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Insula-Retrosplenial Cortex Overconnectivity Increases Internalizing Via Reduced Insight in Autism

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Abstract

BACKGROUND—Internalizing symptoms like anxiety and depression are common and impairing in autism spectrum disorder (ASD). Here, we test the hypothesis that aberrant functional connectivity between three brain networks [salience network (SN), default-mode network (DMN), and frontoparietal network (FPN)] plays a role in the pathophysiology of internalizing in ASD.

METHODS—We examined the association between resting-state functional connectivity and internalizing in 102 adolescents and young adults with ASD (*N*=49) or typical development (TYP; *N*=53). Seed-to-target functional connectivity was contrasted between ASD and TYP using a recent parcellation of the human cerebral cortex, and connections that were aberrant in ASD were analyzed dimensionally as a function of parent-reported internalizing symptoms.

RESULTS—Three connections demonstrated robust *over*connectivity in ASD: i) anterior insula to retrosplenial cortex (i.e. SN-DMN), ii) anterior insula to frontal pole (i.e. SN-FPN), and iii) dorsolateral prefrontal cortex to retrosplenial cortex (i.e. FPN-DMN). These differences remained significant after controlling for age, and no age-related effects survived correction. The SN-DMN connection was associated with greater internalizing in ASD, mediated by a bigger difference between self- and parent-reported internalizing. Control analyses found that the other two connections were not associated with internalizing, and SN-DMN connectivity was not associated with a well-matched control measure (externalizing symptoms).

CONCLUSIONS—The present findings provide novel evidence for a specific link between SN-DMN overconnectivity and internalizing in ASD. Further, the mediation results suggest that intact anterior insula-retrosplenial connectivity may play a role in generating insight into ones' own psychopathology.

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Keywords

Anxiety; Anterior Insula; Autism Spectrum Disorder; Internalizing; Retrosplenial Cortex; Salience Network

The anterior insula (aINS) and anterior cingulate cortex (ACC) have been implicated in several domains of human neuroscience, leading to much speculation about their putative functional roles in cognition and emotion. A common theme across different theories of aINS/ACC function is that these regions are involved in generating awareness of, and coordinating the response to, salient internal or external events (1–5). aINS and ACC anomalies have been reliably reported in individuals with autism spectrum disorder (ASD)–a neurodevelopmental disorder characterized by social difficulties and idiosyncratic patterns of restricted and repetitive behaviors, interests, and activities (6). Structural neuroimaging and postmortem studies have revealed differences in aINS and ACC gray and white matter volumes (7, 8), atypical cortical folding (9) and lamination (10), and atypical maturation between 1.5 and 5 years of age in ASD relative to typical development (TYP; 11). In addition, resting-state functional magnetic resonance imaging (rsfMRI) studies have reliably demonstrated aberrant functional connectivity of neural circuits anchored in the aINS and ACC in ASD (12–14). Though aINS/ACC aberrations appear to be a robust and reliable observation in ASD, the specific role of those aberrations in the clinical symptoms associated with the ASD phenotype has not been established, making it unclear how such findings might inform treatment.

Potential roles of aINS and ACC in the ASD phenotype can be gleaned from the ‘triple network model’ of psychopathology (15). The triple network model integrates evidence from human rsfMRI studies, asserting that aberrant connectivity between aINS/ACC and two large-scale brain networks–frontoparietal network (FPN; dorsolateral prefrontal cortex and posterolateral parietal cortex) and default-mode network (DMN; ventromedial prefrontal cortex, posterior cingulate cortex, and temporoparietal junction)–represents a common component of the pathophysiology of a variety of psychiatric disorders (15). Computationally, the function of the aINS/ACC within this model is of paramount importance: these regions form a ‘salience network’ (SN) that flexibly recruits the DMN for self-referential/stimulus-independent processing and FPN for cognitive control/goal-directed processing in line with one’s goals and ongoing contextual demands (5). Adopting the triple network model, Burrows and colleagues (2017) recently hypothesized that the internalizing symptoms often observed in ASD arise due to SN dysfunction within the DMN-SN-FPN architecture (16). Clinically-significant internalizing symptoms–such as anxiety, depression, social withdrawal, and somatization–are commonly experienced by individuals with ASD, and can disrupt adaptive functioning and exacerbate social difficulties (17–21). However, the empirical evidence offered in support of Burrows and colleagues’ (2017) hypothesis comes primarily from studies of TYP individuals (22–24). In fact, the specific DMN-SN-FPN circuits underlying internalizing symptoms in ASD have not been directly studied. Given the tendency for atypical presentation of internalizing symptoms in individuals with ASD (e.g., unusual specific phobias, excessive worry surrounding rituals or compulsions, etc.; 25), these symptoms may be driven by distinct neural circuitry between ASD and TYP.

The present study used rsfMRI to test the hypothesis that SN dysfunction is associated with internalizing symptoms in ASD. We predicted that functional connectivity across the DMN-SN-FPN architecture would be aberrant in ASD relative to TYP. Critically, we ran dimensional analyses examining the degree to which aberrant functional connectivity is associated with greater internalizing severity in ASD. Additionally, due to the well-established role of the SN in generating conscious emotional awareness (26–28), we predicted that aberrant SN connectivity may promote internalizing by diminishing one's insight into their own level of psychopathology. To quantify this, we computed the discrepancy between self- and other-reported internalizing symptoms, hypothesizing that more aberrant SN functional connectivity in ASD would be associated with a greater discrepancy between self- and other-reported internalizing symptoms. Additionally, based on prior work demonstrating developmental changes in functional connectivity aberrations in ASD (29, 30), we determined whether functional connectivity varied dimensionally as a function of age in ASD.

Methods & Materials

Participants

One-hundred twenty-one individuals (ASD: *N*=60; TYP: *N*=61) participated in the study for financial remuneration. Eleven participants with ASD and five with TYP were excluded due to excessive motion during the structural or rsfMRI scans. Participants were recruited via clinicians, advocacy groups, and the UC Davis MIND Institute's subject tracking system. All participants received a structured interview by a licensed clinical psychologist using either the Structured Clinical Interview for DSM-5 Disorders (SCID, for participants ≥18; 31) or the Kiddie-Sads-Present and Lifetime Version (K-SADS, for participants <18; 32). The SCID and K-SADS involved separate clinical interviews with parent and participant, and diagnoses were determined by consensus by interviewers who achieved research reliability following extensive training. Two TYP participants were ineligible after receiving a clinical diagnosis of attention-deficit hyperactivity disorder (ADHD), and one incorrectly completed the Achenbach System of Empirically Based Assessment (ASEBA). This left a final sample of 102 participants (ASD: *N*=49; TYP: *N*=53; Table 1). Based on the SCID/K-SADS data, 31% of the ASD group met diagnostic criteria for ADHD and 16% met for a comorbid anxiety disorder. The prevalence of anxiety in the current ASD sample is somewhat low relative to recent epidemiological (17) and meta-analytic (19) estimates, which suggest approximately 40% of individuals with ASD have a comorbid anxiety disorder. This is unsurprising as the current study excluded participants taking antipsychotic or antidepressant medications, which are likely to influence functional connectivity estimates (33). Ten participants in the ASD group were taking prescribed psychostimulants, but refrained from taking medications for ≥48 hours in advance of their MRI (34).

Internalizing Assessment

The present study focused on parent-reported internalizing problems on the adult behavior checklist (ABCL; for participants 18 and over) or the child behavior checklist (CBCL; for participants under 18) from the ASEBA. We utilized age- and gender-corrected *T*-Scores[i] on the anxious/depressed subscale, withdrawn subscale, somatic complaints subscale, and the

overall internalizing problems composite score from the ABCL and CBCL. Notably, age was matched between groups (Table 1), the association between age (in months) and internalizing was not significant in either group (ASD: r_{47}=−0.11, p=0.445; TYP: r_{51}=−0.17, p=0.224), and there was no difference between internalizing derived via the CBCL and ABCL in either group (ASD: p=0.211; TYP: p=0.275). In contrast, full scale IQ (FSIQ) was reduced in ASD relative to TYP (Table 1). But, importantly, FSIQ was not associated with internalizing in either ASD (r_{47}=0.07, p=0.610) or TYP (r_{51}=0.14, p=0.329). Therefore, there was no credible evidence that age or FSIQ caused changes in internalizing symptoms, and these variables were not included as covariates in the current study (35).

All but four of the participants in the current study also completed the self-report version of the ASEBA (youth self-report, YSR for participants under 18; adult self-report, ASR for participants 18 and over). The parent-report was more in line with clinician evaluations than the self-report. ASD participants who received an anxiety diagnosis on the SCID or KSADS scored higher on anxious/depressed symptoms on the parent-report (ASD+anxiety=63.62, ASD-anxiety=57.61, t_{47}=−2.07, p=0.044), but not on the self-report (ASD+anxiety=60.57, ASD-anxiety=58.05, t_{47}=−0.69, p=0.491). This is consistent with findings indicating that parent-reported internalizing symptoms are better predictive of clinical referral status than self-report (36), and the suggestion that individuals with ASD possess limited insight into their own psychopathology (37). Therefore, the parent-report was the closest approximation to a dimensional ‘ground truth’ measure of internalizing. Additionally, we computed the discrepancy between self- and parent-report to quantify insight into one’s own internalizing symptoms. This enabled us to investigate whether atypical awareness of one’s internalizing symptoms was associated with SN dysfunction.

Defining Regions-of-Interest (ROIs)

During rsfMRI subjects maintained fixation on a white cross presented on a black background (for complete rsfMRI acquisition and preprocessing details, see Supplementary Materials). Cortical ROIs were defined using an atlas of the human brain’s functional connectivity architecture derived via gradient-weighted Markov Random Field models of functional connectivity data from 1489 participants (38). The resulting atlas is a 400-ROI parcellation divided into 7 intrinsic functional networks (Figure 1A). For the current study, the SN (N=51 nodes), DMN (N=77 nodes), and FPN (N=59 nodes) were selected as *a priori* networks of interest (N=191 total ROIs).

rsfMRI Analysis Approach

Given that the aim of the current study was to determine whether SN dysfunction in ASD is associated with elevated internalizing symptoms, statistical inferences were made in two steps: (i) identifying aberrant connections within the DMN-SN-FPN architecture in ASD relative to TYP, and (ii) examining evidence for correlations between connections identified in step (i) with internalizing symptoms. Accordingly, in step (i) all SN, FPN, and DMN nodes from the cortical atlas were included as seeds and targets in a ROI-ROI connectivity analysis, resulting in 191*191 (36,481) seed-target pairs in the analysis. Functional

[i]The key findings from the current study remain significant when using raw totals instead of age- and gender-corrected *T*-scores

connectivity models were carried out in CONN with regressors representing the intercept (constant=1), clinical diagnosis (1=ASD, −1=TYP), and the average global correlation for each subject (39). A conservative correction for multiple comparisons at the analysis-level was used, keeping the false-discovery rate (FDR) below threshold (q<0.05), two-sided. Therefore, any connections that were significantly aberrant in ASD relative to TYP would be statistically robust.

In step (ii), z-transformed connectivity values for significant results from the ROI-ROI results were extracted from CONN, and analyzed dimensionally as a function of the clinical measures of interest. Robust Bayesian correlation models (40) examined the association between parent-reported internalizing problems and connectivity separately within ASD and TYP. This approach enabled us to subtract the posterior distributions of the correlation coefficient parameter (i.e. *rho*) between groups, to directly quantify the strength of the evidence that these associations were dissociable between ASD and TYP. Next, this approach was repeated for each of the subscales that comprise the internalizing problems composite measure, namely: anxious-depressed symptoms, withdrawn symptoms, and somatic complaints. To determine the potential role of insight into one's internalizing symptoms in these results, the Bayesian correlation models were re-run examining the difference between self- and parent-report measures of internalizing symptoms. Specifically, data were *z*-transformed, and self-reported symptoms were subtracted from parent-reported symptoms. This standardized difference score approach preserves the ranking of parent- and self-reported symptoms, and has been used extensively in past studies (41, 42).

Lastly, a control model with a different ASEBA composite measure–externalizing problems–was conducted to ensure that the hypothesized functional connectivity results were selectively associated with internalizing in ASD. Externalizing represented an ideal comparison measure for three related reasons: i) like internalizing it was assessed via parent-report, ii) the measure was computed using similar procedures to the internalizing composite, and iii) in the current data, externalizing and internalizing were associated in ASD (r_{47}=0.58, p<0.001). Therefore, any internalizing-externalizing dissociations in their dimensional relationships with functional connectivity are likely to control for common methods variance, and represent true incremental variance in their respective latent constructs.

Results

Internalizing Problems in ASD and TYP

Individuals with ASD demonstrated increased internalizing relative to individuals with TYP ($F_{1,100}$=33.20, p<0.001, η^2=0.249; Table 1). An ANOVA with one between-subjects (ASD, TYP) and one within-subjects factor (ASEBA scale: anxious-depressed, withdrawn, and somatic complaints) revealed a main effect of measure ($F_{1.79,179.38}$=12.42, p<0.001, η^2=0.103), a main effect of diagnosis ($F_{1,100}$=22.20, p<0.001, η^2=0.182), and a diagnosis by measure interaction ($F_{1.79,179.38}$=8.33, p<0.001, η^2=0.069). These effects were driven by greater scores on the anxious-depressed (t=3.67, p<0.001, d=0.731) and withdrawn symptoms (t=5.55, p<0.001, d=1.108) subscales in ASD, and relatively matched scores on

the somatization subscale (t=1.84, p=0.069, d=0.365). Thus, in line with previous work, individuals with ASD demonstrated increased internalizing symptoms relative to TYP.

Functional Connectivity

Three ROI-ROI circuits demonstrated overconnectivity in ASD relative to TYP. First, the ASD group demonstrated overconnectivity between a left aINS node within the SN and a left retrosplenial cortex (RSP) node within the DMN (t_{100}=4.83, p_{FDR}=0.0489; Figure 1B.1). Second, the ASD group demonstrated overconnectivity between a right aINS node within the SN and a left frontal pole (FP) node within the FPN (t_{100}=4.76, p_{FDR}=0.0489; Figure 1B.2). Lastly, the ASD group demonstrated overconnectivity between a right dorsolateral prefrontal cortex (dlPFC) node within the FPN and a left RSP node from the DMN (t_{100}=4.71, p_{FDR}=0.0489; Figure 1B.3). Therefore, overconnectivity in the ASD group relative to TYP spanned the entire DMN-SN-FPN neural architecture, but was localized to three specific connections. No within-network connections were aberrant, and no connections were *under*connected in ASD relative to TYP. The three aberrant connections did not vary as a function of age, and no significant connections were revealed when modeling age as a continuous variable across all ROIs.

Dimensional Relationship Between Functional Connectivity and Internalizing

Bayesian correlations evaluated evidence for associations between functional connectivity in the circuits identified in Figure 1B and parent-reported internalizing separately within ASD and TYP. Burrows and colleagues (2017) hypothesized that SN-DMN overconnectivity may be associated with internalizing in ASD (16). In line with this hypothesis, greater aINS-RSP connectivity in ASD was associated with increased internalizing (*rho*=0.31, *95%-HDI*=0.04 to 0.57, p=0.015; Figure 1C). In contrast, this relationship was not present in TYP (*rho*=0.08, p=0.285; *rho difference*=0.23, *95%-HDI*=−0.09 to 0.55, p=0.119). A correlation revealed that aINS-RSP connectivity was not associated with autism symptoms (Social Responsiveness Scale; p=0.154), and a regression with an internalizing*autism interaction term did not reveal evidence that autism symptoms moderated the relationship between internalizing and aINS-RSP connectivity (p=0.523). When considering the individual subfactors that comprise the internalizing problems scale of the ASEBA, aINS-RSP connectivity was associated with higher anxious-depressed symptoms in ASD (*rho*=0.31, *95%-HDI*=0.04 to 0.56, p=0.019), but this relationship was not significant in TYP (*rho*=0.17, p=0.121; *rho difference*=0.14, *95%-HDI*=−0.19 to 0.45, p=0.240). Withdrawn symptoms and somatic complaints were not associated with aINS-RSP connectivity in either ASD (*rho*s≤0.21, ps>0.07) or TYP (*rho*s≤0.03, ps>0.4).

Control models were run to determine 1) whether the observed association between internalizing and functional connectivity in ASD was specific to the aINS-RSP pathway, and 2) that aINS-RSP connectivity was selectively related to internalizing problems, and not just associated with a generalized increase in clinical symptoms in ASD. First, dlPFC-RSP (i.e. FPN-DMN) and aINS-FP (i.e. SN-FPN) connectivity were not associated with any of the ASEBA measures (*rho*s≤0.21, ps≥0.179). Second, externalizing symptoms were not associated with aINS-RSP connectivity in ASD (*rho*=0.14, p=0.184). Therefore, the current results suggest a specific relationship between overconnectivity of a single SN-DMN circuit

(aINS-RSP) is associated with elevated internalizing symptoms–particularly, anxious-depressed symptoms–in participants with ASD.

Dimensional Relationship Between Functional Connectivity and Insight into Internalizing Symptoms

Given the established roles of aINS and RSP in emotional awareness and self-referential processing (26, 27, 43, 44), we predicted that the observed pattern of aINS-RSP overconnectivity in ASD may be associated with atypical insight into one's own internalizing symptoms. Accordingly, we examined the association between the parent-self difference in internalizing symptoms and functional connectivity of the aINS-RSP circuit. The data provided compelling evidence for a significant positive relationship between aINS-RSP connectivity and parent-self difference scores in ASD (*rho*=0.43, *95%-HDI*=0.17 to 0.66, p=0.002; Figure 1D), which was significantly greater than the relationship in TYP (*rho*=−0.009, p=0.474; *rho difference*=0.43, *95%-HDI*=0.11 to 0.74, p=0.015). Similar to the analysis of parent-reported internalizing symptoms, control models were conducted to determine whether this association was specific to aINS-RSP connectivity, or whether it was specific to parent-self difference scores in internalizing. Firstly, neither of the other circuits that demonstrated aberrant connectivity in ASD were associated with reduced parent-self difference scores in internalizing in ASD or TYP (−0.11≥*rho*s≤0.19, ps≥0.11). Second, aINS-RSP connectivity was not significantly associated with parent-self difference scores in externalizing symptoms in ASD (*rho*=0.22, *95%-HDI*=−0.08 to 0.49, p=0.080). Lastly, aINS-RSP connectivity was not significantly associated with self-reported internalizing (*rho*=−0.19, *95%-HDI*=−0.46 to 0.11, p=0.116). Therefore, overconnectivity of the aINS-RSP circuit in individuals with ASD was associated with a tendency to underestimate one's own internalizing symptoms, which was significantly different than the association found in TYP, and may represent a functional connectivity marker of reduced insight into one's own internalizing symptoms in ASD.

Exploratory post-hoc analyses revealed a mediation pattern between internalizing, reduced insight, and aINS-RSP connectivity. In a regression of aINS-RSP connectivity as a function of *both* internalizing and insight, internalizing was not associated with aINS-RSP connectivity (p=0.135), whereas insight predicted greater connectivity (β=0.348, t=2.34, p=0.024), which was not inflated by multicollinearity (*VIF*=1.22). When modeling these variables using path analysis and bootstrap standard errors, the relationship between internalizing and aINS-RSP connectivity was fully mediated by diminished insight (direct effect: c=0.047, se=0.035, z=1.36, p=0.175; indirect effect: ab=0.031, se=0.014, z=2.17, p=0.030; Figure 2A). Conversely, a control model with internalizing as mediator did not reveal an indirect effect (p=0.239; Figure 2B). Therefore, the data suggest that the association between internalizing and aINS-RSP connectivity in ASD was mediated by diminished insight.

Discussion

The current study investigated the neural circuitry underlying internalizing symptoms in individuals with ASD and TYP. Specifically, we tested the hypothesis that salience network

(SN) dysfunction represents part of the pathophysiology of internalizing symptoms in ASD (5, 16). In line with this hypothesis, an aINS node of the SN was overconnected to a caudal posterior cingulate cortex node within the DMN (RSP) in individuals with ASD, and the degree of overconnectivity in this circuit was specifically related to increased internalizing symptoms in this group. Prior task-based fMRI paradigms have reliably demonstrated aINS and RSP BOLD activation during the processing of emotionally-salient stimuli (43, 45), but the current study is the first to suggest that functional connectivity between these regions may play a role in internalizing psychopathology. This association was not moderated by autism symptoms. In contrast, another fMRI marker of anxiety–recruitment of the amygdala during emotional face viewing–is known to change as a function of the *interaction* between anxiety and autism symptoms. Specifically, anxiety is positively associated, while social difficulties are negatively associated, with amygdala activity during emotional face viewing (46). It is possible that aINS-RSP connectivity and amygdala activity reflect distinct processes promoting internalizing symptoms, with only the latter being moderated by autism symptoms. Alternatively, it is possible that recruitment of the aINS-RSP circuit during task-based fMRI would reveal an interaction between internalizing and autism symptoms, similar to prior studies on the amygdala. Future work examining the interaction between internalizing and autism across resting and task-based fMRI are needed to reconcile these discrepant findings.

Internalizing in TYP was not associated with aINS-RSP connectivity, suggesting that the current findings were specific to internalizing in ASD. The identification of brain network aberrations underlying clinical symptoms across traditional diagnostic groups is central to the recent Research Domain Criteria framework advocated by the National Institute of Mental Health (47). In contrast, the current study suggests that aINS-RSP connectivity represents an ASD-specific marker of internalizing. However, variance in the current TYP sample may have been too homogenous to correlate with functional connectivity. Therefore, future studies should examine dimensional internalizing symptoms and SN dysfunction in anxiety enriched samples of ASD and TYP individuals, to determine whether aINS-RSP overconnectivity represents a transdiagnostic or ASD-specific neural marker of clinically-significant anxiety.

Our work adds to an accumulating body of evidence implicating SN dysfunction in psychopathology in ASD (5, 16, 48). Burrows et al. (2017) argue that SN-DMN overconnectivity is associated with negative self-thought in ASD, which perpetuates the development of internalizing symptoms (16). In contrast, here we hypothesized that aINS-RSP overconnectivity is linked to impaired emotional awareness in ASD. Impaired emotional awareness may then perpetuate internalizing by making it more difficult to recruit appropriate emotion regulation strategies (49, 50). A link between aberrant SN-DMN connectivity and impaired emotional awareness was supported by our observation that greater aINS-RSP connectivity was associated with reduced insight into one's own internalizing symptoms in ASD. This hypothesis was supported by our path analysis, which demonstrated that the relationship between internalizing symptoms and aINS-RSP connectivity was fully mediated by reduced insight. These findings are directly compatible with a recent structural equation modeling study that found a high degree of shared variance between alexithymia–a condition characterized by impaired emotional awareness–and

anxiety in ASD (51). However, as the current study used task-free rsfMRI, future task-based fMRI studies are required to provide additional insight into whether a 'repetitive negative thinking' (e.g. using a recently devised social evaluation task; 52) or 'impaired emotional awareness' (e.g. using Lane's Levels of Emotional Awareness task; 28) model provides a better fit for the neural computations underlying internalizing in ASD. Such studies could replicate the current study's path analysis using a measure of emotional insight that is statistically independent from measures of anxiety, providing a stronger test of the mediation relationship proposed in the current study. This work could have tremendous significance for informing intervention design, providing information concerning the active ingredients that might be most helpful for reducing internalizing in ASD (e.g. emphasizing cognitive restructuring to reframe negative thinking, or mindfulness training to improve emotional awareness).

Whereas it has been suggested that aINS demonstrates generalized *under*connectivity in ASD (13), the current study suggests that aINS-RSP *over*connectivity is associated with internalizing symptoms in ASD. Importantly, the current study does not represent the first to demonstrate aINS overconnectivity in ASD. For example, Di Martino and colleagues demonstrated striatal-aINS overconnectivity in ASD (53), and Dajani and Uddin (2016) observed local overconnectivity in aINS in individuals with ASD relative to TYP (54). In fact, it has been suggested that models of ASD as a disorder of generalized over-/underconnectivity are not supported by the empirical data emerging from this field (55). A more likely suggestion based on the data is that ASD may be associated with aberrant or delayed integration within, and segregation between, large-scale brain networks over the course of development (29, 30, 56, 57). The current data provide additional support for this theory: each of the robust overconnectivity patterns were found *between* networks (i.e. SN-DMN, FPN-DMN, and SN-FPN) suggesting reduced network segregation in ASD. Future longitudinal studies over the course of adulthood are required to determine whether network segregation remains aberrant, or if it emerges at a later developmental stage in ASD. Surprisingly, the current study did not find evidence for age-related variation in functional connectivity. This is likely due to the continuous age distribution of the current sample, which did not enable us to conduct high-powered cross-sectional contrasts of non-overlapping age groups (c.f. 29).

Four limitations of the study should be noted. First, the ASD group had a relatively low incidence of comorbid anxiety disorders (16%, relative to prior estimates around 40%; 17, 19), was relatively high-functioning (FSIQ>70), and contained a selective and cross-sectional age range (12≤age≤22). Therefore, future studies are required to determine the representativeness of the present results for ASD samples enriched for anxiety, individuals with underrepresented intellectual abilities, and outside of the present study's age range (i.e. in young children and older adults). Second, parents and clinicians are not privy to many situations commonly faced by adolescents and young adults with ASD (e.g. interactions with peers). Future studies should examine teacher or peer ratings of internalizing to get a comprehensive depiction of presentations in the everyday lives of adolescents and young adults with ASD. Third, longitudinal studies must be conducted to examine how SN-DMN circuitry might develop atypically in ASD. Finally, the difference score approach to self-

other agreement has limitations, which could be addressed in future studies by using approaches that are appropriate with large sample sizes (58).

Based on significant dimensional relationships between internalizing symptoms and aINS-RSP connectivity–and the absence of any relationships in the control analyses–the current study identifies a specific, novel functional connectivity marker of internalizing in ASD. This work is directly in line with a recent model implicating SN dysfunction in the pathophysiology of internalizing in ASD (16). Importantly, it must be noted that the current study focused on aberrant connections to identify neural circuits associated with internalizing *in ASD*. This represented a first pass at an important topic, but future studies should adopt a multivariate, whole-brain approach to replicate and extend the current findings. Such an approach would likely identify other SN-based circuitry associated with internalizing that were not detected in the current dataset. Accordingly, these findings provide insight into the neural bases of internalizing in ASD, and should serve as inspiration for task-evoked functional connectivity analyses to establish the specific cognitive and affective processes associated with aINS-RSP overconnectivity in ASD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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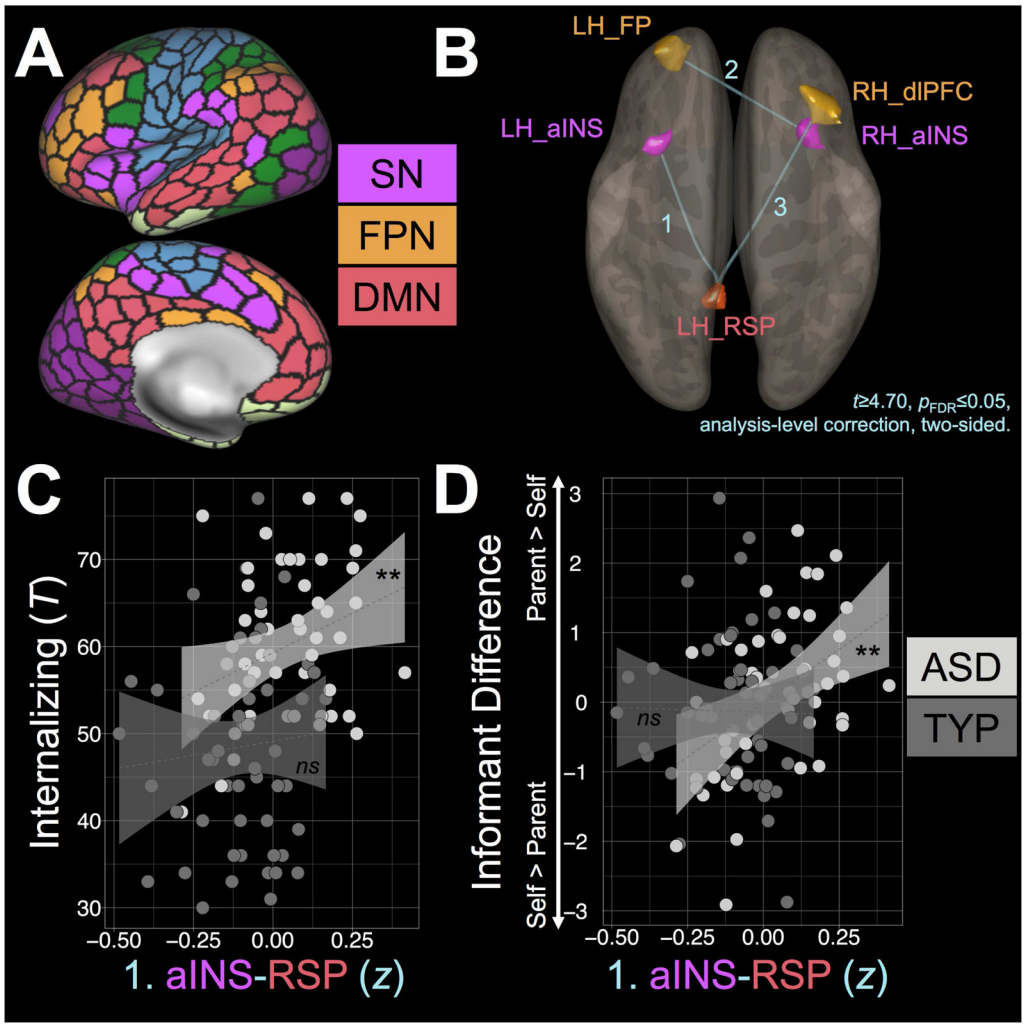

Figure 1.

(A) Schaefer et al. parcellation and three networks of interest. (**B)** ROI-ROI functional connectivity contrast between ASD and TYP. 1) Left anterior insula (LH_aINS) and retrosplenial cortex (LH_RSP), 2) Right anterior insula (RH_aINS) and left frontal pole (LH_FP), and 3) Right dorsolateral prefrontal cortex (RH_dlPFC) and LH_RSP were overconnected in ASD relative to TYP. **(C)** aINS-RSP connectivity was positively associated with parent reported internalizing symptoms in ASD, and **(D)** was positively related to the difference between parent- and self-reported internalizing in ASD. Note: ***: p<0.01, ns: p≥0.29.*

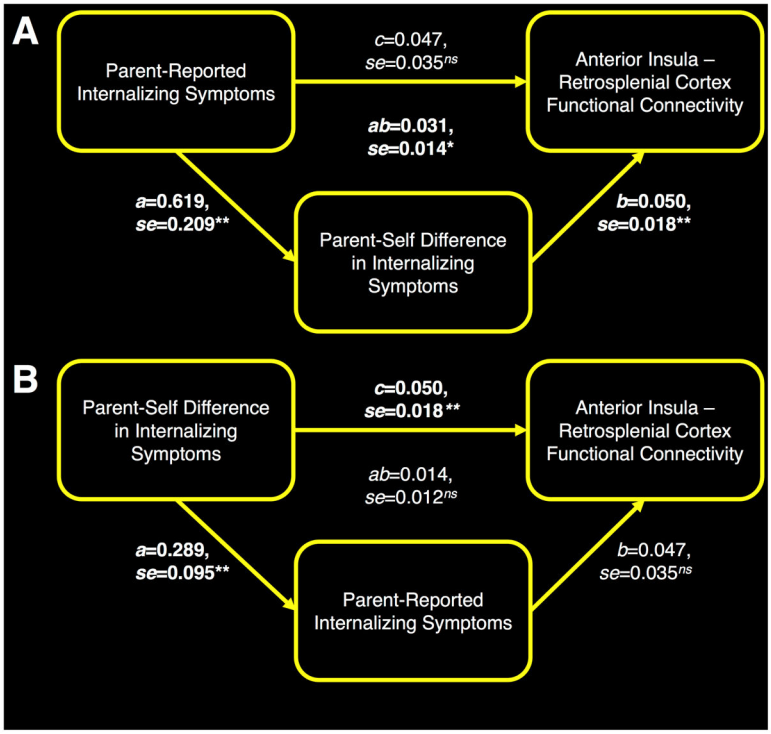

Figure 2.

(**A)** Path analysis with bootstrap standard errors revealed that the association between internalizing symptoms and aINS-RSP connectivity was fully mediated by the degree to which individuals with ASD underestimate their own level of internalizing (i.e. parent-self difference scores). **(B)** In contrast, the reverse model with parent-reported internalizing as the mediating variable did not reveal a significant indirect effect. Note: ***: p*<0.01, *: *p*<0.05, *ns: p*≥0.29.

Table 1

Summary of the current study sample.

Variables	ASD (*N*=49)	TYP (*N*=53)	Groupwise Comparison
Demographic			
Age (years)	17.39 (3.10)	16.80 (2.95)	$t(100)=0.99$, $p=0.322$
Gender (M,F)	(43,6)	(43,10)	$OR=0.603$, $p=0.422$
Control Variables			
Nonverbal IQ	109.94 (16.84)	111.23 (12.77)	$t(100)= -0.44$, $p=0.663$
*Verbal IQ***	97.28 (15.10)	104.41 (11.60)	$t(100)= -2.69$, $p=0.008$
*Full-Scale IQ**	103.65 (14.46)	108.81 (10.76)	$t(100)= -2.05$, $p=0.043$
Clinical Measures			
ADOS (calibrated severity score)	7.84 (1.59)	N/A	N/A
*SCQ (total)****	21.62 (5.78)	3.02 (3.13)	$t(100)=20.32$, $p<0.001$
*Internalizing Problems****	60.14 (9.00)	48.45 (11.25)	$t(100)=5.76$, $p<0.001$
*Anxious/Depressed***	58.59 (7.77)	53.60 (5.72)	$t(100)=3.71$, $p<0.001$
*Withdrawn****	63.51 (9.12)	54.74 (6.51)	$t(100)=5.63$, $p<0.001$
Somatic Complaints	57.02 (7.89)	54.34 (6.71)	$t(100)=1.85$, $p=0.067$
Difference Between Self- and Parent-Report (positive = parent > self; negative = self > parent)			
Internalizing Difference	0.154 (1.13)	−0.131 (1.10)	$t(96)=1.26$, $p=0.212$

* $p<0.05$,

** $p<0.01$,

*** $p<0.001$,

blank: $p\geq0.05$.