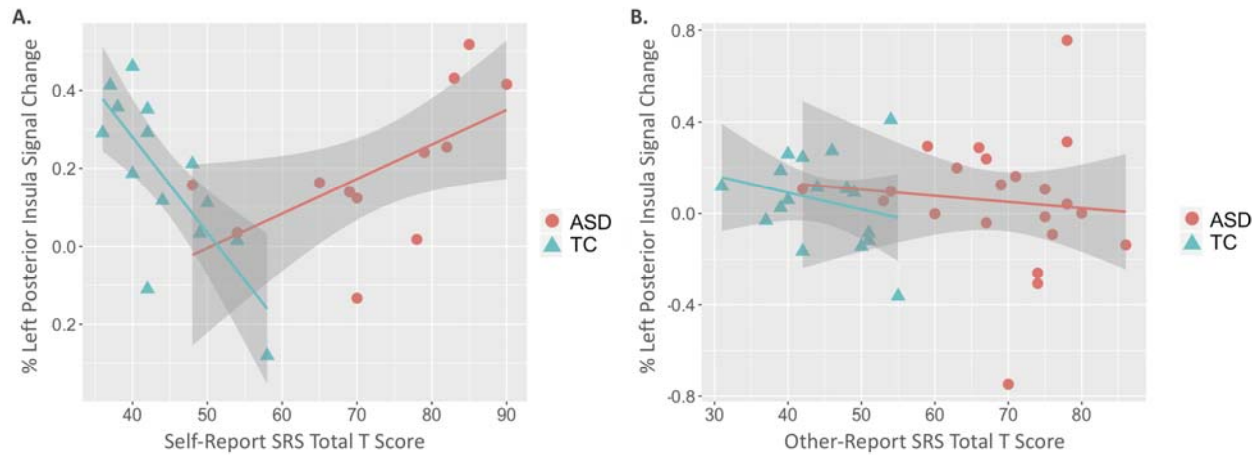
1 linear regression models by self and other report. We chose the left postserior insula for this  
 2 analysis, because posterior insula contains primary interoceptive cortex and left insula may be  
 3 more responsive to social and other emotionally relevant stimuli (54, 55). As age was  
 4 significantly associated with posterior insula response, we included age in our models of SRS  
 5 scores. We also incorporated an interaction between insula response and diagnostic group in  
 6 our models.

7 **Table 3** summarizes models for total SRS scores, separated by self-report and other  
 8 reporters. In the self-report model, there was a significant interaction between percent signal  
 9 change in the left posterior insula and diagnostic group on total SRS T-scores (**Figure 3, Table**  
 10 **3**). There was a positive relationship between left posterior insula response and SRS scores for  
 11 those with ASD, whereas there was a negative relationship between insula response and SRS  
 12 scores in the TC group. In the other-reporting model, diagnostic group was significantly  
 13 associated with SRS scores, but left posterior insula response was not significantly related, nor  
 14 was there a significant interaction between left posterior insula response and diagnostic group.  
 15 Age was not significantly associated with SRS score in either model.

16 **Table 3. Linear regression models of ASD-related social symptomology with left**  
 17 **posterior insula and diagnostic group.**

$R^2$	Variable	$\beta$	Standard Error	t	p-value
<b><i>Self-Report</i></b>					
0.801	Age	0.1224	0.307	0.399	0.6947
	Left Posterior Insula (% Signal Change)	41.407	13.356	3.100	<i>0.0058</i>
	Diagnostic Group	-16.363	4.461	-3.668	<i>0.0016</i>
	Insula x Group	-65.450	17.023	-3.844	<i>0.0011</i>
<b><i>Other-Report</i></b>					
0.591	Age	-0.053	0.202	-0.264	0.7926
	Left Posterior Insula (% Signal Change)	-4.253	7.539	-0.564	0.5754
	Diagnostic Group	-21.840	2.937	-7.435	<i>&lt;0.0001</i>
	Insula x Group	-1.021	13.310	0.077	0.9392

*Note: italicized p-value denotes  $p < 0.05$*



1

2

3 **Figure 3. Associations between autism-relevant symptomology and left posterior insular**

4 **response during an interoceptive task.** In the self-report model (A), there was a significant

5 interaction between percent signal change in the left posterior insula and diagnostic group on

6 total SRS T-scores. In the other-reporting model (B), diagnostic group was significantly

7 associated with SRS scores, but left posterior insula response was not significantly related, nor

8 was there a significant interaction between left posterior insula response and diagnostic group.

9 See **Table 3** for model specifics.

10

## 11 **Discussion**

12 In this study, we report developmental effects on neural response to an interoceptive

13 heartbeat counting task in children and adults with and without ASD. Neural responses

14 throughout the insula during the heartbeat counting task were not different by diagnostic group,

15 consistent with our previous behavioral findings of intact cardiac interoception in ASD (Schauder

16 et al., 2015, Mash et al., 2017). However, we found that the response of primary interoceptive

17 cortex during interoception interacted with group to predict ASD symptoms as measured by

18 SRS self-report. These findings suggest that people with autism may integrate internal sensory

19 cues and external, social information, in a different way. This work reinforces the link between

1 neural processing of internal sensory cues and social-emotional awareness. The size and broad  
2 age range of our sample provided a unique opportunity to detect age-related patterns in  
3 interoception.

4         In this study, there was a significant relationship between interoceptive processing and  
5 age that was different for children compared to adults. There was a positive relationship  
6 between age and insular response to heartbeat counting that continued into early adulthood.  
7 This mirrors the findings of Li and colleagues, (24), who described nonlinearly increasing  
8 interoceptive BOLD responses in the ventral anterior insula with age in a sample aged 10-20  
9 years. Our results corroborate this peak of insular response in late adolescence or early  
10 adulthood, which could suggest maturation of these circuits in this timeframe. Interestingly,  
11 cardiovagal autonomic function peaks around the same time, suggesting maturation of  
12 important autonomic neural mechanisms in parallel (56). While our sample did include up to  
13 middle adulthood, it is not clear how this relationship might extend into later adulthood. There is  
14 evidence of reduced interoceptive accuracy with age in adults (57, 58), but it is not clear how, or  
15 if, the neural circuits underlying interoception change throughout adulthood. Our results suggest  
16 there is a tempered decline in interoceptive responses in the insula, but replication is needed to  
17 confirm this finding.

18         There were no significant differences in cardiac interoceptive neural processing in ASD,  
19 either in insula subdivisions or in the whole brain search, and no group differences in  
20 interoceptive accuracy. Yet, we show a clear relationship between neural processing of  
21 interoceptive signals and reported social responsiveness in ASD. While some previous studies  
22 have reported diminished heartbeat counting accuracy in children (16) and adults with ASD (17,  
23 18), we found no group differences in the current study, which is consistent with the majority of  
24 reports (10, 11, 19–21). These data may suggest that, globally, primary cardiac interoceptive  
25 ability is not dramatically impacted in ASD. However, several previous reports suggest  
26 differences in interoceptive sensitivity and awareness, rather than accuracy, reflecting altered

1 attention to and interpretation of interoceptive cues (9, 17, 18, 59). This mixed profile, potentially  
2 reflecting a mismatch between accuracy and confidence (60) could significantly impact social-  
3 emotional awareness and development by increasing the likelihood of erroneous or inconsistent  
4 interoceptive interpretations. This is supported by our data in adults, where insula response was  
5 related to self-reported social functioning. Yet, we saw no relationship in caregiver reports of  
6 social functioning. Its important to note that self-report was only collected in adults and the  
7 majority of caregiver reports were collected in children and young adults. More research is  
8 needed to understand the direct influence of primary interoceptive insula response on  
9 interoceptive sensitivity and awareness, and how that influence changes during development.

10 In our study, there were no differences in accuracy by diagnostic group, gender, or age.  
11 However, accuracy was significantly related to IQ. This may reflect a general understanding of  
12 task requirements, but more than likely reflects participants' prior knowledge of average resting  
13 heartrate (see (61, 66) for more). As we did not measure beliefs regarding average heartrate,  
14 the role prior knowledge may play is unclear. There is likely a complicated association between  
15 interoceptive accuracy, age (58), IQ (61), and diagnostic group, as demonstrated in our  
16 previous work (10, 11), but our study was likely not powered to detect these relationships. To  
17 our knowledge, no other studies have examined associations between insula percent signal  
18 change and cardiac interoceptive accuracy.

19 There are important strengths and limitations to consider in this study. This is one of a  
20 few studies to experimentally measure interoceptive accuracy and neural correlates in children,  
21 which adds to the novelty of this study. Additionally, we excluded for a number of medications  
22 that could influence autonomic and/or higher-order cognitive or affective function. In a sample  
23 with ASD, this is very difficult given the common use of these types of medications. Yet,  
24 because of the common use of these medications, future studies will need to understand the  
25 interoceptive consequences of these medications on socio-emotional health. As this work is  
26 cross-sectional, longitudinal data are needed to confirm developmental shifts in interoception-

1 related BOLD response. We also only investigated one modality of interoception, however,  
2 there are a number of other internal stimuli highly related to development and ASD, like  
3 gastrointestinal-related cues(62) and pain (63, 64). There is limited evidence that interoceptive  
4 accuracy in one modality like heartbeat is related to accuracy in other modalities (65), yet this  
5 has not been investigated developmentally or in ASD specifically. Similarly, heartbeat counting  
6 accuracy may not be as ‘purely’ interoceptive as it appears; it may be influenced by non-  
7 interoceptive processes such as beliefs about heart rates, estimating heart beats, etc. (see (66–  
8 69)). However, for this study, this task provided a developmentally accessible interoceptive task  
9 to examine neural correlates of interoceptive processes without the additional consideration of  
10 exteroceptive signals as in heartbeat discrimination tasks. Given wide-ranging implications of  
11 interoceptive processing across development and in ASD, it will be important to address these  
12 limitations in future studies.

13 Another important consideration when interpreting interoceptive processing in socio-  
14 emotional functioning is the role of alexithymia. Alexithymia refers to “impaired awareness of  
15 emotions due to a deficit in processing of affective information” (70) and is highly prevalent in  
16 ASD (70). In fact, Bird and colleagues suggest interoceptive deficits are only observable in the  
17 subset of people with ASD who are alexithymic (19, 20, 71). However, recent work suggests  
18 that cardiac interoceptive accuracy is not associated with ASD traits or alexithymia (21). While  
19 we did not measure alexithymia, we did not find differences in cardiac interoceptive accuracy by  
20 diagnostic group. The overlapping features of ASD and alexithymia clearly complicate this  
21 conceptual landscape and likely contribute to mixed findings.

22 Given our current data, we suggest that 1) there is a developmental trajectory of  
23 interoceptive neural processing in the insular cortex that is dynamic throughout early adulthood,  
24 and 2) interoceptive influence on social functioning in ASD is not likely due to primary  
25 interoceptive deficits. Future studies will need to examine connectivity of interoceptive networks,  
26 especially in the face of exteroceptive demands, to understand influences on social behaviors.

1 Given evidence for altered structural connectivity in the insula of children with ASD (12), this will  
2 be an important consideration moving forward. Greater relative involvement of extrastriate visual  
3 cortex in individuals with ASD across the lifespan may further suggest differences in strategies  
4 for bodily perception that could be explored in the context of internalizing, anxiety, and other  
5 mental health variables. Thus, future studies should examine how people access interoceptive  
6 memories or information (72), as this may be highly relevant in social functioning. Finally,  
7 longitudinal studies of interoception will help to shed light on the complex interplay between  
8 visceral sensation, mental health, and social function as these processes evolve over the  
9 lifespan.



1 **References**

- 2 1. Craig AD (2002): How do you feel? Interoception: the sense of the physiological condition of  
3 the body. *Nat Rev Neurosci.* 3: 655–666.
- 4 2. Pollatos O, Schandry R (2008): Emotional processing and emotional memory are modulated  
5 by interoceptive awareness. *Cogn Emot.* 22: 272–287.
- 6 3. Critchley HD, Garfinkel SN (2017): Interoception and emotion. *Curr Opin Psychol.* 17: 7–14.
- 7 4. Palmer CE, Tsakiris M (2018): Going at the heart of social cognition: is there a role for  
8 interoception in self-other distinction? *Curr Opin Psychol.* 24: 21–26.
- 9 5. Murphy J, Brewer R, Catmur C, Bird G (2017): Interoception and psychopathology: A  
10 developmental neuroscience perspective. *Dev Cogn Neurosci.* 23: 45–56.
- 11 6. Maister L, Tang T, Tsakiris M (2017): Neurobehavioral evidence of interoceptive sensitivity in  
12 early infancy. *eLife.* 6. doi: 10.7554/eLife.25318.
- 13 7. Schandry R (1981): Heart Beat Perception and Emotional Experience. *Psychophysiology.* 18:  
14 483–488.
- 15 8. Koch A, Pollatos O (2014): Cardiac sensitivity in children: sex differences and its relationship  
16 to parameters of emotional processing. *Psychophysiology.* 51: 932–941.
- 17 9. Fiene L, Brownlow C (2015): Investigating interoception and body awareness in adults with  
18 and without autism spectrum disorder. *Autism Res Off J Int Soc Autism Res.* 8: 709–  
19 716.
- 20 10. Mash LE, Schauder KB, Cochran C, Park S, Cascio CJ (2017): Associations Between  
21 Interoceptive Cognition and Age in Autism Spectrum Disorder and Typical Development.  
22 *J Cogn Educ Psychol JCEP.* 16: 23–37.
- 23 11. Schauder KB, Mash LE, Bryant LK, Cascio CJ (2015): Interoceptive ability and body  
24 awareness in autism spectrum disorder. *J Exp Child Psychol.* 131: 193–200.

- 1 12. Failla MD, Peters BR, Karbasforoushan H, Foss-Feig JH, Schauder KB, Heflin BH, Cascio  
2 CJ (2017): Intra-insular connectivity and somatosensory responsiveness in young  
3 children with ASD. *Mol Autism*. 8: 25.
- 4 13. Uddin LQ, Menon V (2009): The anterior insula in autism: under-connected and under-  
5 examined. *Neurosci Biobehav Rev*. 33: 1198–1203.
- 6 14. Ondobaka S, Kilner J, Friston K (2017): The role of interoceptive inference in theory of mind.  
7 *Brain Cogn*. 112: 64–68.
- 8 15. Quattrocki E, Friston K (2014): Autism, oxytocin and interoception. *Neurosci Biobehav Rev*.  
9 47: 410–430.
- 10 16. Palser ER, Fotopoulou A, Pellicano E, Kilner JM (2018): The link between interoceptive  
11 processing and anxiety in children diagnosed with autism spectrum disorder: Extending  
12 adult findings into a developmental sample. *Biol Psychol*. 136: 13–21.
- 13 17. Garfinkel SN, Tiley C, O’Keeffe S, Harrison NA, Seth AK, Critchley HD (2016):  
14 Discrepancies between dimensions of interoception in autism: Implications for emotion  
15 and anxiety. *Biol Psychol*. 114: 117–126.
- 16 18. Mul C-L, Stagg SD, Herbelin B, Aspell JE (2018): The Feeling of Me Feeling for You:  
17 Interoception, Alexithymia and Empathy in Autism. *J Autism Dev Disord*. 48: 2953–2967.
- 18 19. Shah P, Hall R, Catmur C, Bird G (2016): Alexithymia, not autism, is associated with  
19 impaired interoception. *Cortex J Devoted Study Nerv Syst Behav*. 81: 215–220.
- 20 20. Shah P, Catmur C, Bird G (2016): Emotional decision-making in autism spectrum disorder:  
21 the roles of interoception and alexithymia. *Mol Autism*. 7: 43.
- 22 21. Nicholson TM, Williams DM, Grainger C, Christensen JF, Calvo-Merino B, Gaigg SB (2018):  
23 Interoceptive impairments do not lie at the heart of autism or alexithymia. *J Abnorm*  
24 *Psychol*. 127: 612–622.

- 1 22. DuBois D, Ameis SH, Lai M-C, Casanova MF, Desarkar P (2016): Interoception in Autism  
2 Spectrum Disorder: A review. *Int J Dev Neurosci Off J Int Soc Dev Neurosci*. 52: 104–  
3 111.
- 4 23. Crone EA, Dahl RE (2012): Understanding adolescence as a period of social–affective  
5 engagement and goal flexibility. *Nat Rev Neurosci*. 13: 636–650.
- 6 24. Li D, Zucker NL, Kragel PA, Covington VE, LaBar KS (2017): Adolescent development of  
7 insula-dependent interoceptive regulation. *Dev Sci*. 20. doi: 10.1111/desc.12438.
- 8 25. Pollatos O, Matthias E, Keller J (2015): When interoception helps to overcome negative  
9 feelings caused by social exclusion. *Front Psychol*. 6. doi: 10.3389/fpsyg.2015.00786.
- 10 26. Schulz SM (2016): Neural correlates of heart-focused interoception: a functional magnetic  
11 resonance imaging meta-analysis. *Philos Trans R Soc B Biol Sci*. 371. doi:  
12 10.1098/rstb.2016.0018.
- 13 27. Craig AD (Bud) (2014): Topographically Organized Projection to Posterior Insular Cortex  
14 from the Posterior Portion of the Ventral Medial Nucleus (VMpo) in the Long-tailed  
15 Macaque Monkey. *J Comp Neurol*. 522: 36–63.
- 16 28. Gu X, Hof PR, Friston KJ, Fan J (2013): Anterior Insular Cortex and Emotional Awareness. *J*  
17 *Comp Neurol*. 521: 3371–3388.
- 18 29. Garfinkel SN, Critchley HD (2013): Interoception, emotion and brain: new insights link  
19 internal physiology to social behaviour. Commentary on: “Anterior insular cortex mediates  
20 bodily sensibility and social anxiety” by Terasawa et al. (2012). *Soc Cogn Affect*  
21 *Neurosci*. 8: 231–234.
- 22 30. Terasawa Y, Shibata M, Moriguchi Y, Umeda S (2013): Anterior insular cortex mediates  
23 bodily sensibility and social anxiety. *Soc Cogn Affect Neurosci*. 8: 259–266.
- 24 31. Dennis EL, Jahanshad N, McMahon KL, de Zubicaray GI, Martin NG, Hickie IB, *et al.*  
25 (2014): Development of Insula Connectivity Between Ages 12 and 30 Revealed by High  
26 Angular Resolution Diffusion Imaging. *Hum Brain Mapp*. 35: 1790–1800.

- 1 32. Lord C, Rutter M, DiLavore P, Risi S, Gotham K, Bishop S (2012): *Autism Diagnostic*  
2 *Observation Schedule, second edition (ADOS-2) manual (Part I): Modules 1-4.*  
3 Torrance, CA: Western Psychological Services.
- 4 33. Constantino JN, Gruber CP (2012): *Social responsiveness scale (SRS).* Western  
5 Psychological Services Torrance, CA.
- 6 34. Harrigan RL, Yvernault BC, Boyd BD, Damon SM, Gibney KD, Conrad BN, *et al.* (2016):  
7 Vanderbilt University Institute of Imaging Science Center for Computational Imaging  
8 XNAT: A multimodal data archive and processing environment. *NeuroImage*. 124: 1097–  
9 1101.
- 10 35. Asman AJ, Landman BA (2013): Non-local statistical label fusion for multi-atlas  
11 segmentation. *Med Image Anal*. 17: 194–208.
- 12 36. Ashburner J, Friston KJ (1999): Nonlinear spatial normalization using basis functions. *Hum*  
13 *Brain Mapp*. 7: 254–266.
- 14 37. Ashburner J, Neelin P, Collins DL, Evans A, Friston K (1997): Incorporating prior knowledge  
15 into image registration. *NeuroImage*. 6: 344–352.
- 16 38. Jenkinson M, Bannister P, Brady M, Smith S (2002): Improved optimization for the robust  
17 and accurate linear registration and motion correction of brain images. *NeuroImage*. 17:  
18 825–841.
- 19 39. Smith SM (2002): Fast robust automated brain extraction. *Hum Brain Mapp*. 17: 143–155.
- 20 40. Woolrich MW, Ripley BD, Brady M, Smith SM (2001): Temporal autocorrelation in univariate  
21 linear modeling of fMRI data. *NeuroImage*. 14: 1370–1386.
- 22 41. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE (2012): Spurious but  
23 systematic correlations in functional connectivity MRI networks arise from subject  
24 motion. *Neuroimage*. 59: 2142–2154.
- 25 42. Beckmann CF, Jenkinson M, Smith SM (2003): General multilevel linear modeling for group  
26 analysis in fMRI. *NeuroImage*. 20: 1052–1063.

- 1 43. Woolrich M (2008): Robust group analysis using outlier inference. *NeuroImage*. 41: 286–  
2 301.
- 3 44. Woolrich MW, Behrens TEJ, Beckmann CF, Jenkinson M, Smith SM (2004): Multilevel linear  
4 modelling for fMRI group analysis using Bayesian inference. *NeuroImage*. 21: 1732–  
5 1747.
- 6 45. Farb NAS, Segal ZV, Anderson AK (2013): Attentional modulation of primary interoceptive  
7 and exteroceptive cortices. *Cereb Cortex N Y N 1991*. 23: 114–126.
- 8 46. Barrett LF, Simmons WK (2015): Interoceptive predictions in the brain. *Nat Rev Neurosci*.  
9 16: 419–429.
- 10 47. Worsley KJ (2001): Statistical analysis of activation images. Ch 14, in *Functional MRI: An*  
11 *Introduction to Methods*. In: Jezzard P, Matthews PM, Smith SM, editors. New York,  
12 NY: Oxford University Press, pp 251–270.
- 13 48. Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE (2014): Permutation  
14 inference for the general linear model. *NeuroImage*. 92: 381–397.
- 15 49. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG (2009): Research  
16 electronic data capture (REDCap)--a metadata-driven methodology and workflow  
17 process for providing translational research informatics support. *J Biomed Inform*. 42:  
18 377–381.
- 19 50. Holm S (1979): A Simple Sequentially Rejective Multiple Test Procedure. *Scand J Stat*. 6:  
20 65–70.
- 21 51. Azur MJ, Stuart EA, Frangakis C, Leaf PJ (2011): Multiple Imputation by Chained Equations:  
22 What is it and how does it work? *Int J Methods Psychiatr Res*. 20: 40–49.
- 23 52. Buuren S van, Groothuis-Oudshoorn K (2011): mice: Multivariate Imputation by Chained  
24 Equations in R. *J Stat Softw*. 45: 1–67.
- 25 53. Telzer EH, McCormick EM, Peters S, Cosme D, Pfeifer JH, van Duijvenvoorde ACK (2018):  
26 Methodological considerations for developmental longitudinal fMRI research. *Dev Cogn*

- 1            *Neurosci*, Methodological Challenges in Developmental Neuroimaging: Contemporary  
2            Approaches and Solutions. 33: 149–160.
- 3    54. Craig KD (2009): The social communication model of pain. *Can Psychol Can.* 50: 22–32.
- 4    55. Uddin LQ, Nomi JS, Hebert-Seropian B, Ghaziri J, Boucher O (2017): Structure and function  
5            of the human insula. *J Clin Neurophysiol Off Publ Am Electroencephalogr Soc.* 34: 300–  
6            306.
- 7    56. Lenard Z, Studinger P, Mersich B, Kocsis L, Kollai M (2004): Maturation of cardiovagal  
8            autonomic function from childhood to young adult age. *Circulation.* 110: 2307–2312.
- 9    57. Khalsa S, Rudrauf D, Tranel D (2009): Interoceptive awareness declines with age.  
10           *Psychophysiology.* 46: 1130–1136.
- 11   58. Murphy J, Geary H, Millgate E, Catmur C, Bird G (2017): Direct and indirect effects of age  
12           on interoceptive accuracy and awareness across the adult lifespan. *Psychon Bull Rev.*  
13           1–10.
- 14   59. Fiene L, Ireland MJ, Brownlow C (2018): The Interoception Sensory Questionnaire (ISQ): A  
15           Scale to Measure Interoceptive Challenges in Adults. *J Autism Dev Disord.* 48: 3354–  
16           3366.
- 17   60. Garfinkel SN, Seth AK, Barrett AB, Suzuki K, Critchley HD (2015): Knowing your own heart:  
18           Distinguishing interoceptive accuracy from interoceptive awareness. *Biol Psychol.* 104:  
19           65–74.
- 20   61. Murphy J, Millgate E, Geary H, Ichijo E, Coll M-P, Brewer R, *et al.* (2018): Knowledge of  
21           resting heart rate mediates the relationship between intelligence and the heartbeat  
22           counting task. *Biol Psychol.* 133: 1–3.
- 23   62. Mazurek MO, Vasa RA, Kalb LG, Kanne SM, Rosenberg D, Keefer A, *et al.* (2013): Anxiety,  
24           Sensory Over-Responsivity, and Gastrointestinal Problems in Children with Autism  
25           Spectrum Disorders. *J Abnorm Child Psychol.* 41: 165–176.

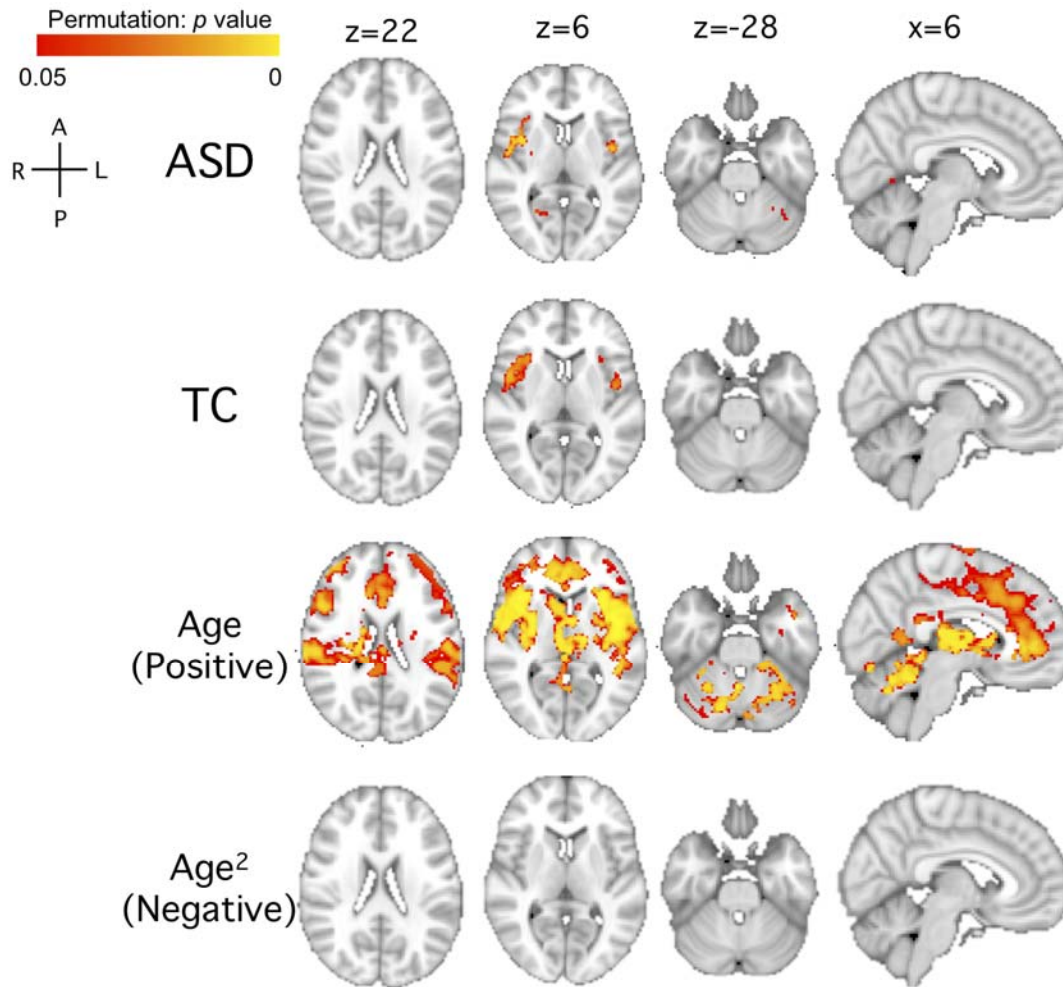
- 1 63. Failla MD, Moana-Filho EJ, Essick GK, Baranek GT, Rogers BP, Cascio CJ (2017): Initially  
2 intact neural responses to pain in autism are diminished during sustained pain. *Autism*  
3 *Int J Res Pract.* 1362361317696043.
- 4 64. Moore DJ (2015): Acute pain experience in individuals with autism spectrum disorders: a  
5 review. *Autism Int J Res Pract.* 19: 387–399.
- 6 65. Herbert BM, Muth ER, Pollatos O, Herbert C (2012): Interoception across Modalities: On the  
7 Relationship between Cardiac Awareness and the Sensitivity for Gastric Functions.  
8 *PLOS ONE.* 7: e36646.
- 9 66. Ring C, Brener J, Knapp K, Mailloux J (2015): Effects of heartbeat feedback on beliefs about  
10 heart rate and heartbeat counting: a cautionary tale about interoceptive awareness. *Biol*  
11 *Psychol.* 104: 193–198.
- 12 67. Desmedt O, Luminet O, Corneille O (2018): The heartbeat counting task largely involves  
13 non-interoceptive processes: Evidence from both the original and an adapted counting  
14 task. *Biol Psychol.* 138: 185–188.
- 15 68. Murphy J, Brewer R, Hobson H, Catmur C, Bird G (2018): Is alexithymia characterised by  
16 impaired interoception? Further evidence, the importance of control variables, and the  
17 problems with the Heartbeat Counting Task. *Biol Psychol.* 136: 189–197.
- 18 69. Zamariola G, Maurage P, Luminet O, Corneille O (2018): Interoceptive accuracy scores  
19 from the heartbeat counting task are problematic: Evidence from simple bivariate  
20 correlations. *Biol Psychol.* 137: 12–17.
- 21 70. Poquérousse J, Pastore L, Dellantonio S, Esposito G (2018): Alexithymia and Autism  
22 Spectrum Disorder: A Complex Relationship. *Front Psychol.* 9. doi:  
23 10.3389/fpsyg.2018.01196.
- 24 71. Bird G, Cook R (2013): Mixed emotions: the contribution of alexithymia to the emotional  
25 symptoms of autism. *Transl Psychiatry.* 3: e285.

- 1 72. DeVile DC, Kerr KL, Avery JA, Burrows K, Bodurka J, Feinstein JS, *et al.* (2018): The
- 2 Neural Bases of Interoceptive Encoding and Recall in Healthy Adults and Adults With
- 3 Depression. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 3: 546–554.

4

5





1  
2  
3 **Supplemental Figure 1. Whole brain heartbeat counting-visual counting in the**  
4 **total sample permutation tested.** Group maps are shown for the autism spectrum  
5 disorders (ASD) group, typical comparison (TC) group, and linear and nonlinear  
6 (quadratic) age associations. Group maps were calculated with  $z > 3$  and  $p < 0.01$ . We  
7 also tested group comparison of ASD > TC and TC > ASD (thresholded at  $z > 2.3$  and  
8  $p < 0.05$ ) but there were no significant clusters. There were no negative linear age  
9 associations and no positive nonlinear age<sup>2</sup> associations. Nonlinear permutation tested  
10 clusters were calculated using *fsl randomise*. Reported clusters represent  $p < 0.05$  after  
11 5000 iterations.

1 **Supplemental Table 1.**  
2

<b>Excluded Psychotropic Medications</b>			
<b>Antidepressants</b>	SSRI	Prozac	fluoxetine
		Zoloft	sertraline
		Luvox	fluvoxamine
		Paxil	paroxetine
		Celexa	citalopram
		Lexapro	escitalopram
	SNRI	Effexor	venlafaxine
		Cymbalta	duloxetine
	NASSA	Remeron	mirtrazapine
	NADRI	Wellbutrin	bupropion
	Tricyclics	Elavil	amitrptyline
		Tofranil	imipramine
		Anafranil	clomipramine
	<b>Other</b>	atypical antipsychotics	Risperdal
Seroquel			quetiapine
Abilify			aripiprazole
Geodon			ziprasidone
Zyprexa			olanzapine
mood stabilizers and/or anticonvulsants		Lithium	lithium carbonate
		Tegretol	carbamazepine
		Trileptal	oxcarbazepine
		Depakote	divalproex sodium
		Lamictal	lamotrigine
		Neurontin	gabapentin
		Topamax	topiramate
anti-hypertensive		Keppra	levetiracetam
		Clonidine	clonidine
		Catapres	clonidine
		Intuniv	guanfacine
anti-anxiety		Tenex	guanfacine
		Xanax	alprazolam
		Klonopin	clonazepam
		Ativan	lorazepam
		Valium	diazepam

Note: SSRI, selective serotonin reuptake inhibitors; SNRI, serotonin and noradrenergic reuptake inhibitors; NASSA, noradrenergic and specific serotonergic antidepressants; NADRI, norepinephrine-dopamine reuptake inhibitors.