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Regular Article

Perceptual alterations in the relationship between sensory reactivity, intolerance of uncertainty, and anxiety in autistic children with and without ADHD

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Abstract

Sensory differences and anxiety disorders are highly prevalent in autistic individuals with and without ADHD. Studies have shown that sensory differences and anxiety are associated and that intolerance of uncertainty (IU) plays an important role in this relationship. However, it is unclear as to how different levels of the sensory processing pathway (i.e., perceptual, affective, or behavioral) contribute. Here, we used psychophysics to assess how alterations in tactile perception contribute to questionnaire measures of sensory reactivity, IU, and anxiety. Thirty-eight autistic children (aged 8-12 years; 27 with co-occurring ADHD) were included. Consistent with previous findings, mediation analyses showed that child-reported IU fully mediated an association between parent-reported sensory reactivity and parent-reported anxiety and that anxiety partially mediated an association between sensory reactivity and IU. Of the vibrotactile thresholds, only simultaneous frequency discrimination (SFD) thresholds correlated with sensory reactivity. Interestingly, we found that sensory reactivity fully mediated an association between SFD threshold and anxiety, and between SFD threshold and IU. Taken together, those findings suggest a mechanistic pathway whereby tactile perceptual alterations contribute to sensory reactivity at the affective level, leading in turn to increased IU and anxiety. This stepwise association can inform potential interventions for IU and anxiety in autism.

Keywords: autism; anxiety; intolerance of uncertainty; perception; sensory

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Introduction

Autism is a life-long, neurodevelopmental condition that is clinically defined by differences in social communication and a range of non-social characteristics, including restrictive, repetitive behaviors and atypical responses to sensory stimuli (American Psychiatric Association, 2013). Sensory differences are estimated to be present in up to 97% of autistic individuals[1] (Baranek et al., 2006; Crane et al., 2009; Dellapiazza et al., 2018; Leekam et al., 2007). The importance of sensory differences as a defining feature of autism was highlighted following its addition to the DSM-5 criteria in 2013 (American Psychiatric Association, 2013). As with other core features of autism, sensory processing can vary widely, both among and within individuals. Sensory differences in autism have been observed from as early as the first year of life and are known to persist throughout adulthood (Baranek et al., 2006; Crane et al., 2009; Leekam et al., 2007). While some sensory experiences can be positive, others can be distressing, and can negatively impact school performance, social and adaptive behaviors, and developmental outcomes (Butera et al., 2020; Dellapiazza et al., 2018; Howe & Stagg, 2016; Kojovic et al., 2019; Lane et al., 2010).

It is estimated that around 70% of autistic individuals will be diagnosed with at least one other co-occurring psychiatric disorder, of which ADHD and anxiety disorders are among the most common (Joshi et al., 2012; Lai et al., 2019; Leyfer et al., 2006; Simonoff et al., 2008). Anxiety disorders present unique challenges to autistic individuals and can be a great source of distress and

[1]While we acknowledge that individual preferences are preferred in practice, we use identity-first language here following recent recommendations (Bottema-Beutel et al., 2021)

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impairment (T. E. Davis et al., 2011; Keefer et al., 2018; Mazurek et al., 2012; McVey et al., 2018; Rodgers & Ofield, 2018; Vasa et al., 2020). Studies of sensory differences in autistic individuals with co-occurring ADHD are comparatively sparse in relation to those studying the conditions separately. However, evidence suggests that those with a dual diagnosis may experience more sensory problems and have higher rates of other psychiatric conditions compared with either condition alone (Chen et al., 2015; Mattard-Labrecque erg et al., 2013; Sanz-Cervera et al., 2017; Simonoff et al., 2008). As well as showing links with other autistic features, sensory differences have increasingly been found to be associated with anxiety disorders (Amos et al., 2019; Carpenter et al., 2019; Green et al., 2012; South & Rodgers, 2017). This relationship between sensory differences and anxiety in autisim is well documented and has also been shown to exist in other developmental disorders such as ADHD, as well as in neurotypical children (Carpenter et al., 2019; Lane et al., 2010a, 2010b; MacLennan et al., 2021; Neil et al., 2016; Wigham et al., 2015). Although the directionality of the association between sensory differences and anxiety requires further elucidation and is likely to be complex in nature, two longitudinal studies in groups with and without autism found a unidirectional relationship between sensory hyper-reactivity and anxiety (Carpenter et al., 2019; Green & Ben-Sasson, 2010). Thus, sensory hyper-reactivity appears to be an early and important risk factor in the development of anxiety.

In efforts to define the mechanisms underlying the link between sensory differences and anxiety, several studies have highlighted the important role of intolerance of uncertainty (IU). IU is a multidimensional construct that is broadly defined as having a negative perception and/or reaction to situations or events that are deemed uncertain or unpredictable (Dugas et al., 1997). IU is known to be a dispositional risk factor for the development and maintenance of multiple anxiety disorders, including generalized anxiety disorder, social anxiety, OCD, and panic disorder (Mahoney & McEvoy, 2012; Carleton et al., 2013). However, the role of IU in autism and sensory processing is less understood. Though IU is an independent construct, it shares similarities with some of the core features of autism, such as a desire for sameness and rigidity in routine (Joyce et al., 2017). Thus, many studies have sought to understand the relationship between IU and clinical features of autism (Vasa et al., 2018), as well as its role in anxiety in autism (Boulter & Freeston, 2014; Hodgson et al., 2017; Jenkinson et al., 2020; Keefer et al., 2017). Importantly, IU has been shown to be implicated in the relationship between sensory differences and anxiety, in both pediatric and adult autistic samples (Glod et al., 2019; Hwang et al., 2020; MacLennan et al., 2021; Neil et al., 2016; Wigham et al., 2015).

Thus far, studies investigating sensory reactivity, IU, and anxiety have predominantly assessed sensory differences using questionnaire-based measures (e.g., parent/caregiver- or self-report). While informative, questionnaires often do not distinguish between different levels of the sensory processing pathway, including low-level perceptual sensitivity (i.e., how well an individual can detect or discriminate sensory stimuli) and sensory reactivity (i.e., how an individual feels or reacts towards sensory stimuli). Increasingly, evidence from prior studies in the visual and auditory domains has shown that objective measures of perceptual sensitivity (i.e., threshold data) and self-reported measures of sensory reactivity do not always correlate, thus highlighting that perceptual sensitivity and sensory reactivity are distinct constructs (Kuiper et al., 2019; Sapey-Triomphe et al., 2023; Schulz & Stevenson, 2022). To our knowledge, only one study has assessed the associations of perceptual sensitivity and self-reported sensory reactivity with IU and anxiety (Sapey-Triomphe et al., 2023). Using a comprehensive set of assessments in a sample of autistic and neurotypical adults, Sapey-Triomphe et al. (2023) showed that higher self-reported sensory reactivity, and to a lesser extent, visual perceptual sensitivity, were associated with more IU and anxiety, particularly in the autism group. However, thresholds for detection (visual perceptual sensitivity) and affective responses to visual stimuli did not correlate in either autistic or neurotypical adults. Those findings suggest that perceptual sensitivity and sensory reactivity have important, but separate, contributions to IU and anxiety. Thus, it remains unclear as to how the contributions of sensory differences to IU and anxiety are driven by alterations at the basic perceptual level and/or at the affective/behavioral level. Understanding the mechanisms for how different levels of sensory processing impact anxiety could help to guide specific interventions if appropriate and may lessen the development of additional co-occurring challenges.

Unlike questionnaire-based measures, psychophysical approaches can offer insight into the biological processes that underlie sensory processing, owing to their ability to probe lower-level perceptual function, and therefore link to neural responding patterns (Read, 2015). Since different perceptual processes such as detection and discrimination involve distinct mechanisms, their unique associations to behavioral measures allow a better understanding of the specific biological processes that govern those relationships. Studies using psychophysics have revealed altered sensory processing in autism across multiple sensory domains (e.g., tactile, auditory, and visual), as well as different sub-domains (e.g., detection and discrimination; Bonnel et al., 2010; Dwyer et al., 2022; He et al., 2021b; Heaton et al., 2008; Puts et al., 2014, 2017; Tavassoli et al., 2011). Recent findings within the tactile domain have shown that differences in perceptual ability were related to several core traits of autism, such as social and communication differences (Bryant et al., 2019; He et al., 2021b; Ide et al., 2019). Furthermore, psychophysical studies have shown that low-level perceptual sensitivity is associated with differences in cortical excitation/inhibition balance, enabling inferences to the potential neurophysiological substrates of these differences (He et al., 2021; Puts et al., 2011; Read, 2015). Thus, psychophysical approaches are a useful objective measure that can complement questionnaire-based assessments of sensory differences and their contributions to anxiety and IU.

In the current study, we aimed to investigate the associations between low-level tactile perception, sensory reactivity, anxiety, and IU, in a cohort of 8–12-year-old children with an autism diagnosis. We focused on low-level perception within the tactile domain as differences in touch processing are among the most widely observed in autism, and because alterations in tactile processing are known to contribute to other core features of autism, such as social and communicative problems (Cascio et al., 2016; Foss-Feig et al., 2012; Rogers et al., 2003). As autism and ADHD commonly co-occur, and sensory differences are likely to be exacerbated in autistic individuals with co-occurring ADHD, we included participants with both an ASD only or ASD and ADHD diagnosis. First, we used parent- and child-report questionnaires to understand the relationships previously observed in the literature. We used the Sensory Processing Measure (SPM; Palmer et al., 2007) to assess sensory reactivity, the Screen for Child Anxiety Related Emotional Disorders (SCARED; Birmaher et al., 1997) to assess anxiety, and the Intolerance of Uncertainty Scale for Children (IUSC; Comer et al., 2009), to assess IU. We then used a

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well-validated battery of performance-based psychophysical tasks to assess whether individuals' tactile detection and discrimination thresholds were associated with questionnaire measures of sensory reactivity, anxiety, and IU. We hypothesized that the relationship between sensory differences and anxiety would be mediated by intolerance of uncertainty, as previously shown in the literature. We further hypothesized that both tactile perceptual alterations and sensory reactivity would be associated with increased IU and anxiety.

Methods

Participants

Data from 38 children aged 8-12 years, were included in the analyses. Of the 38 children, 27 also had a clinical diagnosis of ADHD (11 had a diagnosis of autism only). Though the sample was predominantly male (32 male; 6 female), there were no apparent differences between male and female participants in age, IQ, or any of the study measures (Supplementary Table 1). Informed consent was provided by the caregiver for each child. Studies from which these data were collected received ethical approval from an Institutional Review Board. Relevant descriptive variables including age, IQ, and sex, are summarized in Table 1.

Participants were principally recruited through local schools and additionally through community advertisements, advocacy organizations, and pediatricians. Screening was done via telephone interview with the child's parent or caregiver. Children were excluded if they had a history of intellectual disability, seizures, brain injury, known causes of ASD (e.g., Fragile X), or other neurological disorders (e.g., Tourette's syndrome), or were taking psychotropic medications other than stimulants. Children taking stimulants temporarily ceased their stimulant medication on the day before, and on, the day of testing. To check for possible deviations in attention or focus that could result from ceasing stimulant medication, we visually examined participants' response tracking (evaluating correct/incorrect responses across the duration of trials) for each of the vibrotactile tasks. Response tracking and reaction times for all participants appeared normal.

Children were excluded if they had a history of, or met criteria for, major depressive disorder, conduct disorder, bipolar disorder, mania, adjustment disorder, or schizophrenia, as assessed by the Diagnostic Interview for Children and Adolescents Fourth Edition (DICA; Reich, 2000) and/or the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS; Kaufman et al., 1997). Children with a co-occurring diagnosis of anxiety disorders, obsessive compulsive disorder (OCD), or oppositional defiance disorder were not excluded. Intellectual ability was assessed using the Wechsler Intelligence Scale of Children Fourth edition (WISC; Wechsler, 2003). Children with full-scale IQ scores below 80 (based on the lower band for clinical average scores [80-119]) were excluded unless there was an index discrepancy of equal or greater than 12, in which case one of either the Verbal Comprehension or Perceptual Reasoning Index scores had to exceed 80 and the lower of the two had to exceed 65.

Children included in the study met the Diagnostic and Statistical Manual of Mental Disorders Fourth (DSM-IV) and/or Fifth (DSM-5) edition criteria for ASD (American Psychiatric Association, 2000, 2013). An ASD diagnosis was confirmed using the Autism Diagnostic Observation Schedule-Generic or 2 (ADOS; Lord et al., 2000, 2012) and Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994). ASD children were also included if they met additional DSM-IV or DSM-5 criteria for ADHD. ADHD diagnosis was confirmed through the Conners or Conners 3 Parent and Teacher Rating Scales-Revised: Long Form (ADHD-specific broad behavior rating scales and the ADHD Rating Scale-IV, home and school versions [ADHD-RS or DuPaul scale]; Conners et al., 1998a; 1998b Conners, 2008). Diagnostic information was reviewed and verified by an experienced child neurologist.

Measures

Screen for child anxiety-related emotional disorders

The Screen for Child Anxiety Related Emotional Disorders (SCARED; Birmaher et al., 1997) was used to measure child anxiety symptoms. The SCARED is a 41-item dual-informant scale with identical parent and child versions that ask about various DSM anxiety symptoms. A total score of $\geq$ 25 may indicate the presence of an anxiety disorder (Birmaher et al., 1997). The SCARED has been found to have good internal consistency in autistic children (Carruthers et al., 2020; Stern et al., 2014). Total parent-reported SCARED scores were reported here.

Intolerance of uncertainty scale children

The Intolerance of Uncertainty Scale for Children (IUSC) is a 27-item questionnaire that assesses children's tendency to react negatively to uncertain situations or events (Comer et al., 2009). The IUSC includes a child-report form and parent-report form, which were adapted from the original Intolerance of Uncertainty Scale for adults (Buhr & Dugas, 2002). Higher scores indicate higher IU (Comer et al., 2009). Total IUSC scores from the child report were used in this study owing to its stronger convergent validity with measures of anxiety in typically developing children compared with the parent-report version (Comer et al., 2009).

Sensory processing measure

The Sensory Processing Measure (SPM) is a norm-referenced parent/caregiver-report measure that assesses sensory processing, praxis, and social participation (Palmer et al., 2007). Higher scores indicate more difficulties with the associated scales. The SPM has shown excellent reliability and validity, including convergent validity with the more commonly adopted Sensory Profile (Brown et al., 2010). T-scores for the Total Sensory Systems Scale of the SPM (TOT; combining scores from the vision, hearing, touch, body awareness and balance, and motion scales) were used in the analyses. Analyses using TOT raw scores (unadjusted for age or sex), allowing age to be included as a confounder, yielded similar results (Supplementary Table 2; Supplementary Figure 1; Supplementary Table 5).

Psychophysical assessment of tactile perception

Children's tactile perceptual thresholds were assessed using a Cortical Metrics Braingauge stimulator (Fig. 1a) using a vibrotactile battery of tasks originally developed by Puts et al. (2013). All participants underwent psychophysical assessment. Children were asked to place their left hand on the stimulator, which delivered vibrotactile stimuli to the participants' left index and middle fingers, with an amplitude of 0–350 μm and frequency in the flutter range of 0-50 Hz. Participants responded to each protocol using a computer mouse in their right hand. Vibrotactile stimulus parameters (i.e., amplitude and frequency) were controlled, and data collected and saved, by running a cortical metrics script on a computer. To ensure participants understood the protocols correctly, they had to pass three consecutive practice trials before starting each of the tasks. A stepwise adaptive tracking

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Table 1. Descriptive statistics of participant demographics and study variables

	Whole cohort				ASD only				ASD + ADHD				Group differences
	N	*Range*	*Mean*	*SD*	*N*	*Range*	*Mean*	*SD*	*N*	*Range*	*Mean*	*SD*	*p-value*
Age (years)	38	8.1–12.6	10.4	1.4	11	8.1–12.2	10.0	1.4	27	8.2–12.6	10.5	1.3	.264
IQ	38	63.0–132.0	100.9	16.0	11	89.0–132.0	112.8	13.5	27	63.0–125.0	96.1	14.6	**.005**
SCARED	38	0.0–56.0	16.2	12.7	11	0–29.0	11.3	8.2	27	4.0–56.0	18.2	13.7	.151
SPM	38	44.0–75.0	63.9	6.7	11	44.0–65.0	58.8	7.2	27	53.0–75.0	66.0	5.3	**.003**
IUSC	38	28.0–101.0	53.8	15.8	11	28.0–63.0	47.0	10.7	27	35.0–101.0	56.6	16.8	.166
SFD threshold	37	2.8–17.6	11.1	3.4	11	6.4–14.4	10.4	2.9	26[a]	2.8–17.6	11.1	3.4	.280
	N	*Percentage*			*N*	*Percentage*			*N*	*Percentage*			
Sex													
Male	32	84.2%			8	72.7%			24	88.9%			
Female	6	15.8%			3	27.3%			3	11.1%			
Ethnicity													
White	31	81.6%			10	90.9%			21	77.8%			
Black or African American	2	5.3%			0	0.0%			2	7.4%			
Hispanic	1	2.6%			0	0.0%			1	3.7%			
Other	4	10.5%			1	9.1%			3	11.1%			
Handedness													
Right	36	94.7%			10	90.9%			26	96.3%			
Left	2	5.3%			1	9.1%			1	3.7%			

IUSC = intolerance of uncertainty scale for children; SPM = sensory processing measure; SCARED = screen for child anxiety related emotional disorders; SFD = simultaneous frequency discrimination; SD = standard deviation. Sensory reactivity, intolerance of uncertainty, and anxiety correspond to SPM Total Sensory Systems T-scores, IUSC (child-report) total scores, and SCARED (parent-report) total scores, respectively.
[a] Data from one participant was missing as they did not complete the SFD task.

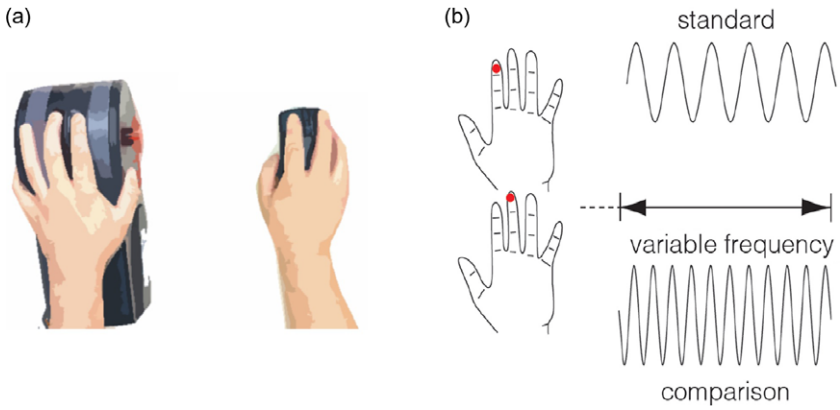

Figure 1. ***a***. Illustration of a cortical metrics braingauge vibrotactile stimulator. Children were asked to place their left hand on the stimulator and their right hand on a computer mouse. Two 5 mm cylindrical probes delivered sinusoidal pulses to the index and middle fingers. Participants responded with their right index and middle finger. Stimuli amplitude and frequency were between 0–350 μm and 0–50 Hz, respectively. ***b***. Visual schematic of the simultaneous frequency discrimination task. Using a two-alternative forced choice design, both fingers are stimulated, one of which receives a standard stimulus of constant frequency (30Hz), and the other receives a comparison stimulus where the frequency can vary (initial frequency was 40 Hz). Children were asked to indicate on which finger they felt the higher stimulus. Following a staircase approach, the comparison stimulus decreased in frequency after a correct response and increased in frequency after an incorrect response. Details of the other vibrotactile tasks can be found in the supplementary methods.

strategy was used for all conditions (detailed below and in the Supplementary Methods for each protocol). Participants were allowed to take breaks as needed between the protocols, except for protocols within the same domain. Data were visualized and analyzed using a custom-made software package in the programing language R (available at: https://github.com/HeJasonL/BATD).

Data from a total of five tasks (simultaneous frequency discrimination, static detection, dynamic detection, amplitude discrimination, and temporal order judgment) in the vibrotactile battery were included in the correlational analyses. As meaningful correlations were found only between the simultaneous frequency discrimination (SFD) task and our questionnaire measures, only the SFD task (Fig. 1b) is described here in detail. Brief descriptions for the remaining tasks are given below. Further details of each task are reported in the Supporting Information.

Simultaneous frequency discrimination

During the simultaneous frequency discrimination (SFD) task (Fig. 1b), vibrotactile stimuli were delivered simultaneously to both fingers (duration: 500 ms; amplitude: 200 μm). On one finger, a standard vibration (frequency = 30 Hz) was delivered, while a comparative vibration (starting frequency = 40 Hz) was delivered

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to the other. The order of the standard and comparative stimuli was pseudorandomized between each finger, in which the order was never the same in five or more consecutive trials. Using a two-alternative forced choice design, participants were asked to indicate which finger they thought had received the higher frequency stimulation, as soon as they felt it. The protocol included 20 trials, which were inter-spaced with a period of 5 seconds. In the first 10 trials, the comparative stimulus frequency was decreased for each correct response that the participant gave and increased for each incorrect response (one–up–one-down tracking paradigm). For the remaining trials, the comparative stimulus frequency was decreased for every two correct answers and increased for one incorrect answer (two–up–one-down tracking paradigm). SFD threshold for each participant was determined by taking the frequency mean of the last five trials, by which point the threshold (i.e., the minimum difference in frequency the participant can perceive) was assumed to have been reached. Higher thresholds are indicative of lower tactile perceptual sensitivity.

Other vibrotactile tasks

For the static and dynamic detection tasks, vibrotactile stimuli were delivered to a single fingertip, alternating between each finger in a pseudorandomized order, at either a constant (static) or gradually increasing (dynamic) amplitude. Participants were asked to indicate on which finger they felt the stimulus, as soon as they felt it. For the amplitude discrimination task, stimuli were delivered simultaneously to both fingertips, of which one differed in amplitude. Participants were asked to indicate on which finger they felt the stronger stimulus. In the temporal order judgment task, participants received stimuli to both fingertips at differing time intervals. Participants were asked to indicate on which finger they felt the first stimulus. Further detail of the vibrotactile tasks can be found in the Supporting Information.

Statistical analyses

Data were analyzed using R (version 4.0.3). All data and code for the analyses and figures can be found on the Open Science Framework (OSF; https://osf.io/uw8vb/).

Differences in age, IQ, SFD thresholds, SPM, SCARED, and IUSC scores between participants with a single diagnosis of autism (ASD only) and participants with a dual diagnosis of autism and ADHD (ASD + ADHD), and between male and female participants (Supplementary Table 1), were assessed using a series of Wilcoxon Rank Sum tests. Significance level was set at $p \leq .008$ following Bonferroni's correction for multiple comparisons (.05/6 tests).

Pearson correlation analyses were run between the parent-reported Total Sensory Systems (TOT) T-scores of the SPM (termed from now on as 'sensory reactivity'), parent-reported total SCARED scores, child-reported IUSC total scores, the vibrotactile thresholds, and age. Cook's Distance with a D_i cutoff of 0.2 (below which values are not considered to have major influence) was used to identify potential outliers. Results did not significantly differ between Pearson correlations with and without outliers, therefore data analyses are shown here without the outliers removed. Results of all Pearson correlation analyses, including those without outliers as identified by Cook's Distance, can be found in Supplementary Table 2 and Supplementary Table 3. Levene's tests showed homoscedasticity between all variables. Inspection of histogram and Q-Q plots showed that distributions for IUSC scores and SFD thresholds appeared normal, however, the distributions for SCARED and SPM scores were skewed. Therefore, we also investigated relationships using Spearman rank correlations (Supplementary Table 3). As the results between Spearman and Pearson correlations were similar, only the Pearson correlations are shown here. Significance levels were set at $p \leq .05$, except for analyses involving the vibrotactile thresholds, which were adjusted to $p \leq .006$ after Bonferroni's correction (.05/9 tests).

Mediation analyses using the non-parametric percentile bootstrap method were run using the *mediation* package in R for Causal Mediation Analysis (Tingley et al., 2014). Evidence of mediation is indicated by a significant indirect effect (the association between the predictor and outcome variable runs through the mediator). Full mediation exists when the indirect effect is significant, but the direct effect (the effect of the predictor on the outcome variable, controlling for the mediator) is not. If both indirect and direct effects are significant, the result is partial mediation. Unstandardized indirect effects were computed for each of the 10,000 bootstrapped samples, and the 95% confidence interval was computed by determining the indirect effects at the 2.5th and 97.5th percentiles. Interaction effects between predictor and mediator were not examined as we did not have a sufficiently large sample size. To check for the influence of outliers, we also compared the mediation results from the percentile bootstrap method with those of a bootstrap method using data cleaning via multivariate winsorization and coefficient estimation via maximum likelihood estimation of the covariance matrix (Zu & Yuan, 2010). Results from the multivariate winsorization bootstrap method and the percentile bootstrap method yielded almost identical results (Supplementary Table 6). As an assumption of causal mediation analysis is the correct causal ordering of independent, mediator, and dependent variables, we also tested the significance of indirect effects for different path combinations, which are shown in Supplementary Tables 4 and 5.

Results

Group differences between autism-only participants and those with co-occurring ADHD

To test for differences between participants with a single autism (ASD only) diagnosis and those with co-occurring ADHD (ASD + ADHD), a series of Wilcoxon rank sum tests were run. There were no significant differences between the two groups for age, SFD thresholds, anxiety scores or intolerance of uncertainty (IU) scores (Table 1). However, IQ scores were lower, and SPM scores higher, in the ASD + ADHD group compared with the ASD-only group ($p = .005$ and .003, respectively), concordant with the literature (Mattard-Labrecque erg et al., 2013; Sanz-Cervera et al., 2017). IQ scores for the ASD + ADHD group were still within clinical average range.

Correlational analyses

Pearson's correlation analyses showed that parent-reported sensory reactivity was positively correlated with parent-reported anxiety (Fig. 2a; $R = .37$; $p = .023$) and child-reported intolerance of uncertainty (Fig. 2b; $R = .40$; $p = .013$). Intolerance of uncertainty (IU) was also positively correlated with anxiety (Fig. 2c; $R = .48$; $p = .002$). When investigating the associations between tactile perceptual thresholds determined using vibrotactile psychophysics, we found that only simultaneous frequency discrimination (SFD) threshold significantly correlated with parent-reported sensory reactivity (Fig. 2d; $R = .41$; $p = .011$),

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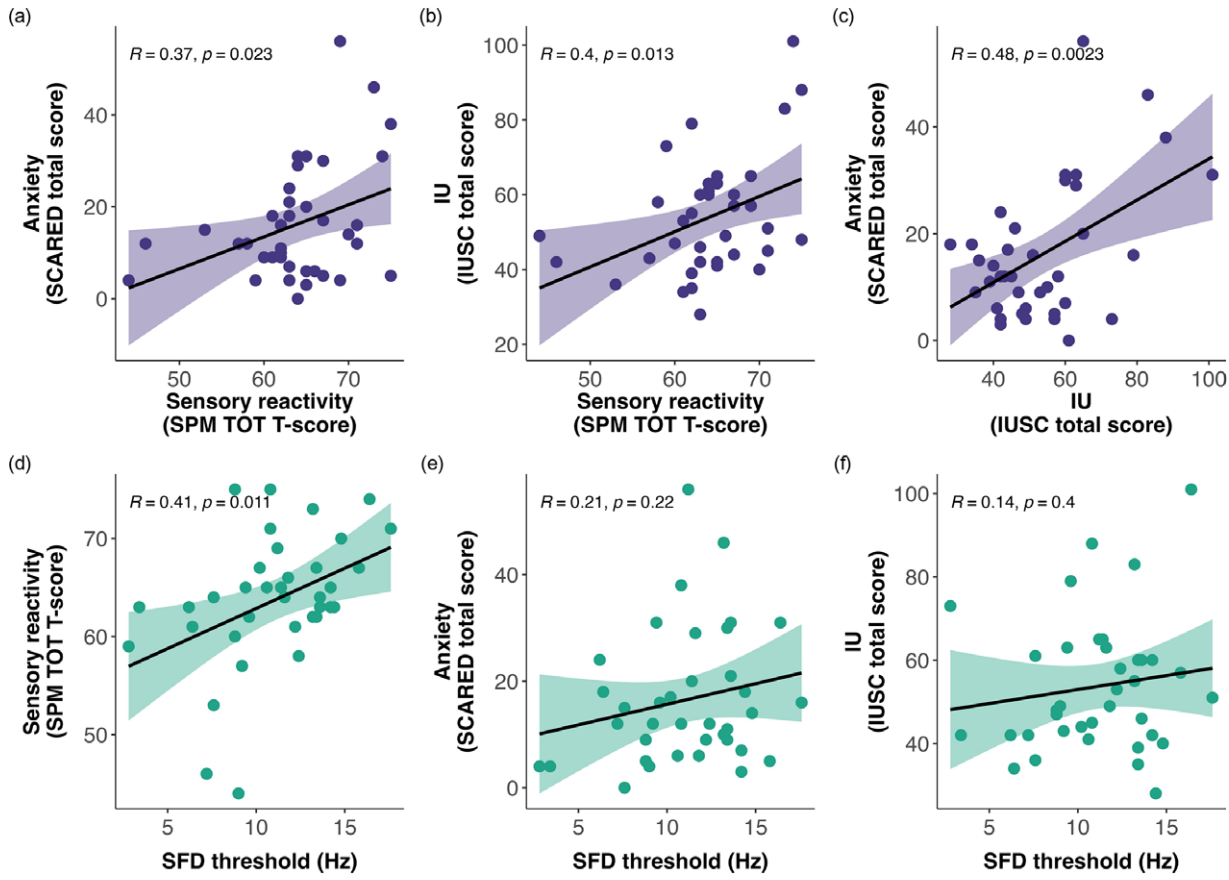

Figure 2. Pearson's correlations between sensory reactivity, IU, anxiety and SFD threshold. **a-c**. There were moderate positive associations between all three measures of sensory reactivity, IU, and anxiety. **d-f**. There was a moderate positive association between SFD threshold and sensory reactivity. No significant associations were found between SFD threshold and anxiety or IU. IU = intolerance of uncertainty; IUSC = intolerance of uncertainty scale for children; SCARED = screen for child anxiety related emotional disorders; SFD = simultaneous frequency discrimination; SPM TOT = sensory processing measure total sensory systems.

although after Bonferroni's correction (p = .006), this significance was lost. In addition, contrary to our hypothesis, no significant associations were found between SFD threshold and anxiety or IU (Fig. 2e, f; $R = .21$; $p = .22$ and $R = .14$; $p = .40$, respectively). There were no significant correlations between age and the study variables or between the raw scores of the SPM that were unadjusted for age and sex (Supplementary Table 2). Child-reported SCARED total scores were also investigated, however, despite a significant correlation between child-reported SCARED and IUSC scores ($R = .45$; $p = .005$; Supplementary Table 2), there were no significant correlations observed between child-reported SCARED total scores and SPM scores or SFD thresholds. Results of all correlational analyses can be found in Supplementary Table 2.

Mediation analyses

IU mediates the relationship between sensory reactivity and anxiety

To replicate findings from previous studies in which IU has commonly been shown to mediate the relationship between sensory reactivity and anxiety, we first ran mediation analyses between these constructs. As a recent study by MacLennan et al. (2021) also found a full mediation effect between sensory hyper-reactivity and IU mediated through anxiety, in addition to a full mediation effect between sensory hyper-reactivity and anxiety through IU, we aimed to test both these models (MacLennan et al., 2021). Mediation analyses revealed a significant full mediation effect of child-reported IU on the association between parent-reported sensory reactivity and parent-reported anxiety ($\beta = 0.30$; 95% CI [0.03, 0.76]; $p = .02$; Fig. 3a). We also found a significant, partial mediation effect of anxiety on the association between sensory reactivity and IU ($\beta = 0.34$; 95% CI [0.04, 0.74]; $p = .02$; Fig. 3b). No significant mediation effects were found for different mediation paths (Supplementary Table 4). These results suggest that in our sample of autistic children, the impact of sensory reactivity on anxiety appears to be fully driven by intolerance of uncertainty. As higher anxiety also appears to contribute to a higher IU, our findings suggest that IU and anxiety could be reinforcing factors of each other in the presence of augmented sensory reactivity. To test whether mediation effects were influenced by ADHD traits such as inattention, Conners and DuPaul scores were added to the mediation analyses as covariates. The indirect effects for both models remained significant (Supplementary Table 7), indicating that attentional difficulties or other ADHD traits did not influence the relationship between sensory reactivity and anxiety.

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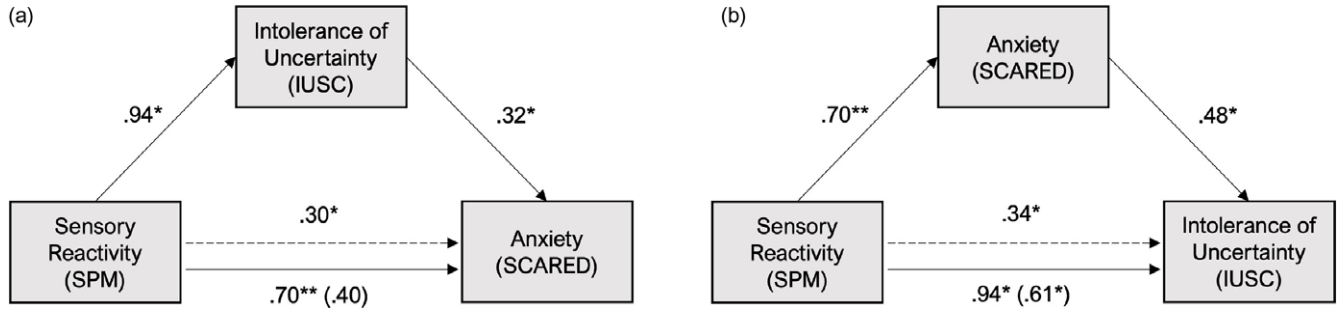

Figure 3. Visual schematic of the mediation path models between sensory reactivity (SPM total sensory systems T-scores), intolerance of uncertainty (IU; IUSC total scores), and anxiety (SCARED total scores). Dashed arrows indicate the indirect effect between the predictor and outcome that is mediated by the mediator. Solid arrows between the predictor and outcome indicate the total effect, with the direct effect in parentheses. **a**. Full mediation effect of IU on the relationship between sensory reactivity and anxiety. **b**. Partial mediation effect of anxiety on the relationship between sensory reactivity and IU. IUSC = intolerance of uncertainty scale for children; SPM = sensory processing measure; SCARED = screen for child anxiety related emotional disorders. $^{**}p \leq .01$; $^{*}p \leq .05$.

Sensory reactivity mediates the relationship between tactile perceptual sensitivity and anxiety

We next investigated two additional mediation models to assess the relationship between SFD threshold, IU, and anxiety, with sensory reactivity as the mediator. We hypothesized that SFD threshold would be a predictor of sensory reactivity, given that lower-level perception of sensory input occurs before higher-order processing. We also hypothesized that sensory reactivity is likely to precede anxiety and IU, as this has been the predominant finding in literature, and is consistent with our results from the previous two mediation models. For both mediation models, we found that parent-reported sensory reactivity significantly mediated the association between SFD threshold and parent-reported anxiety ($\beta = 0.58$; 95% CI [0.06, 1.56]; $p = .02$; Figure 4a), as well as between SFD threshold and child-reported IU ($\beta = 0.80$; 95% CI [0.11, 1.99]; $p = .01$; Figure 4b). Analyses of the reverse of those relationships yielded significant indirect effects, however, the corresponding regression coefficients were close to zero ($\beta = 0.04$; Supplementary Table 4), suggesting that those results are unlikely to be meaningful. Taken together, our results suggest that differences in tactile perceptual sensitivity impact anxiety through differences in sensory reactivity at the affective level. When Conners and DuPaul scores were added as covariates to the mediation analyses, the indirect effects for both mediation models remained significant (Supplementary Table 7), suggesting that ADHD-related traits did not contribute to the outcomes.

Discussion

Here we assessed the role of tactile perceptual alterations on the relationship between sensory reactivity, intolerance of uncertainty (IU), and anxiety, in a sample of 8-12-year-old autistic children with and without co-occurring ADHD. Consistent with previous findings, our mediation analyses revealed that child-reported IU fully mediated an association between parent-reported sensory reactivity and parent-reported anxiety and that anxiety partially mediated an association between sensory reactivity and IU. When investigating the association between tactile perceptual sensitivity and questionnaire measures, we found that simultaneous frequency discrimination (SFD) was the only vibrotactile task for which thresholds significantly correlated with sensory reactivity. However, we found no direct correlations between SFD threshold and anxiety or IU. Interestingly, we found that sensory reactivity fully mediated an association between SFD threshold and anxiety, and between SFD threshold and IU. These results suggest a stepwise relationship whereby increased SFD threshold, in which higher thresholds indicate lower perceptual sensitivity towards frequency discrimination of tactile stimuli, contribute to increased prevalence of sensory reactivity, leading in turn to increased anxiety and IU. It is possible that the absence of a direct correlation between SFD threshold and anxiety and IU could therefore be explained by the fact that the effects of a higher SFD threshold on anxiety and IU may only occur when they are mediated by sensory reactivity at the affective level. Although these findings warrant replication in a larger, more sex-balanced cohort, our results suggest a bottom-up perceptual mechanism underlying the relationship between sensory differences and anxiety in autism.

Implications of SFD threshold in the associations between sensory reactivity, IU, and anxiety

Owing to the complexity and heterogeneity of both sensory differences and anxiety in autism, understanding how alterations in low-level neural functioning contribute to sensory differences at the affective level is crucial. In the current study, we found that individuals with higher (i.e., worse) SFD thresholds had higher sensory reactivity. The mechanisms underlying the relationship between perceptual processing and sensory reactivity have not been clearly established. However, in the context of this study, it is possible that individuals with altered SFD thresholds who are worse at discriminating stimuli could experience difficulties with interpreting or integrating sensory information, thus creating a sensory environment that is uncertain or 'noisy' (Pellicano & Burr, 2012; van de Cruys et al., 2014). Persistent unpredictability of sensory stimuli could therefore lead to greater negative affective responses and/or sensory avoidance. Over time, this difficulty with regulating sensory information at the perceptual and affective level could worsen one's intolerance to perceived uncertainty of stimuli, which could further result in distress or anxiety.

Interestingly, there were no significant correlations between any of the other vibrotactile tasks and the questionnaire measures for sensory reactivity, anxiety, or IU. Though we cannot rule out that the absence of associations between other tasks and sensory reactivity could be because of insufficient power, it is possible that the specific mechanisms that underpin frequency discrimination are most accordant with those driving sensory-related anxiety and IU. Increasingly, studies have shown differences in only some perceptual sub-domains (e.g., amplitude, frequency, or temporal discrimination) but not others, suggesting that symptom presentations could have sub-domain-specific mechanisms, rather

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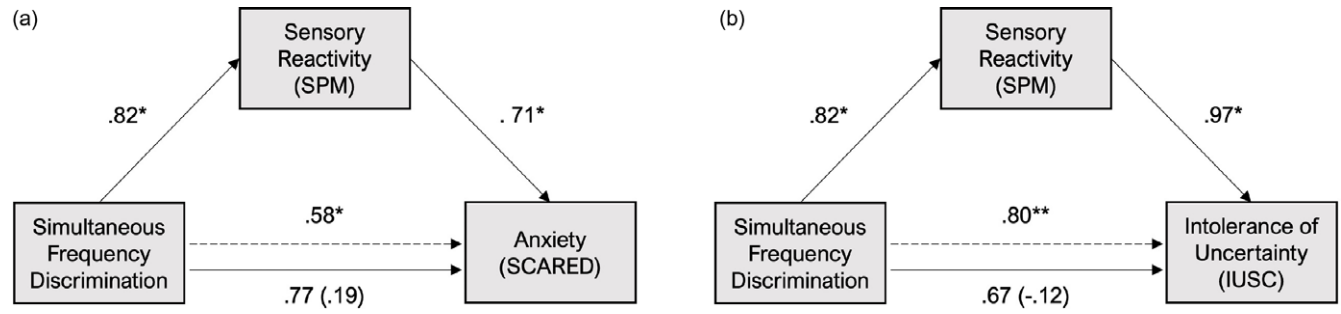

Figure 4. Visual schematic of the mediation path models between SFD threshold, sensory reactivity (SPM total sensory systems T-scores), intolerance of uncertainty (IU; IUSC total scores), and anxiety (SCARED total scores). Dashed arrows indicate the indirect effect between the predictor and outcome that is mediated by the mediator. Solid arrows between the predictor and outcome indicate the total effect, with the direct effect in parentheses. **a.** Full mediation effect of sensory reactivity on the relationship between SFD threshold and anxiety. **b.** Full mediation effect of sensory reactivity on the relationship between SFD threshold and IU. IUSC = intolerance of uncertainty scale for children; SPM = sensory processing measure; SCARED = screen for child anxiety related emotional disorders; SFD = simultaneous frequency discrimination. $^{**}p \leq .01$; $^{*}p \leq .05$.

than associations to "perception" as a whole (Bryant et al., 2019; He et al., 2021b; Ide et al., 2019). Furthermore, our previous work has shown that autistic children with co-occurring ADHD had significantly more difficulty with discriminating the frequency and amplitude of simultaneously delivered vibrotactile stimuli compared with typically developing children and those with ADHD only (He et al., 2021b). Thresholds for both tasks were correlated with sub-scales of the Autism Diagnostic Observation Scale (ADOS), but only SFD, and not amplitude discrimination, showed additional associations with some sub-scales of the Sensory Experiences Questionnaire (He et al., 2021b). Those findings suggest that SFD may play a more important role in sensory reactivity at the affective level and that this may be specific to autism.

SFD requires complex encoding of temporal, intensity, and spatial information, and has previously been reported to be more difficult than other vibrotactile tasks, including simultaneous amplitude discrimination (Puts et al., 2013). As such, SFD might encapsulate the complexity of tactile perceptual processing more so than other tasks. As the ability to discriminate sensory stimuli involves GABA-mediated lateral inhibition of cortical minicolumns (Casanova et al., 2003), SFD may also require a higher demand for GABA (Cardin et al., 2009; Harris et al., 2006). In alignment with this, our previous work using magnetic resonance spectroscopy showed that higher vibrotactile SFD thresholds were associated with lower GABA and higher glutamate/glutamine concentrations in the sensorimotor region and thalamus, respectively (He et al., 2021a; Puts et al., 2011). This could suggest that poorer frequency discrimination may be linked to excessive neural excitation or reduced inhibition. The concept of an excitation/inhibition (E/I) imbalance has long been proposed as a theoretical framework underlying neural processes associated with autism, including those related to sensory differences (Rubenstein & Merzenich, 2003). In the context of anxiety, as GABAergic signaling in the amygdala is known to play a crucial role in regulating anxiety and threat responses (Davis, 1992), we speculate that the cortical and cognitive processes that govern poorer frequency discrimination may also be related to those contributing to anxiety.

In the current study, we used vibrotactile stimuli for our measure of perceptual sensitivity, but the extent to which the perceptual contributions found here could translate to other sensory domains (e.g., visual, auditory) remains unclear. As signals encoding sensory input are known to converge in both cortical and subcortical regions, despite distinct receptive origins, it is possible that psychophysical measures could provide insight into task-specific biological mechanisms that are domain-general (Stein & Stanford, 2008). However, further study comparing perceptual contributions to IU and anxiety across different sensory domains would be needed.

Perceptual sensitivity and hypo- vs. hyper-reactivity

Our finding of an association between higher vibrotactile thresholds (i.e., hyposensitivity) and increased parent-reported sensory reactivity is consistent with that of a previous study, in which higher auditory detection thresholds were found to associate with increased self-reported auditory reactivity (Kuiper et al., 2019). However, our results contrast with work by Sapey-Triomphe et al. (2023), who reported an association between heightened visual perceptual sensitivity and increased self-reported responsivity (Sapey-Triomphe et al., 2023). Together, those findings suggest that both hypo- and hyper-sensitivities at the perceptual level can relate to more sensory reactivity at the affective level.

In MacLennan et al. (2021)'s study, sensory hyper-reactivity, but not hypo-reactivity or sensory seeking, specifically predicted outcomes of IU and anxiety. Conversely, other studies, including one study involving autistic children with a similar age range to those in the current study (Wigham et al., 2015), have highlighted relationships between sensory reactivity, IU and anxiety involving *both* hypo- and hyper-reactivity (Hwang et al., 2020; Sapey-Triomphe et al., 2023; Wigham et al., 2015). As the SPM does not provide specific sub-scores for hypo- or hyper-reactivity, we could not test for associations between those phenotypes directly. However, in previous work, higher tactile perceptual thresholds were shown to positively associate with both hypo- and hyper-reactivity sub-scores (He et al., 2021b). As individuals can experience both hypo-reactivity and hyper-reactivity to different stimuli across and *within* sensory domains, we hypothesize that higher SFD thresholds could relate to affective responses to sensory stimuli that manifest as either hypo-reactive or hyper-reactive phenotypes.

IU in the context of sensory differences and anxiety

Our findings of a full mediation effect between sensory reactivity and anxiety through IU, and a partial mediation effect between sensory reactivity and IU through anxiety are like those of MacLennan et al., (2021)'s study, who found significant mediating roles for both IU and anxiety with sensory hyper-reactivity as the predictor in preschool-aged children. While the issue of reverse

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causality cannot be ruled out as our data was cross-sectional, our findings provide support for the notion that sensory-related IU and anxiety could be involved in a feedback loop, both contributing to the development and maintenance of anxiety and IU. Green & BenSasson (2010) originally hypothesized in their 'Primary Sensory Over-responsivity Model' that individuals could develop anxiety through an increased aversity towards unpredictable or uncontrollable sensory stimuli within the environment (Green & Ben-Sasson, 2010). As IU could lead to a state of hypervigilance, which could worsen one's anxiety and ability to regulate affective and behavioral responses to sensory input (i.e., sensory reactivity), this could further reinforce intolerance towards unpredictable sensory stimuli and/or the associated environment.

Perceptual sensitivity and sensory reactivity are related, but separate, constructs

As our questionnaire measure for sensory reactivity was significantly correlated with anxiety and IU, but SFD threshold was not, our findings support the view that low-level perceptual sensitivity and sensory reactivity are related, but separate constructs, with unique contributions to IU and anxiety. These results highlight that a possible reason why some studies may fail to demonstrate direct associations between low-level physiological measures and clinical symptoms is that associations may only exist when they are mediated by an external factor. These findings further highlight the need for measures that differentiate between perceptual and affective/behavioral responses to sensory stimuli. As most clinical measures for assessing sensory processing in children are reliant on teacher-, parent-, and/or self-report, the sensory differences that are captured in the assessments are often restricted to affective or behavioral responses to sensory stimuli (note, where some studies refer to "behavioral" responses as objective measures of perceptual sensitivity, here we use the term "behavioral" to mean a subjective response or reaction to sensory stimuli [e.g., avoidance]). Few questionnaire measures include questions relating to sensory differences at the lower level (i.e., how well an individual perceives stimuli), and if present, do not provide this information as separate scores. Being able to specify the level of sensory processing being measured, whether perceptual, affective, behavioral, or neural, would improve our understanding of the mechanisms involved, and facilitate clearer comparisons between studies (He et al., 2023; Schulz & Stevenson, 2022; Ward, 2018).

Limitations

Several limitations must be considered when interpreting the findings from our study. First, the sample size in our study was relatively small. It is therefore possible that we did not have enough power to detect certain relationships, e.g., between vibrotactile tasks other than SFD. Moreover, our sample had a larger proportion of male relative to female participants. Consequently, our findings presented here may principally apply to male autistic children. As evidence for sex differences in sensory processing of non-painful stimuli in autism have been observed at the affective/behavioral level (Osório et al., 2021; Tavassoli et al., 2014), but not at the perceptual level (Asaridou et al., 2022), future studies, using a larger cohort with a higher representation of female participants, would be valuable to replicate the associations observed in this study and evaluate potential sex differences. A larger sample size would also allow the use of more complex modeling techniques, such as path analysis or structural equational modeling, to better define the relationships between SFD threshold, sensory differences, IU, and anxiety by combining them into a single model. However, such techniques require access to large data sets, which can be challenging in clinical populations.

Furthermore, the data that we used in the study was cross-sectional. Therefore, the stepwise associations that we demonstrate must be interpreted with caution. Ideally, mediation analyses should assess variables that are measured at separate timepoints, so that any reverse relationships can be ruled out. While we found non-significant mediation effects for different path combinations outside the paths of interest, future work would benefit from assessing the relationships between perceptual alterations, sensory reactivity, IU, and anxiety using longitudinal or temporally-ordered data.

We note that our study had a high percentage of ASD individuals with co-occurring ADHD (n = 27; 71%). While the prevalence of co-occurring ADHD in autistic individuals varies in the literature, the rate of co-existing ADHD in our sample was generally consistent with rates previously reported (40-80%; Rau et al., 2020; Rong et al., 2021; Sinzig et al., 2004). Evidence for both distinct and overlapping sensory processing patterns has been found in autism and ADHD, though these have not been clearly defined (Bijlenga et al., 2017; Dellapiazza et al., 2021; He et al., 2021b; Little et al., 2018; Scheerer et al., 2022). However, some results from neurophysiological studies suggest that the two conditions may share underlying mechanisms (Itahashi et al., 2020). Though studies have previously been limited by the preclusion of a dual diagnosis of autism and ADHD prior to the DSM-5, more recent studies suggest that sensory differences in those with both ASD and ADHD traits are similar, or more severe than in either condition alone (Mattard-Labrecque erg et al., 2013; Sanz-Cervera et al., 2017). Thus, there is a critical need to understand the neural mechanisms that contribute to phenotypic sensory differences in autistic individuals with and without co-occurring ADHD. In this exploratory study, we did not have the statistical power to run separate mediation analyses for those with and without a co-occurring ADHD diagnosis, however as all mediation effects remained significant when Conners and DuPauls scores were added as covariates, the results suggest that the outcomes are unlikely to be an effect of ADHD-related traits. In addition, except for higher SPM scores in the ASD + ADHD group, there were no statistical differences in SFD thresholds, anxiety, or IU between the two groups. Thus, we believe our findings are clinically relevant to autistic individuals with and without co-occurring ADHD. Nevertheless, studies evaluating how atypical low-level sensory perception contributes to clinical symptoms of anxiety and IU in individuals with ASD, ADHD, and co-occurring ASD and ADHD, would be useful for understanding how underlying neurophysiological mechanisms might differ between them.

A final limitation is that we used parent-report measures from the SCARED assessment and not child reports. While child-reported SCARED scores were significantly correlated with child-reported IUSC scores (Supplementary Results), no significant correlations were observed between child-reported SCARED and SPM scores. As the SPM is an assessment that is completed by the parent or caregiver, it is possible that no significant correlations were found between the SPM score and child-reported SCARED score owing to child-parent report inconsistencies, a phenomenon that is often observed in psychology studies (De Los Reyes et al., 2015). Using a combination of both parent and child reports where

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appropriate, and reports within different contexts, are likely to be the most informative.

Conclusion

In conclusion, our study showed that parent-reported sensory reactivity fully mediated an association between tactile perceptual alterations and parent-reported anxiety, and between tactile perceptual alterations and child-reported IU, in autistic children with and without co-occurring ADHD. Our findings add to current literature by suggesting that bottom-up perceptual alterations in relation to frequency discrimination of vibrotactile stimuli, contribute to sensory reactivity at the affective level, which could in turn lead to increased anxiety and IU. Understanding how differences in perceptual, affective, and behavioral levels of the sensory processing pathway, as well as differences in specific subdomains, contribute to behavioral challenges that commonly co-occur in autism, such as anxiety and IU, could help to guide more specific, individualized interventions.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0954579423001360.

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Competing interests. None.

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