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Amy Ursitti April 12, 2022

A multifaceted psychophysical approach to assessment of sensory sensitivity and pain in adolescents with autism

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Abstract

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Autism spectrum disorder (ASD) encompasses a spectrum of conditions that are fairly common and heterogeneous in presentation. One core symptom of ASD as defined in the DSM-5 is altered sensory sensitivity, which can include hyper- or hyposensitivity to various environmental stimuli as well as diminished or heightened responses to pain. Previous studies have reported unusual sensory responses in 42-88% of older children with ASD that can greatly interfere in their abilities to participate in everyday activities; however, few clinical measures exist to adequately characterize sensory sensitivity and pain perception in ASD, especially in adolescent and adult populations. Few studies have employed quantitative sensory testing (QST) to assess neurophysiological underpinnings of sensory sensitivity in ASD, but these have yielded mixed results across age groups. The aim of this study was to utilize QST to better understand how ASD affects adolescents' sensitivity to both noxious and innocuous thermal, pressure, mechanical, and auditory stimuli. We compared pain and detection thresholds measured in adolescents with ASD (n = 10) to published reference data collected from 13-16-year-old neurotypical adolescents (n = 64). Additionally, we utilized devices and methods that have yet to be tested in ASD populations, including the thermal grill illusion (TGI) and a hedonic rating scale, to provide a richer characterization of sensory sensitivity in ASD. Our findings indicate overall hypersensitivity specifically to noxious stimuli in ASD, and hyposensitivity to innocuous stimuli. Further comparison of individual threshold values to the reference data as well as exploratory examination of TGI and hedonic ratings revealed inter- and intra-individual variability of sensory responses in ASD, which may be reflective of heterogeneity inherent in the

symptoms of the disorder. These findings indicate the value of QST as a tool for providing a detailed assessment of sensory sensitivity in ASD across numerous modalities.

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Table of Contents

Tables and Figures

INTRODUCTION

Autism Spectrum Disorder (ASD) and Sensory Sensitivity

Autism spectrum disorder (ASD), initially illustrated as a rare, narrowly-defined childhood disorder, is now acknowledged as a spectrum of conditions that are lifelong and relatively common (Lord et al., 2018). According to the most recent data from the CDC's Autism and Developmental Disabilities Monitoring (ADDM) Network, about 1 in 44 children have ASD, with diagnosis being 4 times more common in boys than in girls (Maenner et al., 2021). Onset of symptoms usually occurs by age 3, but in some individuals symptoms may not manifest until school age or later, while in others symptoms may be identified between 6 and 18 months of age (Szatmari et al., 2016).

Although presentations of ASD are quite heterogeneous, all individuals diagnosed with ASD according to the criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) must demonstrate difficulties in three social communication domains as well as at least two clearly defined repetitive sensorimotor behaviors (APA, 2013). Social communication symptoms can include difficulties in social-emotional reciprocity and back-and-forth conversation, abnormal nonverbal communicative behaviors (e.g., eye contact, facial expression, use of gestures), and maintaining and understanding interpersonal relationships. Repetitive behaviors may manifest as stereotyped motor movements (e.g., hand-flapping), difficulty with transitions and changes in routine, or highly fixated interests, for example. ASD severity levels are further defined by support need in the categories of social communication and restricted, repetitive behaviors, with some individuals with ASD requiring support, substantial support, or very substantial support in these two areas. Common comorbidities further complicate the heterogeneous presentation of ASD, with the most common

co-occurring diagnoses in a group of 112 10- to 14-year-old children with ASD being social anxiety disorder (29.2%), attention-deficit/hyperactivity disorder (28.2%), and oppositional defiant disorder (28.1%) (Simonoff et al., 2008). The extreme variation in presentation of symptoms and developmental trajectory in ASD makes identification of risk factors and effective treatment options difficult (Wiggins et al., 2021).

As outlined in the DSM-5, altered sensory sensitivity is considered a new defining feature of ASD under the category of restricted, repetitive behaviors and may be relevant in the formation of diagnostic tools and targeted treatment options (APA, 2013). Specifically, individuals with ASD may exhibit "hyper- or hyporeactivity to sensory input or unusual interests in sensory aspects of the environment (e.g., apparent indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling or touching of objects, visual fascination with lights or movement)" (APA, 2013, p. 28). Hypersensitivity can be characterized by intensified behavioral responses such as covering ears in response to loud noises or avoiding touch, while hyposensitivity can lead to unique issues such as self-injurious behaviors and undertreatment of medical conditions (Baranek et al., 2006; Cascio et al., 2008; Dubois et al., 2020; Hirstein et al., 2001; McAlonan et al., 2002; Miller et al., 2001). Unusual sensory responses (including both hyper- and hyposensitivity) have been reported in 42 to 88% of older children with autism in various studies (Baranek, 2002; Weitlauf et al., 2017). Sensory sensitivities can lead to difficulty in tolerating everyday stimuli, such as bright lights, certain clothing and food textures, and loud noises, and they can further interfere in the abilities of individuals with ASD to participate in social situations, care for themselves, or even simply to leave the home (Weitlauf et al., 2017). Despite the addition of a diagnostic criterion and the overwhelming effects that sensory sensitivities can have on individuals, recent reports highlight

incomplete understanding of sensory sensitivity in ASD, as well as a lack of assessment tools that are effective past childhood, particularly those that assess neurophysiological underpinnings (Moore, 2015; DuBois et al., 2017).

According to a recent review, most studies (78.78% of 66) on sensory sensitivity in ASD still rely on self- and proxy-report questionnaires alone (DuBois et al., 2017). Similarly, in clinical practice, results from such questionnaires are regularly the sole source of information guiding clinicians in making diagnosis and treatment decisions. A number of validated questionnaires exist to assess sensory sensitivity in pediatric populations; however, very few of the existing measures targeted toward adolescent and adult populations are clinically available and have norms, even though sensory symptoms of ASD have been shown to persist past childhood (DuBois et al., 2017). Until recently, the Adolescent and Adult Sensory Profile (AASP) was the only clinical measure for sensory processing in adolescents and adults with published psychometric properties (DuBois et al., 2017). Despite its wide use, this measure has shown a number of limitations, including that it may miss repetitive sensory seeking behaviors in individuals with ASD, especially since it was developed using samples from the general population and thus is not spectrum-specific (Elwin et al., 2012). Another measure designed to assess sensory symptoms in detail in both children and adults with ASD is the Diagnostic Interview for Social and Communication Disorders (DISCO) (Leekam et al., 2002), but this interview is conducted with a parent or caregiver and thus may not reflect the personal experiences of the individual with ASD with complete accuracy. The strengths of the aforementioned self- and proxy-report measures lie in the ease with which they can be implemented and the breadth of information that they can provide in a short amount of time; however, Cascio et al. (2016) noted that these measures “often reflect attentional and affective

aspects of perception and behavioral response that are less likely to correlate directly with basic sensory processes assessed in the controlled laboratory setting" (p. 923).

Other techniques of assessing sensory sensitivity in ASD include direct behavioral observation, qualitative interview techniques, and neuroimaging. Direct behavioral observation can take place in both naturalistic and controlled settings (e.g., during a functional behavioral assessment), but focus is placed on specific behaviors such as repetitive movements or spinning rather than the neurophysiological underpinnings of the observed behavior (DuBois et al., 2017). Qualitative interviews allow for more free-flowing, personal responses from individuals with ASD about their sensory experiences; however, clinical interviews are limited by the skills of the clinician and can be challenging and time-consuming to implement, especially for individuals with ASD whose communication abilities are limited. Finally, neuroimaging has its own set of major limitations related to cost, availability, and training. Symptoms inherent in ASD also make participation in neuroimaging procedures challenging, namely deficits in social communication, anxiety surrounding new experiences, impaired language abilities, and even sensory sensitivity itself (Smith et al., 2019).

Given the wide-ranging heterogeneity in ASD symptoms, DuBois et al. (2017) posited that a comprehensive body of approaches is necessary for clinical assessment of core ASD symptoms such as sensory sensitivity. For example, it has been suggested that in conjunction with questionnaires, quantitative sensory testing (QST) that assesses dysfunction in specific peripheral nerve fibers may provide a useful clinical picture in deficits regarding basic perception and self-reported experience of peripheral sensory input (Elwin et al., 2012). These assessments may have direct implications related to safety (e.g., temperature/pain, falls) and identification of useful interventions (e.g., body awareness training, desensitization therapy)

(DuBois et al., 2017). Despite the acknowledgement of the detailed information that it can provide to complement questionnaire results, QST has been investigated and applied in fewer studies compared to other sensory sensitivity assessment methods, and there is still no gold standard on how it should be utilized as an assessment tool in ASD.

Quantitative Sensory Testing (QST) in ASD Populations

Quantitative sensory testing (QST), a subjective psychophysical method, has been used in few studies to examine threshold detection and pain hyper- or hyposensitivity in ASD. In QST, thermal, static, and dynamic stimuli are used to assess functions of specific types of receptors, peripheral nerve fibers, and central nervous system (CNS) pathways (Hansson et al., 2007). Specific QST parameters can examine large fiber function (Aβ), namely the measurement of mechanical detection thresholds (MDT) and vibration detection thresholds (VDT) (Rolke et al., 2006; Blankenburg et al., 2010). Others, like the measurement of cool detection thresholds (CDT) and mechanical pain thresholds (MPT), characterize non-nociceptive and nociceptive small fiber (Aδ) function. Further, warm detection thresholds (WDT), heat pain thresholds (HPT), and cold pain thresholds (CPT) represent the function of non-nociceptive and nociceptive small fiber (C) function. Individual roles for Aδ and C fibers in pressure pain thresholds are not as well understood; however, when the aforementioned QST parameters are performed together on an individual, a holistic picture of Aβ, Aδ, and C fiber function can be pieced together (Rolke et al., 2006). QST has been found to be feasible for children over five years of age, and it may be a promising tool in assessing sensory sensitivity in ASD at the level of fiber dysfunction as it can account for both hyper- and hyposensitivity in these specific pathways (Blankenburg et al., 2010).

QST measures designed to test tactile submodalities (touch, pressure, temperature, and pain) are especially relevant in ASD as tactile hypersensitivity has been hypothesized to lead to aversion to social touch and subsequent social withdrawal (Cascio et al., 2008). However, previous studies have yielded mixed results regarding differences in ASD response in all of these submodalities across different age groups.

Varying observations regarding differences in mechanical detection thresholds between ASD groups and controls exemplify the extremely mixed results of studies employing QST in ASD populations to date. Studies in both children (O'Riordan & Passetti, 2006) and adults (Cascio et al., 2008; Fründt et al., 2017) have found comparable mechanical detection thresholds between ASD groups and controls, suggesting normal Aβ fiber function in ASD. However, in their study employing the entire standardized QST protocol of the German Research Network on Neuropathic Pain (Deutscher Forschungsverbund Neuropathischer Schmerz, DFNS), Vaughan et al. (2019) found that a group of 13 ASD adults had significantly higher mechanical detection thresholds (i.e., hyposensitivity) compared to controls. Further, the ASD group mean value fell outside of the normal distribution of healthy individuals established by the DFNS, suggesting some clinical significance of this hyposensitivity. In contrast, Riquelme et al. (2016) observed decreased mechanical detection thresholds (i.e., hypersensitivity) in children with ASD (n = 27) specifically in the C tactile (CT) afferent-innervated areas of the face and hand dorsum as compared to the palm.

CT afferents have been a particular topic of interest in studies on sensory sensitivity in ASD, especially those employing QST. These are unmyelinated, low-threshold mechanoreceptive units that have been found in peripheral nerves from many skin areas (Olausson et al., 2002). As previously mentioned, they are present in human hairy skin, such as

that in the face and hand dorsum, but not in glabrous (hairless) skin such as that on the palm. In two patients lacking Aβ afferents that are normally responsible for detection of innocuous tactile stimuli, soft brush stroking was still able to be faintly detected on the forearm where CT afferents are abundant, while this same sensation was not detected on the glabrous skin of the palm (Olausson et al., 2002, 2008; Cole, 2006). Further, these patients were unable to detect the application of vibrotactile stimuli at both locations, which have been shown to elicit poor responses in CT afferents but strong activation of Aβ afferents as demonstrated by single-fiber recordings from cats and monkeys, in which CT afferents were first discovered (Iggo, 1960; Bessou et al., 1971, Kumazawa & Perl, 1977). These observations suggest a role for CT afferents in detecting stroking, pleasant touch that may be relevant in social situations. In fact, considering these findings, Olausson et al. (2010) posited what they named their "social touch hypothesis" that "CT afferents have a particular potential to elicit pleasant subjective experience alongside behavioral, hormonal, and autonomic responses during gentle touch between individuals" (p. 185).

Interestingly, children and adolescents with ASD have exhibited reduced activity in the insula and other brain regions involved in social-emotional processing in response to continuous brushing on the forearm (CT afferent-innervated) compared to neurotypical controls, suggesting alterations in this social touch pathway (Kaiser et al., 2015). The findings of Riquelme et al. (2016) in their psychophysical study further support those of Kaiser et al. (2015); however, in contradiction to the CT afferent hypothesis, Cascio et al. (2008) found that hedonic (i.e., pleasantness) ratings of various textured stimuli, including a coarse burlap fabric, plastic mesh, and a soft cosmetic brush that were stroked across the skin did not differ significantly between forearm and palm test sites in a group of ASD adults. The paucity of studies on this pathway in

ASD and the contradictory evidence presented thus far indicates the need for further research, particularly that examines responses to QST parameters both in CT afferent-innervated and non-innervated areas, to solidify the role of CT afferents in altered sensory sensitivity in ASD.

Differing responses to vibration, which is generally thought to be mediated by Aβ fibers much like light touch, have also been observed specifically in CT afferent-innervated areas, with ASD adults exhibiting significantly lower vibration detection thresholds (i.e., hypersensitivity) in the forearm compared to controls, but not in the palm (Cascio et al., 2008). While the finding that ASD participants displayed greater vibrotactile sensitivity where CT afferents are present is fascinating, Cascio et al. (2008) acknowledged that CT afferents are recognized to be particularly poor at responding to vibrotactile stimuli, and that the Aβ fibers that innervate the forearm also make their own contributions to discriminative sensitivity, so the role of CT afferents in this differential response remains unclear. They also highlighted the interesting point that while this site-specific difference in vibrotactile sensitivity was observed, ASD adults did not demonstrate differences compared to controls in mechanical detection, which is also Aβ-mediated, at either test site, and they attempted to reconcile this discrepancy with the explanation that vibrotactile stimuli specifically activate rapidly-adapting mechanoreceptive afferents, while light touch stimuli do not. Further, an earlier study by Blakemore et al. (2006) found greater vibrotactile sensitivity in adults with ASD in response to high-frequency (200 Hz) stimulation, but not low-frequency (30 Hz) stimulation. They chose these two frequencies because they are known to stimulate different mechanoreceptors, with high-frequency vibration stimulating Pacinian corpuscles and activating type II rapidly-adapting afferents, and low-frequency vibration stimulating Meissner corpuscles and activating type I rapidly-adapting afferents (Weisenberger, 2005). In contrast, many studies have found no significant differences between ASD groups and

controls regarding vibration detection thresholds, further supporting the concept of normal Aβ fiber function in ASD and suggesting that effects seen in other studies may be due to higher-order processes rather than fiber dysfunction (Güçlü et al., 2007; Fründt et al., 2017; Vaughan et al, 2019).

Beyond mechanical and vibration detection, mechanical pain thresholds have scarcely been investigated in ASD. The two recent studies by Fründt et al. (2017) and Vaughan et al. (2019) employing the entire standardized DFNS protocol measured mechanical pain threshold values, which represent small, unmyelinated Aδ fiber function. Fründt et al. (2017) found no significant difference in mechanical pain thresholds between 13 ASD adults and 13 matched healthy controls. Vaughan et al. (2019), on the other hand, found that ASD adults exhibited significantly increased mechanical pain thresholds (i.e., hyposensitivity) compared to controls. However, mean threshold values for both groups fell within the normative range established by the DFNS, indicating that differences were not clinically significant.

While the findings from these two particular studies regarding mechanical detection thresholds and mechanical pain thresholds in ASD adults were clearly divergent, Fründt et al. (2017) and Vaughan et al. (2019) both curiously observed dynamic mechanical allodynia (DMA) in two ASD participants, a phenomenon that was not present in controls. In DMA, participants perceive innocuous, moving touch stimuli, such as gentle stroking, as painful. DMA has been widely suggested to be mediated by low-threshold Aβ fibers (Landerholm & Hansson, 2011; Jensen & Finnerup, 2014); however, some have attributed DMA to alterations in central processes rather than fiber dysfunction (Baron & Sauger, 1995), or even to dysfunction in the previously mentioned CT afferents (Liljencrantz et al., 2013). Given that Fründt et al. (2017) and Vaughan et al. (2019) did not examine differences between ASD groups and controls regarding

other Aβ fiber-mediated processes (i.e., mechanical detection, vibration detection), they suggested that DMA could likely be ascribed to central processes in ASD, or possibly by CT fiber dysfunction, but that further research is needed to pinpoint a mechanism for DMA in ASD.

Further, while these two groups also observed no significant differences in thermal detection or thermal pain thresholds between ASD groups and controls, previous studies demonstrated varying results across different age groups for these QST parameters. Cascio et al. (2008) found that ASD adults did not differ significantly in warm detection thresholds or cool detection thresholds compared to controls in both palm and forearm sites, but they demonstrated increased sensitivity to heat and cold pain. Another study on 83 ASD individuals and 59 controls aged 7-54 years also found comparable warm detection thresholds and cool detection thresholds between groups (Williams et al., 2019). However, significant relationships between IQ and thermal detection thresholds were observed, with lower performance IQ scores predicting higher detection thresholds (i.e., hyposensitivity). In contrast, Duerden et al. (2015) found that adolescents with ASD differed significantly from controls in the way that they were hyposensitive to both warm and cool, while they demonstrated normal thermal pain thresholds. In concurrence with Williams et al. (2019), however, they also observed significant relationships between IQ and thermal detection thresholds.

One study that focused specifically on heat pain thresholds in ASD adults is particularly worth noting, as no differences in heat pain thresholds were observed between ASD adults and controls when a method of limits protocol (i.e., one in which the magnitude of the stimulus was gradually increased) was used; however, in suprathreshold heat pain tasks in which participants rated their pain using a visual analogue scale (VAS) (No Pain, Worst Pain anchors) when (1) 40, 42, 44, 45, 46, 47, and 48°C stimuli were presented for 5 seconds each across 5 trials in a

pseudorandom order, and (2) alternating low (42℃) and high (46℃) temperatures were applied for 21 seconds each across 6 trials, ASD participants exhibited higher mean pain ratings (Failla et al., 2020). Failla et al. (2020) noted that previous studies that found no differences in heat pain thresholds in ASD did not employ reaction time-independent methods for measuring these thresholds and argued that the time that it takes for the individual to process the stimulus, decide that it is painful, and then execute the appropriate response could all be slowed in ASD and confound results determined using the method of limits. Specifically, individuals with ASD have been observed to have significantly larger variabilities in reaction time compared to controls in multiple studies, especially in cases of ADHD comorbidity (Geurts et al., 2008; Karalunas et al., 2014; Hwang-Gu et al., 2018; Karalunas et al., 2018). The contrasting results between reaction time-dependent and reaction time-independent methods in this study highlight the importance of taking effects of reaction time into account when interpreting results and crafting methods to minimize these effects when possible.

Beyond reaction time-independent methods, there are additional methods and devices designed to test various aspects of pain perception, including sensory integration stages of pain processing, that have been used in few, if any, studies on sensory sensitivity in ASD to date. One of these devices is the thermal grill device, which consists of adjacent metal bars that are controlled to alternating innocuous warm and cool temperatures; the simultaneous warm and cool stimulation produces an illusion of a surface that feels both much hotter than the temperature of the warm bars and uniformly hot, and thus the presentation of this stimulus has the potential to reveal alterations in sensory integration pathways. When a cool stimulus alone is presented, both Aδ fibers that respond to cool temperatures (A-cool fibers) and C fibers that respond to noxious cold temperatures but are partially activated at cool temperatures (C-cold

fibers) are activated (Prescott et al., 2014). A-cool fiber pathways inhibit C-cold fiber pathways and prevent cool temperatures from being perceived as painful. In the thermal grill illusion (TGI), however, the interleaving of warm and cool stimuli causes A-cool fiber pathways to be inhibited in the spinal cord by warm-sensitive fiber input, leading to disinhibition of the C-cold fiber pathway and the unmasking of a burning sensation (Craig & Bushnell, 1994). In the ASD population in general, sensory processing differences have yet to be assessed using this device, although it may be useful in identifying alterations in pathways involving integration of multiple sensory stimuli.

Further, of the few studies on QST in ASD to date, only one (Duerden et al., 2015) focused specifically on sensory sensitivity in adolescents. Sensory sensitivity has been shown to change with age both in ASD and in the general population (Kern et al., 2006; Blankenburg et al., 2010). Adolescence in particular can present new sensory challenges for individuals with ASD that make participation in everyday activities difficult. For example, Humphrey & Lewis (2008) reported anxiety in a group of adolescents with ASD in relation to moving through school hallways full of people pushing into each other. Issues with auditory filtering, sensory underresponsivity, and sensory seeking in adolescents with ASD have also been shown to interfere with academic performance and social interaction in school settings (Ashburner et al., 2008; Hilton et al., 2010). More generally, adolescence marks a critical time period during which individuals with ASD prepare for the transition to adulthood, when the availability of services and supports changes drastically and they might be pursuing further education, employment, or even living independently for the first time. As adolescents with ASD and their families prepare for this major transition that likely will involve encountering many new environments that present potentially overwhelming sensory stimuli, it is crucial for sensory sensitivities to be

characterized in detail and treated when necessary to minimize the negative impact that these sensitivities may have on daily life.

Even among the more commonly studied child and adult populations, the existing literature is evidently inconsistent regarding QST responses in ASD populations compared to controls across a number of parameters. Further, in the one study that did specifically focus on the adolescent population, only sensitivity to thermal stimuli were tested, while touch, pressure, and auditory sensitivity, for example, were not assessed (Duerden et al., 2015). It has been acknowledged that sensory sensitivities may differ across modalities in ASD, and it is critical to assess these sensitivities individually as they may make their own contributions to behavior and quality of life (Cascio et al., 2008).

The primary objective of the present study was to collect preliminary data regarding differences in response to various QST parameters between adolescents with ASD and neurotypical controls to better understand how ASD affects individuals' sensitivity to both noxious and innocuous stimuli, including thermal, pressure, mechanical, and auditory stimuli. A secondary objective included utilizing devices and methods of sensory sensitivity assessment that, to our knowledge, have not been tested in ASD populations to date, including the thermal grill device and a hedonic scale used to rate the pleasantness/unpleasantness of stimuli covering a wide range of modalities, including thermal, tactile, and auditory. Ultimately, an overarching motivation for testing such objectives lies in the hope that results from these types of experiments may inform creation of more targeted diagnostic and treatment tools for this pervasive symptom of ASD.

Hypotheses

Compared to controls, ASD participants will demonstrate: (1) hypersensitivity to thermal, mechanical, and pressure pain stimuli, indicating altered Aδ and C nociceptor function or possibly heightened central processing mechanisms; (2) hypersensitivity to dynamic tactile stimuli, indicating altered CT afferent function that may be related to aversion to social touch in ASD; (3) similar thresholds for thermal and mechanical detection, indicating typical A-cool, C-warm, and Aβ fiber function; (4) higher pain ratings for suprathreshold heat stimuli and the thermal grill illusion; (5) higher loudness ratings for auditory stimuli; and (6) more extreme hedonic ratings of stimuli.

Methods

Participants

All study procedures were approved by the Emory Institutional Review Board. Adolescent participants (ages 12-17) with ASD were recruited from the greater Atlanta community via paper flyers and social media advertisements, particularly in local autism parent support groups. Parents of the adolescent participants provided written informed consent prior to study procedures beginning, and all participants provided written informed assent as well. When requested, participants were shown a social story that included images of the various QST devices during the informed consent process to better familiarize them with and prepare them for the study procedures.

This study included a total of 10 adolescents with ASD and 1 neurotypical control participant. The mean age for the ASD group was 14.70 years (range = 13-17 years), and the group included 5 females and 5 males (**Table 1**). The neurotypical control participant was a 15-

year-old female. Potential study participants who lacked verbal communication abilities that would allow them to indicate pain ratings or who had a verbal IQ below 65 were excluded from this study. Participants with comorbid psychiatric conditions (e.g., generalized anxiety disorder, major depressive disorder, attention-deficit/hyperactivity disorder) were not excluded. Informed consent procedures and assessment of eligibility took place during an initial virtual screening visit.

Screening Measures

During a one-hour screening visit conducted via Zoom (Zoom Video Communications, Inc., San Jose, CA, USA), potential participants were screened for verbal IQ ≥ 65 and ASD diagnosis. The Differential Ability Scales, Second Edition (DAS-II) Verbal Ability Subtests (Word Definitions and Verbal Similarities) were used to assess verbal IQ. A 20-minute clinical interview was conducted to initially screen for ASD symptoms. This interview consisted of questions about current relationships, social behaviors and cognition, academic experiences, repetitive behaviors, and sensory processing. If potential participants passed the screening visit, they were then invited for a 3-5 hour in-person lab visit to complete QST procedures. All 10 ASD participants and the 1 neurotypical control who completed the Zoom visit passed the screening.

ASD Diagnosis Confirmation

During the second in-person lab visit, additional questionnaires were administered to confirm ASD diagnosis. The Social Communication Questionnaire (SCQ; Rutter et al., 2003), a first-level screening measure for ASD based on the Autism Diagnostic Interview-Revised (ADI-

R; Lord et al., 1994), was used as an initial assessment of ASD symptoms in participants. It is a 40-item parent report consisting of yes/no questions about autistic behavior both currently and at the age of 4 to 5 years, and it has shown strong discrimination between ASD and non-ASD cases (Chandler et al., 2007). Rutter et al. (2003) suggested a cutoff score of 15 to indicate a high likelihood of ASD and the need for the child to receive comprehensive ASD evaluation. However, several studies have suggested that optimal cutoff scores may vary based on a child's age and other characteristics (e.g., ASD symptom severity), so these optimal cutoff scores may be closer to 11-12 in some cases (Allen et al., 2007; Corsello et al., 2007; Schanding et al., 2012).

The Social Responsiveness Scale, Second Edition (SRS-2; Constantino & Gruber, 2012) was an additional measure used to assess ASD symptoms in greater detail. This measure is another parent report that includes 65 questions about the ability of the individual to engage in reciprocal social interactions, deficits in communication, and restricted/stereotypic behaviors or interests. Total raw scores of 52 and above in girls and 58 and above in boys indicate clinically significant deficits in reciprocal social behavior that may be associated with an ASD diagnosis. This assessment also includes specific subscale scores for social motivation, social awareness, social cognition, social communication, and restricted interests and repetitive behavior. The SRS-2 is a valid quantitative measure of autistic traits and is useful in clinical settings and large-scale research studies (Constantino et al., 2003).

The Childhood Autism Rating Scale, Second Edition (CARS-2; Schopler et al., 2010) was used as a final measure to assess ASD symptoms. Participant behavior was observed in the settings of both the Zoom screening visit, particularly during the interview portion, and the lab visit and was subsequently rated on 15 items from 1 (age-appropriate behavior) to 4 (severely

autistic behavior). 9 ASD participants were scored using the CARS-2 High-Functioning Version, which is used when an individual's overall IQ is ≥ 80, while 1 ASD participant was scored using the CARS-2 Standard Version. Scores of 28 and above in the High-Functioning Version and 30 and above in the Standard Version indicate clinically significant symptoms of ASD. When necessary, responses from the CARS-2 Questionnaire for Parents or Caregivers were used to assist in determining scores for certain items.

Sensory Sensitivity Self-Report Questionnaire

The Adolescent/Adult Sensory Profile (AASP; Brown & Dunn, 2002a) was used to assess sensory sensitivity in adolescents through the lens of a self-report questionnaire. This questionnaire consists of 60 items describing behaviors related to taste/smell, movement, visual, touch, and auditory processing, as well as activity level. Each item specifically represents one of four quadrants: low registration, sensation seeking, sensory sensitivity, or sensation avoiding. Participants rated how often they performed each behavior from 1 (almost never) to 5 (almost always), and sums of scores out of 75 total possible points in each quadrant were calculated to determine whether participants exhibited the described sensory response much less than, less than, similar to, more than, or much more than most people, with specific cutoff scores varying slightly depending on the quadrant. Adults with ASD have been shown to score significantly higher than neurotypical controls on low registration, sensory sensitivity, and sensation avoidance quadrants, but significantly lower on the sensation seeking quadrant (Crane et al., 2009). Mean scores for the ASD group and neurotypical control participant for all questionnaires administered in the present study are listed in **Table 1**, along with additional demographic information.

Characteristic	ASD Participants (*n* = 10)	NT Controls (*n* = 1)
Age	14.70 ± 1.70	15
Gender		
Male	5 (50%)	0 (0%)
Female	5 (50%)	1 (100%)
Comorbidities		
Anxiety	3 (30%)	0 (0%)
Depression	2 (20%)	0 (0%)
ADHD	3 (30%)	0 (0%)
OCD	3 (30%)	0 (0%)
Dyslexia	1 (10%)	0 (0%)
Verbal IQ	113.50 ± 19.42	109
CARS-2	35.50 ± 5.34	15
SCQ	15.10 ± 6.31	0
SRS-2	81.50 ± 22.02	5
AASP		
Low Registration	34.67 ± 9.89	23
Sensation Seeking	40.78 ± 8.24	50
Sensory Sensitivity	39.56 ± 12.89	26
Sensation Avoiding	37.33 ± 12.07	25

Table 1: Participant demographics and questionnaire scores. Values for all continuous variables are represented as mean ± standard deviation. ASD screening measures included the Childhood Autism Rating Scale, Second Edition (CARS-2), the Social Communication Questionnaire (SCQ), and the Social Responsiveness Scale, Second Edition (SRS-2). The ASD group mean CARS-2 score (35.50 ± 5.34) indicates that on average, ASD participants exhibited severe symptoms of ASD, while the NT control score (15) indicates minimal-to-no symptoms of ASD. The Adolescent/Adult Sensory Profile (AASP) subscale scores indicate that the ASD group exhibits low registration, sensory sensitivity, and sensory avoiding behaviors at a similar frequency to that of most people. However, they exhibit sensation seeking behaviors less than most people.

QST Procedures

The majority of QST procedures performed were adopted from the protocol of the German Research Network on Neuropathic Pain (DFNS) (Rolke et al., 2006). These include measurement of cool detection thresholds (CDT), warm detection thresholds (WDT), cold pain thresholds (CPT), heat pain thresholds (HPT), pressure pain thresholds (PPT), mechanical detection thresholds (MDT), mechanical pain thresholds (MPT), mechanical pain sensitivity (MPS), and dynamic mechanical allodynia (DMA). This standardized protocol has published reference values and normal distributions available for healthy, neurotypical adults (Rolke et al., 2006), adolescents, and children (Blankenburg et al., 2010), which allow for comparisons to be

made between these values and those measured in various clinical populations. All of the testing procedures in the present study were designed to mimic those outlined in the reference article for children and adolescents (Blankenburg et al., 2010) as closely as possible.

To further characterize sensory sensitivity in ASD, additional tests that are not part of the standardized DFNS protocol and have yet to be tested in adolescents with ASD were performed. These include tests for auditory sensitivity, suprathreshold heat pain, the thermal grill illusion (TGI), and a test of response to various stimuli (e.g., thermal, tactile, auditory) using a hedonic rating scale. Because these tests do not have reference values and normal distributions readily available, analyses regarding the results of these tests were exploratory in nature.

All QST procedures are described below in the order in which they were performed during the in-person lab visit. Summary tables of both standardized tests from the DFNS protocol (**Table 2**) and exploratory measures (**Table 3**) and the sensory processing pathways assessed in each are also included at the end of this section.

Cool Detection Thresholds (CDT) and Warm Detection Thresholds (WDT)

All tests involving thermal stimuli were designed and run using LabVIEW 2019 (National Instruments, Austin, TX, USA). Thermal stimuli were delivered via a 9.0 cm^2 thermode (TSA II, Medoc, Israel), with one experimenter holding the thermode to the skin at the test site. In light of the findings from Failla et al. (2020), thermal detection thresholds were assessed using both reaction time-dependent methods outlined in the standardized DFNS protocol and reaction time-independent methods. First, participants were asked to complete a two-alternative forced-choice task in which pairs of 5-second intervals (A and B) occurred: one during which a 3-second cool or warm stimulus was presented on the volar forearm of the

nondominant side, and the other during which the thermode did not change temperature from baseline (32°C). Participants were tasked with choosing the interval during which they felt the stimulus using a keypad labeled A and B. This test was performed using a three-down, one-up staircase procedure, such that when a participant correctly identified the stimulus interval for three (not necessarily consecutive) trials, the following stimulus would decrease in amplitude (i.e. the task difficulty was increased). On the other hand, each time a participant provided an incorrect answer, the following stimulus would increase in amplitude, making the task easier. The step value was 0.4°C for the first ten trials and 0.2°C for the last ten trials, and thresholds were calculated as the mean temperature for the last four trials. Given the previously observed increased variability in reaction time in ASD populations and the acceptance of the forced-choice method as being free of response bias, this method was an important one to employ in addition to the reaction time-dependent methods employed by the DFNS (Bertelsmann et al., 1985; Geurts et al., 2008; Karalunas et al., 2014; Hwang-Gu et al., 2018; Karalunas et al., 2018).

Next, cool and warm detection thresholds were assessed on the nondominant hand dorsum using a method of limits protocol. The thermode was set to a baseline temperature of 32°C and was slowly cooled or warmed at a rate of 1°C/s. Participants were instructed to press an arrow button on a keypad as soon as they perceived a change in temperature either to cooler or warmer for the first time, at which point the temperature was recorded and the thermode quickly returned to the baseline temperature. For each test, three consecutive threshold measurements were recorded, with a 10 s inter-stimulus interval passing between each stimulus presentation. The three measurements were averaged to determine final cool and warm detection thresholds. The cool and warm detection thresholds of one participant who pressed the button when the

thermode had not yet changed temperature from baseline over multiple trials were excluded from analysis.

Cold Pain Thresholds (CPT) and Heat Pain Thresholds (HPT)

Assessment of cold pain and heat pain thresholds followed the same method of limits protocol as that used for assessment of thermal detection thresholds, with the thermode being held by an experimenter to the nondominant hand dorsum of participants at a baseline temperature of 32°C. The thermode was then slowly cooled or warmed at a rate of 1°C/s; however, during this test, participants were instructed to press the arrow button as soon as they perceived the cooling or warming of the thermode to be *painful*, rather than simply perceiving a change in temperature, at which point the temperature was recorded and the thermode quickly returned to baseline temperature. Again, three consecutive threshold measurements were recorded, with a 10 s inter-stimulus interval passing between each stimulus presentation. The three measurements were averaged to determine final cold pain and heat pain thresholds.

Pressure Pain Thresholds (PPT)

A handheld pressure algometer (Somedic Sales, Hörby, Sweden) with a contact area of 1 cm^2 was placed above the adductor pollicis muscle of the nondominant hand. Participants held a "stop" button and were instructed to press the button as soon as the pressure stimulus became painful. One experimenter gradually increased the intensity of the applied pressure at a rate of approximately 0.5 kg/s (corresponding to 50 kPa/s) until the participant indicated pain, at which point a second experimenter recorded the pressure pain threshold (measured in kPa). This test

was repeated three times, and final pressure pain thresholds were calculated as the geometric mean of the three threshold measurements.

Mechanical Detection Thresholds (MDT)

A set of 12 small plastic filaments, modified von Frey hairs (Optihair2-Set, Marstock Nervtest, Germany), with fixed intensities of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32, 64, 128, 256, and 512 mN were used to determine mechanical detection thresholds with the method of limits Participants were instructed to close their eyes and say "yes" any time they perceived the sensation of light touch. Starting with 16.0 mN, these von Frey hairs were bent on the skin of the nondominant hand dorsum site for 1 s (contact area of 0.5 mm in diameter) and applied in a descending order until participants could no longer perceive the sensation of light touch. At this time, a descending threshold was recorded, and the application of a series of ascending stimuli began until participants first perceived the sensation of touch again, at which point an ascending threshold was recorded. Five series of descending and ascending stimuli were administered, and final mechanical detection thresholds were calculated as the geometric mean of these series. Mechanical detection thresholds of the first two study participants were excluded from analysis because they were assessed using a separate set of von Frey hairs that is similar to, but not exactly the same as, the set used by the reference group, and thus the intensity values were not compatible for mean calculation and further statistical analysis.

Mechanical Pain Thresholds (MPT)

A set of seven weighted pinpricks (MRC Systems, Heidelberg, Germany) with fixed intensities of 8, 16, 32, 64, 128, 256, and 512 mN were used to determine mechanical pain

thresholds with the method of limits at the nondominant hand dorsum site. Participants were instructed to say “blunt” when they felt only the perception of non-painful touch, and then to say “sharp” as soon as they perceived a stimulus presented to be painful. Ascending stimuli (duration of 2 s) were presented until participants first reported the sensation of sharpness, at which point an ascending threshold was recorded. Then, the application of a series of descending stimuli began until participants reported the sensation of bluntness, at which point a descending threshold was recorded. Five series of ascending and descending stimuli were administered, and final mechanical pain thresholds were calculated as the geometric mean of these series.

Auditory Sensitivity

Auditory sensitivity was tested using an MA41 audiometer (MAICO Diagnostics, Eden Prairie, MN, USA). First, participants completed a hearing screening in which 25 dB auditory stimuli of 1000 Hz, 2000 Hz, and 4000 Hz were administered to each ear to ensure proper hearing ability.

At this point in the series of tests, participants were introduced to the process of using a numeric rating scale (NRS) to rate loudness, and they were told that they would use similar scales in following tests as well (e.g., to rate pain and pleasantness/unpleasantness of stimuli). To allow participants to become familiar with using the NRS, a brief mock run of 3-4 2000 Hz auditory stimuli in the 40-60 dB range was administered, and participants were asked to rate the loudness of each stimulus on a scale of 0-100, with 0 meaning no sound and 100 meaning the loudest sound imaginable. They were given the opportunity to ask any clarifying questions about using the NRS to rate their sensory experience.

Once participants were familiar with the NRS, 2000 Hz auditory stimuli between 40-90 dB were administered binaurally for 3 s for the auditory testing. Participants rated the loudness of each stimulus using the NRS. Testing began with an ascending series in which the stimulus intensity increased in 10 dB increments for each trial, and two randomized series immediately followed. Mean loudness ratings were calculated for each individual sound intensity.

Stimulus/Response Functions–Mechanical Pain Sensitivity (MPS) and Dynamic Mechanical Allodynia (DMA)

To further assess mechanical pain sensitivity, each of the seven weighted pinprick stimuli was presented again in a pseudorandomized sequence over three trials. Interleaved in these pinprick stimuli were presentations of three moving tactile stimuli (brush, cotton wisp, and Q-tip) for the purpose of identifying dynamic mechanical allodynia (DMA). Each of these stimuli was stroked across the skin over ~2 cm. Upon the presentation of each stimulus, participants were instructed to provide a pain rating using another NRS, with 0 meaning no pain and 100 meaning the most intense pain imaginable. Mechanical pain sensitivity was calculated as the geometric mean of numerical ratings for all of the pinprick stimuli, while dynamic mechanical allodynia was calculated as the geometric mean of numerical ratings across all three dynamic tactile stimuli. This test was performed twice: once at the nondominant hand dorsum site, and once at the nondominant thenar eminence (palm) site, so that potential differences in response to gentle, stroking stimuli at CT afferent-innervated and non-innervated areas could be examined. For the thenar eminence site, one participant rated the first two stimuli but stated that his palm was especially sensitive, so he asked us not to continue the testing. Data from this participant were not included for any analysis specifically regarding this test site.

Suprathreshold Heat Pain

To further assess sensitivity to heat pain, brief (5 s) heat stimuli between 43-47℃ were delivered to the nondominant hand dorsum site using the thermode. Beginning at baseline temperature (32℃) for each trial, the thermode temperature was rapidly raised (~10℃/s) to the target temperature, and after the 5 s noxious stimulus, it was rapidly cooled back to the baseline temperature, with a 15 s inter-stimulus interval passing before the presentation of the next stimulus. First, stimuli were presented in ascending order in 1℃ increments, and participants were instructed to provide a pain rating using the same NRS previously used, with 0 meaning no pain and 100 meaning the most intense pain imaginable. Subsequently, three randomized series of these stimulus levels were presented, subject to the constraint that no temperature was presented twice in succession. Mean pain ratings given throughout the three randomized trials were calculated for each individual temperature.

Thermal Grill Illusion (TGI)

Participants were instructed to place their forearm on a thermal grill device and hold it in place for 10 seconds to test for alterations in sensory integration. The device consists of six adjacent metal bars, of which the even-numbered bars were controlled to an innocuous warm temperature (41℃), and the odd-numbered bars were controlled to an innocuous cool temperature (18℃).

After placing their forearm on the device for 10 seconds, participants were instructed to provide a pain rating using the same NRS previously used, with 0 meaning no pain and 100 meaning the most intense pain imaginable. Then, participants were presented with a list of nine characteristics, of which they could choose as many as they wanted to describe the sensation they

experienced while their forearm was placed on the bars. These characteristics included neutral, burning, cool, stinging, hot, cold, sharp, warm, and aching. Data from only eight participants was collected for this parameter due to technical difficulties with the thermal grill device during two of the in-person lab visits.

Hedonics Testing

While some sensations might not have necessarily been painful, they might have been pleasant or unpleasant to the participants. To assess this specific aspect of somatosensation, participants were instructed to provide a single numerical response to rate the pleasantness/unpleasantness of a number of stimuli using a new NRS, with -100 meaning the most unpleasant sensation imaginable, 0 meaning neutral, and +100 meaning the most pleasant sensation imaginable. Stimuli presented during this test included innocuous cool (22°C), innocuous warm (38°C), noxious cold (10°C), noxious heat (45°C), innocuous touch (16 mN von Frey hair), stroking touch (brush), noxious pressure (256 mN pinprick), auditory (70 dB), and the thermal grill illusion. This test was conducted over three trials, with each stimulus being presented once during each trial. This test was performed on the volar forearm of the nondominant side, and each trial took place at a different site on the forearm (i.e., upper, middle, or lower) to minimize sensitization to the stimuli presented. Mean pleasantness/unpleasantness ratings given over the three trials were calculated for each stimulus type.

Test Name	*Description*	*Test Site*(s)	*Main Peripheral Sensory Fiber(s)*
Cool Detection Thresholds (CDT)	Thermode slowly cooled from baseline (32°C) until participant pressed button to indicate sensation of cool	Hand dorsum	Aδ
Warm Detection Thresholds (WDT)	Thermode slowly warmed from baseline (32°C) until participant pressed button to indicate sensation of warm	Hand dorsum	C
Cold Pain Thresholds (CPT)	Thermode slowly cooled from baseline (32°C) until participant pressed button to indicate sensation of cold pain	Hand dorsum	C, Aδ nociceptors
Heat Pain Thresholds (HPT)	Thermode slowly warmed from baseline (32°C) until participant pressed button to indicate sensation of heat pain	Hand dorsum	C, Aδ nociceptors
Pressure Pain Thresholds (PPT)	Experimenter gradually increased pressure intensity applied using handheld algometer until participant pressed button to indicate sensation of pressure pain	Adductor pollicis muscle	C, Aδ nociceptors
Mechanical Detection Thresholds (MDT)	von Frey hairs (plastic filaments) of decreasing intensity applied until participant did not respond, then hairs of increasing intensity applied until participant responded "yes"	Hand dorsum	Aβ
Mechanical Pain Thresholds (MPT)	Weighted pinpricks of increasing intensity applied until participant responded "sharp" to indicate painful sensation, then pinpricks of decreasing intensity applied until participant responded "blunt" to indicate nonpainful touch sensation	Hand dorsum	C, Aδ nociceptors
Mechanical Pain Sensitivity (MPS)	Weighted pinpricks applied in pseudorandom order, participant rated pain for each stimulus using pain NRS (0 = no pain, 100 = most intense pain imaginable)	Thenar eminence, hand dorsum	C, Aδ nociceptors
Dynamic Mechanical Allodynia (DMA)	Dynamic tactile stimuli (brush, cotton wisp, and Q-tip) stroked over skin, participant rated pain for each stimulus using pain NRS (0 = no pain, 100 = most intense pain imaginable)	Thenar eminence, hand dorsum	CT, Aβ

Table 2: Summary of QST procedures adopted from the German Research Network on Neuropathic Pain (DFNS) and the peripheral sensory fiber function that they assess.

Test Name	*Description*	*Test Site*	*Sensory Processing Mechanism*
Auditory Sensitivity	Participant rated auditory stimuli (40-90 dB) using loudness NRS (0 = no sound, 100 = loudest sound imaginable)	Ears (binaural)	Cochlear afferent fibers
Suprathreshold Heat Pain	Suprathreshold heat pain stimuli (43-47°C) presented in pseudorandom order, participant rated pain for each stimulus using pain NRS (0 = no pain, 100 = most intense pain imaginable)	Hand dorsum	C, Aδ nociceptors
Thermal Grill Illusion (TGI)	Participant placed forearm on thermal grill device, rated pain using pain NRS (0 = no pain, 100 = most intense pain imaginable), and was given opportunity to choose adjectives to describe sensation	Volar forearm	Sensory integration (C-warm, A-cool fibers)
Hedonics Testing	Participant rated innocuous cool/warm, noxious cold/heat, innocuous touch (von Frey hair), stroking touch (brush), noxious pressure (pinprick), auditory, and thermal grill stimuli using hedonic NRS (-100 = most unpleasant sensation imaginable, 0 = neutral, +100 = most pleasant sensation imaginable)	Volar forearm (upper, middle, and lower sites)	See sensory processing mechanisms for individual stimulus types above

Table 3: Summary of exploratory QST procedures and their associated sensory processing mechanisms.

Data Analysis

For the core QST measures of cool detection thresholds, warm detection thresholds, cold pain thresholds, heat pain thresholds, pressure pain thresholds, mechanical detection thresholds, mechanical pain thresholds, mechanical pain sensitivity, and dynamic mechanical allodynia, the measured thresholds of ASD participants were compared to a set of published reference data for children and adolescents, which included reference values specific to body site, gender, and age group (Blankenburg et al., 2010). Differences of means between the ASD participants and the reference group (specifically the values measured at the hand dorsum site for 13-16-year-olds [n = 64]) were assessed.

Prior to statistical analysis, data were transformed following the guidance of the reference group to allow for comparisons to be made. For many of the QST parameters, raw reference data were not distributed normally, so data were log-transformed. In these cases, data in the present study were log-transformed accordingly. Following the methods of the reference group, cool detection thresholds were calculated as the difference from baseline temperature (mean of threshold measurements - 32℃), multiplied by -1, and then log-transformed. Warm detection thresholds were also calculated as the difference from baseline (mean of threshold measurements - 32℃) and then log-transformed. The geometric means calculated to determine pressure pain thresholds, mechanical detection thresholds, mechanical pain thresholds, and mechanical pain sensitivity were also log-transformed. Importantly, prior to geometric mean calculation and log transformation of ratings for mechanical pain sensitivity, a small constant (+0.1) was added to each rating to avoid loss of zero rating values. Cold pain and heat pain thresholds were distributed normally without log transformation and thus were reported as raw values. Following the appropriate transformation, each parameter was assessed for normality using the Shapiro-Wilk test. None of the variables exhibited a distribution that differed significantly from normal, thus unpaired t-tests were used to compare means for each QST parameter.

For exploratory purposes, Z-scores of individual ASD participants for all QST parameters were calculated to directly compare individual data to reference values. Z-scores were calculated by the following formula: $Z\text{-}score = (X_{ASD} - Mean_{Reference})/SD_{Reference}$.

Further, because the exploratory measures of auditory sensitivity testing, suprathreshold heat pain testing, the thermal grill illusion, and hedonics testing did not have published reference values that were readily available and the number of control participants (n = 1) was very clearly not adequate for any comparisons involving inferential statistics, data from these measures were

largely represented as mean ratings provided for given intensities in the ASD group alongside the rating provided by the one control participant. Data from the auditory sensitivity testing are the one exception to this pattern, however, as ratings were log transformed.

Simple mean and standard deviation calculations, log transformations, and Z-score calculations were performed using Excel (Microsoft Corporation, Redmond, WA, USA). Normality of each variable was assessed by the Shapiro-Wilk test run through SPSS Statistics v.27 (International Business Machines Corporation, Armonk, NY, USA). Unpaired t-tests were performed by inputting the means, standard deviations, and sample sizes of the ASD participant group and the reference group into the GraphPad online t-test calculator (GraphPad Software, San Diego, CA, USA). All figures were designed using SigmaPlot v.14.5 (Systat Software Inc., San Jose, CA, USA).

Results

Core QST Measures

Across multiple measures, pain thresholds were decreased in the ASD group compared to the reference group, indicating hypersensitivity specifically to noxious stimuli. On the other hand, thresholds for detection of innocuous stimuli were largely increased in the ASD group, indicating hyposensitivity to these measures. (See **Table 4** below for a summary of QST results.)

QST parameter	ASD raw data (n = 10)	ASD log-transformed data (n = 10)	Reference data (n = 64)	Significance	Gain/ loss of function
CDT^{log} (°C from baseline)*	-2.793 ± 1.543	0.371 ± 0.291	-0.003 ± 0.200	$p < 0.0001$	Loss
WDT^{log} (°C from baseline)*	3.070 ± 1.511	0.443 ± 0.204	0.178 ± 0.185	$p = 0.0002$	Loss
CPT (°C)	19.950 ± 9.035	-	18.090 ± 8.527	$p = 0.5265$	-
HPT (°C)	39.258 ± 3.790	-	42.365 ± 3.695	$p = 0.0161$	Gain
PPT^{log} (kPa)	295.106 ± 80.362	2.454 ± 0.129	2.726 ± 0.130	$p < 0.0001$	Gain
MDT^{log} (mN)	2.507 ± 2.392	0.263 ± 0.357	-0.556 ± 0.241	$p < 0.0001$	Loss
MPT^{log} (mN)	136.961 ± 134.206	1.903 ± 0.523	1.578 ± 0.285	$p = 0.0044$	Loss
MPS^{log} (NRS 0-100)	6.558 ± 12.227	0.244 ± 0.754	-0.451 ± 0.419	$p < 0.0001$	Gain

Table 4: Summary of QST results from parameters with published reference values available for adolescents ages 13-16. Raw and log transformed data for cold detection thresholds (CDT), warm detection thresholds (WDT), pressure pain thresholds (PPT), mechanical detection thresholds (MDT), mechanical pain thresholds (MPT), and mechanical pain sensitivity (MPS) are included. Gain of function indicates hypersensitivity to a given measure, while loss of function indicates hyposensitivity. (* n = 9)

Pressure Pain Thresholds

First, ASD participants exhibited decreased pressure pain thresholds (295.11 kPa ± 80.36) compared to the reference group ($p < 0.0001$, **Figure 1**), indicating hypersensitivity to pressure pain. Raw mean pressure pain thresholds of the reference group of 13-16-year-old adolescents amounted to 536 kPa, indicating a 45% decrease in the amount of pressure that needed to be applied for ASD participants in the present study to perceive the pressure stimulus as painful.

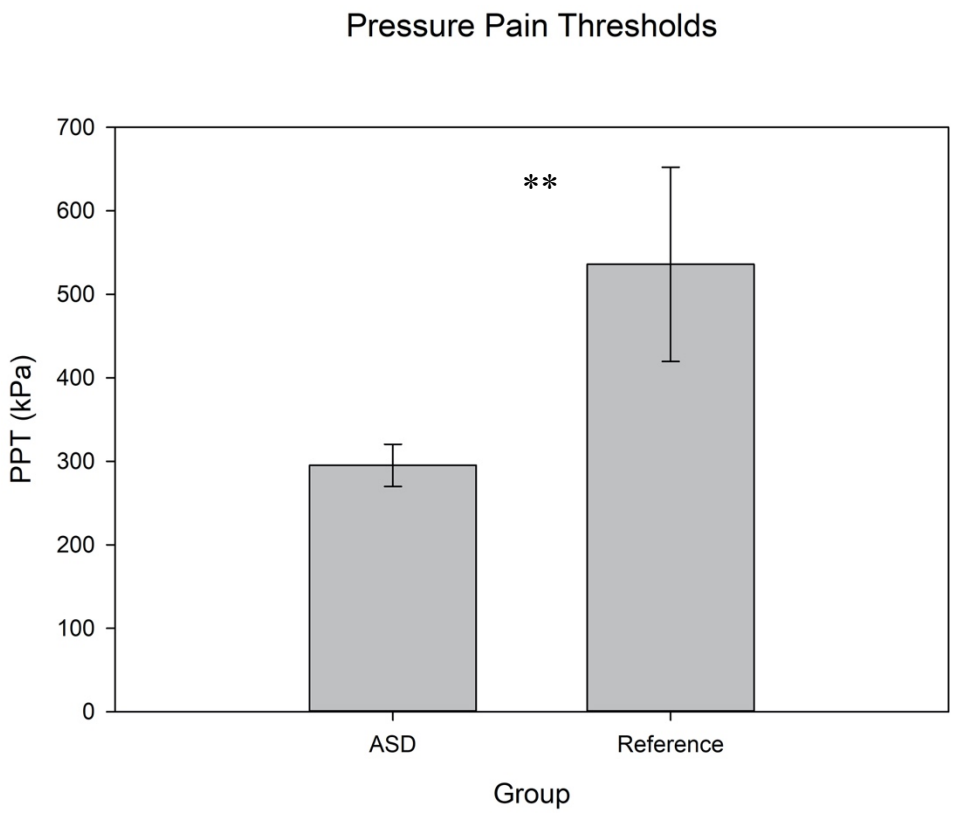

Figure 1: Mean pressure pain thresholds (kPa) of ASD (n = 10) and reference (n = 64) groups. Bars represent average geometric means of three threshold measurements that were calculated for individual participants in each group. Error bars represent standard error of the mean. Statistical significance (** $p < 0.01$) was determined using an unpaired t-test to assess differences between means of log-transformed data from each group, as the raw data were not normally distributed and thus violated the assumptions of the unpaired t-test. However, for clarity, raw geometric means are represented here.

Heat Pain and Cold Pain Thresholds

ASD participants also displayed decreased heat pain thresholds (39.26℃ ± 3.79) compared to the reference group (42.37℃ ± 3.70, p = 0.0161, **Figure 2A**), indicating hypersensitivity to a second noxious stimulus type. Heat pain thresholds ranging from 34.96-45.07℃ in the ASD group suggest differing sensitivity to this stimulus type within the group. For the individual whose heat pain threshold was 34.96℃, the thermode only had to change 2.96℃ from baseline (32℃) for the sensation of heat to be perceived as painful, and this case illustrates one example of extreme sensitivity to heat stimuli.

Regarding cold pain thresholds, however, ASD participants (19.95℃ ± 9.03) did not differ significantly from the reference group (18.09℃ ± 8.53, $p = 0.5265$, **Figure 2B**). As can be ascertained from the higher standard deviations, greater variability was observed both in the ASD group and the reference group in measurements of cold pain thresholds compared to heat pain thresholds, which may partially account for the lack of significance observed in this particular parameter.

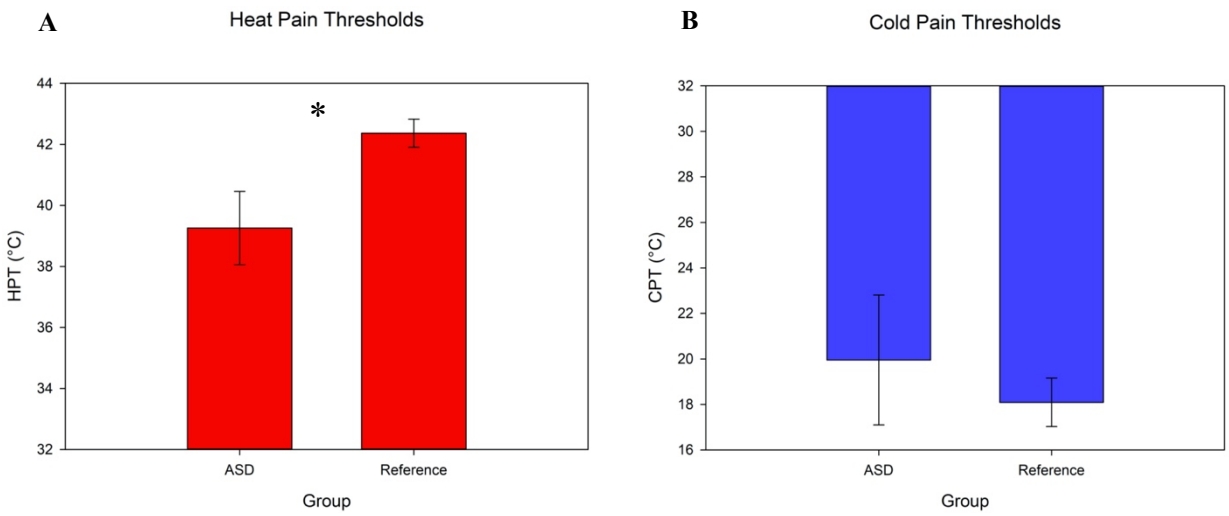

Figure 2: Mean thermal pain thresholds of ASD ($n = 10$) and reference ($n = 64$) groups. Bars represent mean thresholds for heat pain (A) and cold pain (B) thresholds as the difference from baseline temperature of the thermode (32℃). Error bars represent standard error of the mean. (* $p < 0.05$)

Mechanical Pain Sensitivity

In another parameter involving noxious stimuli, significantly increased mechanical pain sensitivity was observed in adolescents with ASD compared to the reference group ($p < 0.0001$, **Figure 3**). The increased mechanical pain sensitivity seen here falls in line with the hypersensitivity observed in response to pressure pain and heat pain and contributes to the

characterization of the adolescent ASD group as generally displaying hypersensitivity to noxious stimuli.

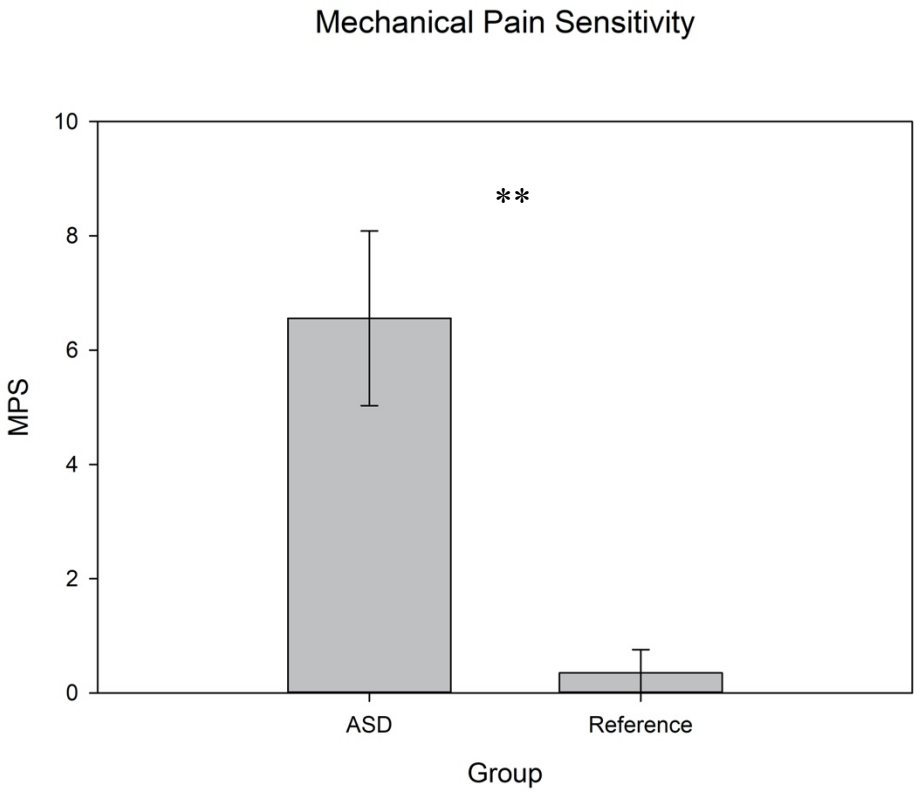

Figure 3: Raw mechanical pain sensitivity of ASD (n = 10) and reference (n = 64) groups. Bars represent average geometric means of pain ratings provided in response to pinprick stimuli (8-512 mN) that were presented in a pseudorandom order, with each intensity being presented once in each of three trials. Error bars represent standard error of the mean. Statistical significance (** $p < 0.01$) was determined using an unpaired t-test to assess differences between means of log-transformed data from each group, as the raw data were not normally distributed and thus violated the assumptions of the unpaired t-test. However, for clarity, raw geometric means are represented here.

Mechanical Pain Thresholds

In contrast with other pain measures, however, mechanical pain thresholds in the ASD group (136.96 mN ± 134.21) were significantly higher than those reported in the reference group (p = 0.0044, **Figure 4**), indicating hyposensitivity to mechanical pain stimuli at the threshold level. This finding was especially surprising given the significantly increased mechanical pain sensitivity observed in the ASD group. Here, the discrepancy between sensitivity as assessed by

mechanical pain thresholds and mechanical pain sensitivity tests may indicate alterations in higher-order functions that take place during pain processing in ASD rather than specific fiber dysfunction.

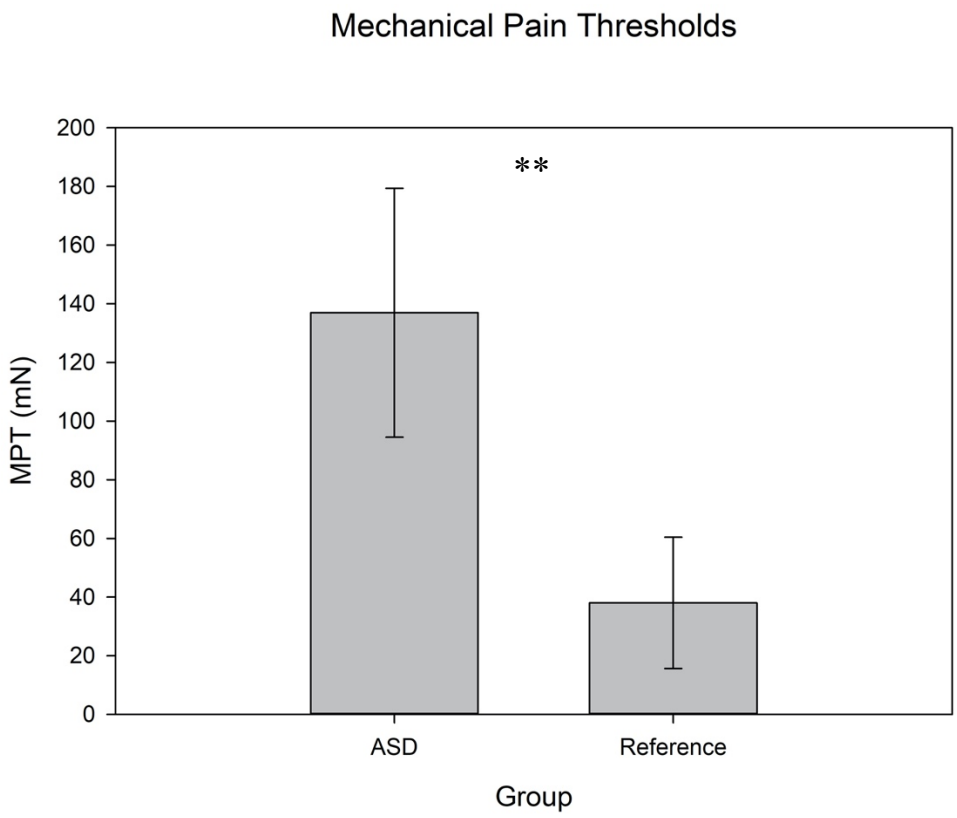

Figure 4: Raw mechanical pain thresholds of ASD (n = 10) and reference groups (n = 64). Bars represent average geometric means of five ascending and descending threshold measurements that were calculated for individuals in each group. Error bars represent standard error of the mean. Statistical significance (** $p < 0.01$) was determined using an unpaired t-test to assess differences between means of log-transformed data from each group, as the raw data were not normally distributed and thus violated the assumptions of the unpaired t-test. However, for clarity, raw geometric means are represented here.

Stimulus/Response Functions and Dynamic Mechanical Allodynia

Unlike Fründt et al. (2017) and Vaughan et al. (2019), the current study did not identify the presence of dynamic mechanical allodynia in any of the ASD participants. Of the ten ASD participants, none rated the brush, cotton wisp, or Q-tip stimuli as being painful when tested on

the hand dorsum (CT afferent-innervated) or the thenar eminence (non-CT afferent-innervated) (**Figure 5**).

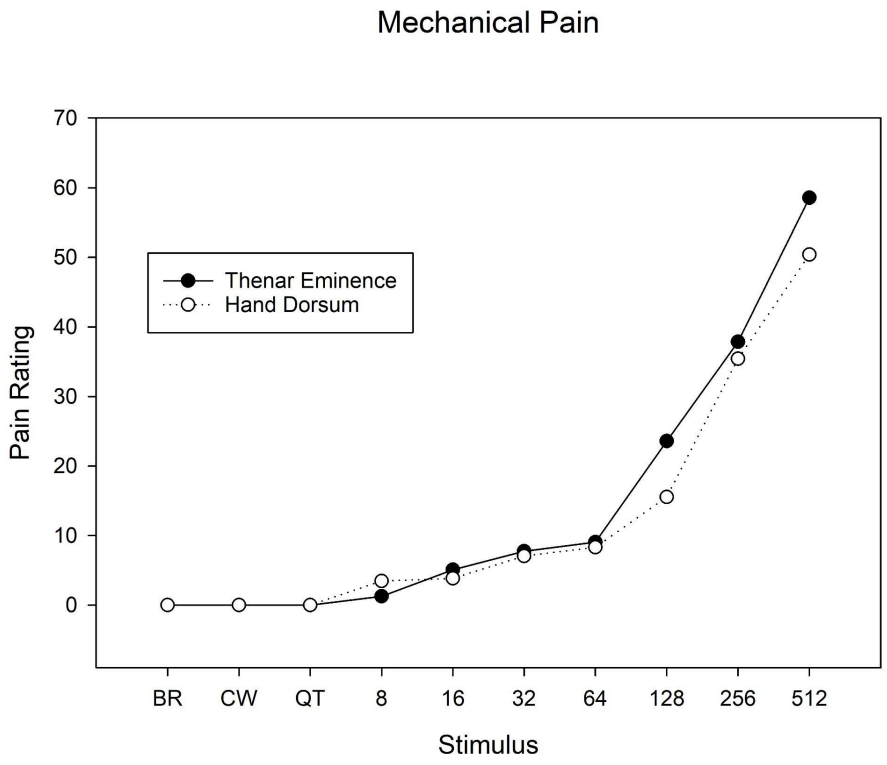

Figure 5: ASD group mean pain ratings for tactile (brush, cotton wisp, Q-tip) and mechanical pain (pinprick) stimuli at thenar eminence (n = 9) and hand dorsum (n = 10) sites. Notably, mean pain ratings for all tactile stimuli were 0. (BR = brush; CW = cotton wisp; QT = Q-tip. Numbers on the x-axis indicate the varying intensities [mN] of pinprick stimuli presented during this test.)

Warm and Cool Detection Thresholds

Interestingly, ASD participants demonstrated increased warm detection thresholds (35.07℃ ± 1.51, p = 0.0002, **Figure 6A**) and decreased cool detection thresholds (29.21℃ ± 1.54, p < 0.0001, **Figure 6B**) compared to the reference group, both of which indicate hyposensitivity to thermal detection. These thresholds represent those measured using the method of limits, since the two-alternative forced-choice task that was also used to determine warm and cool detection thresholds did not have published reference values available. Therefore, reaction time differences could also potentially account for the longer time that it apparently took

for the ASD group to indicate the sensation of warm or cool, and this possibility will be further explored in the discussion section.

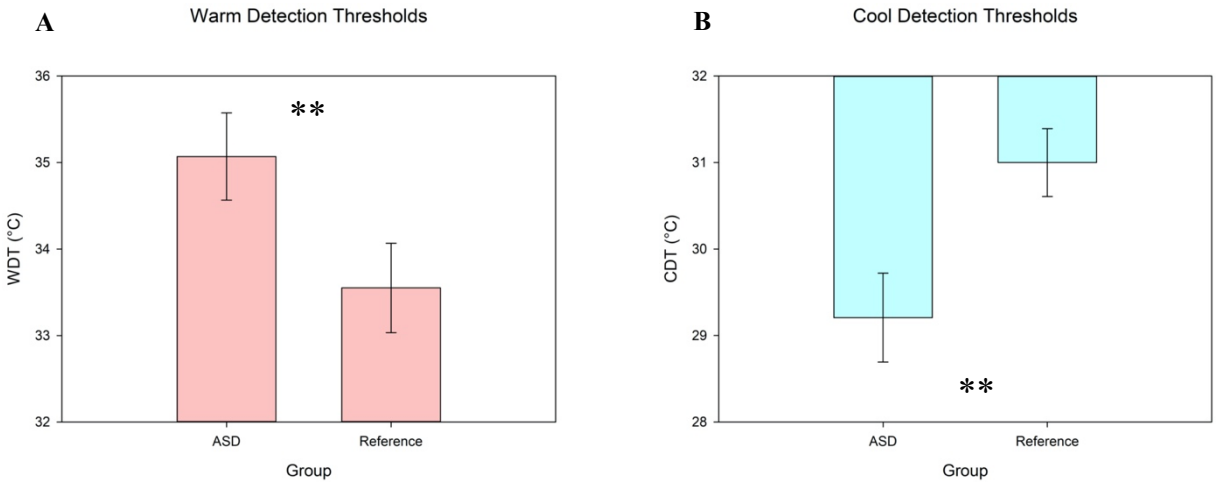

Figure 6: Mean thermal detection thresholds of ASD (n = 9) and reference (n = 64) groups. Bars represent mean thresholds for warm detection (A) and cool detection (B) thresholds as the difference from baseline temperature of the thermode (32°C). Error bars represent standard error of the mean. Statistical significance (** $p < 0.01$) was determined using an unpaired t-test to assess differences between means of log-transformed data from each group, as the raw data were not normally distributed and thus violated the assumptions of the unpaired t-test. However, for clarity, raw means are represented here.

Mechanical Detection Thresholds

Finally, ASD participants exhibited increased mechanical detection thresholds (2.51 mN ± 2.39) compared to the reference group ($p < 0.0001$, **Figure 7**), suggesting hyposensitivity to light touch in adolescents with ASD. Considering the findings from the QST parameters previously mentioned, increased mechanical detection thresholds further characterize the ASD group as being particularly sensitive to painful stimuli, and not as sensitive to innocuous stimuli.

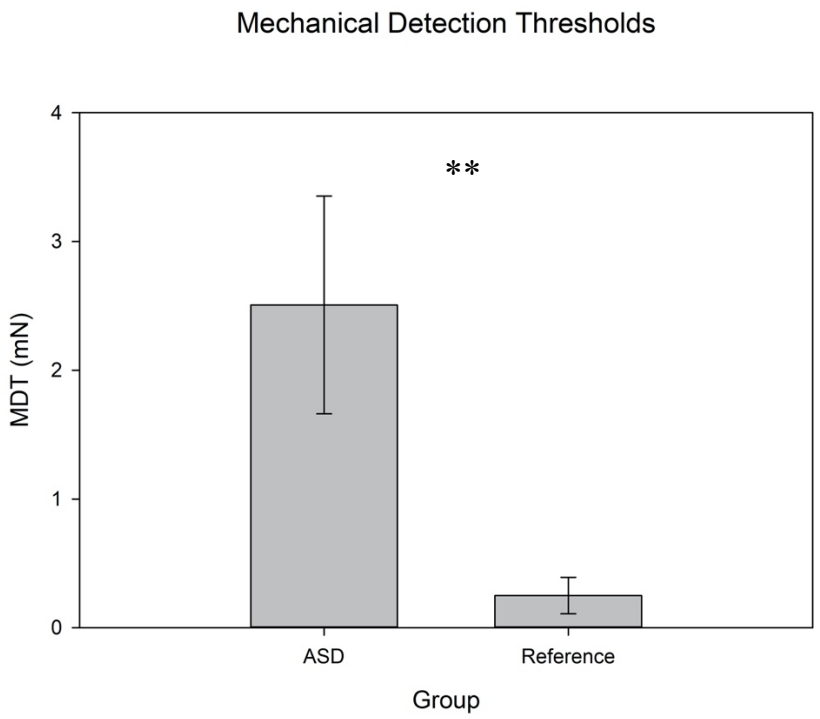

Figure 7: Raw mechanical detection thresholds of ASD (n = 8) and reference (n = 64) groups. Bars represent average geometric means of five descending and ascending threshold measurements that were calculated for individuals in each group. Error bars represent standard error of the mean. Statistical significance (** $p < 0.01$) was determined using an unpaired t-test to assess differences between means of log-transformed data from each group, as the raw data were not normally distributed and thus violated the assumptions of the unpaired t-test. However, for clarity, raw geometric means are represented here.

Individual Z-score Calculations

For exploratory purposes, individual Z-scores for each QST parameter were calculated to compare data from individual participants to the reference data and to further assess clinical significance of these findings. Z-scores that fell outside of the 95% confidence interval established by the reference data set (i.e., greater than 1.96 standard deviations from the mean) were considered to indicate clinically significant gain or loss of function. Calculations of individual Z-scores allow for a visualization of the heterogeneity that existed in the results for each test within the ASD population. For some tests, individual results were quite reflective of the differences of means between the ASD and reference groups determined through t-tests. For example, in line with the apparent group hypersensitivity to pressure pain, five of ten (50%)

ASD participants demonstrated individual pressure pain threshold Z-scores outside of the normal range established by the reference data set (**Figure 8**). In contrast, however, calculation of individual Z-scores reveals that while mechanical pain thresholds were determined to be significantly higher in the ASD group compared to the reference group, only three of ten (30%) individuals fell below the normal range to indicate clinically significant hyposensitivity, and one participant (10%) even displayed clinically significant hypersensitivity (**Figure 8**).

Even among the three pain measurements for which hypersensitivity was indicated in the ASD group (HPT, PPT, and MPS), not every participant necessarily exhibited increased sensitivity across all three measures, pointing toward another source of heterogeneity within this group regarding their sensitivity to different types of pain. For example, one participant displayed decreased heat pain (34.96℃, Z = 2.004) and pressure pain (227.94 kPa, Z = 2.836) thresholds compared to the reference group, suggesting hypersensitivity to these measures, but decreased mechanical pain sensitivity (0.137, Z = -0.986), suggesting hyposensitivity that is inconsistent with the hypersensitivity observed in the ASD group as a whole. On the other hand, another participant displayed decreased pressure pain thresholds (324.91 kPa, Z = 1.65) and increased mechanical pain sensitivity (2.88, Z = 2.17), but slightly increased heat pain thresholds (43.45℃, Z = -0.29), indicating some hyposensitivity to heat pain. Of the ten ASD participants, only six (60%) showed increased sensitivity across all three measures. Inter- and intra-individual variability regarding pain thresholds within this small sample highlights the importance of utilizing measures of sensory sensitivity that can pinpoint these differences, and it also suggests that mechanisms of pain processing beyond the receptor level may account for some of the differences in sensory sensitivity seen across the autism spectrum.

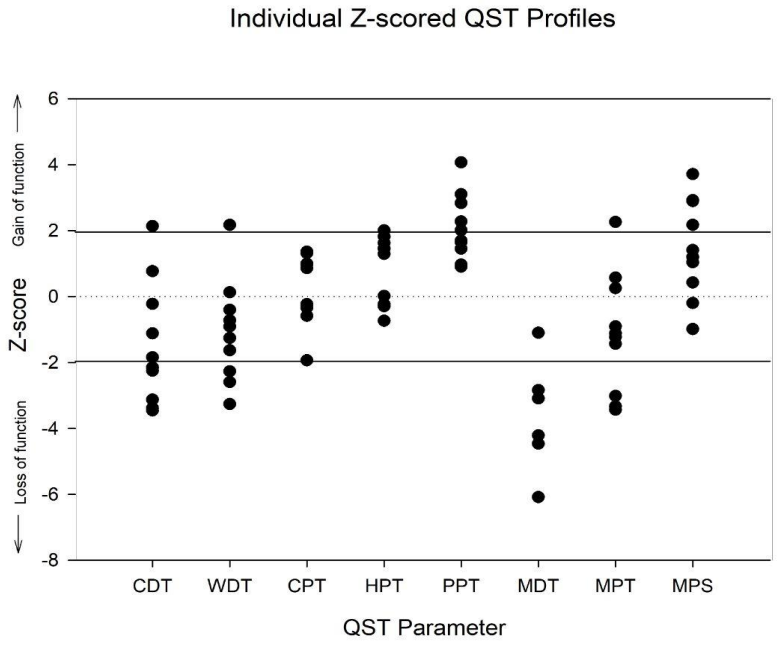

Figure 8: Z-scores of individual ASD participants for core QST parameters. Z-scores were calculated to compare individual data to the published reference values by the following formula: $Z\text{-}score = (X_{ASD} - Mean_{Reference})/SD_{Reference}$.

Exploratory Measures

All results from exploratory measures should be interpreted with extreme reservation, as data was collected from only 1 control participant to compare to that collected from the 10 ASD participants. However, some speculation regarding differences between ASD and neurotypical adolescent groups can be made from the current results, especially when considered alongside results from the QST tests for which published reference values are available.

Auditory Sensitivity

For each sound intensity tested in the auditory sensitivity test, mean ratings of the ASD group were higher than the rating from the control participant (**Figure 9**). Results from this test support previous findings from numerous studies across multiple age groups reporting increased

auditory sensitivity as one of the most common sensory sensitivities present in ASD (Kientz & Dunn, 1997; Baranek et al., 2006; Kern et al., 2006; Jones et al., 2009). Notably, many of these previous studies reported increased auditory sensitivity based on self- and proxy-report measures (e.g., the Sensory Profile). Upon collection of additional data from neurotypical controls, results from this test could provide a quantitative account that reflects commonly observed sensory sensitivities in ASD.

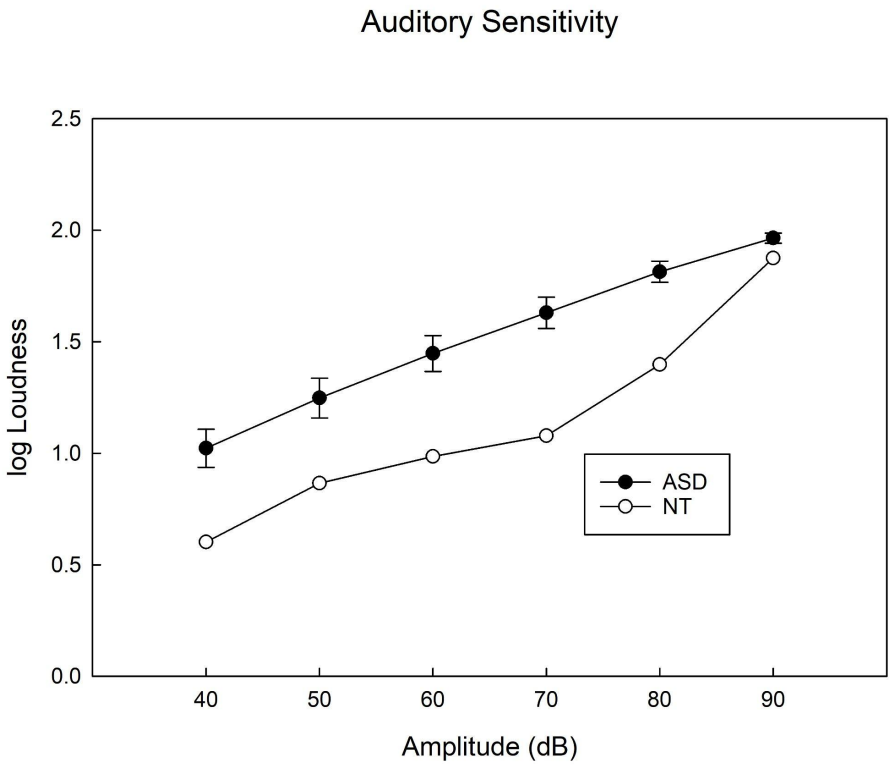

Figure 9: Auditory sensitivity of ASD group (*n* = 10) and neurotypical (NT) control participant (*n* = 1). As raw loudness rating data was not normally distributed, the means of log-transformed values are represented here. Error bars represent standard error of the mean.

Suprathreshold Heat Pain

At all temperatures tested in the suprathreshold heat pain task (43-47°C), ASD participants rated the painfulness of the stimulus to be higher than the control participant did (**Figure 10**). Considering that ASD participants exhibited decreased heat pain thresholds in the

earlier test employing the method of limits (**Figure 2A**), results from this test further indicate increased heat pain sensitivity in ASD that can be attributed to alteration at some point along the pain processing pathway, rather than factors such as anxiety due to lack of familiarity with the testing environment or procedures, for example. Again, collection of data from additional control participants is needed to strengthen the assertions made regarding these test results.

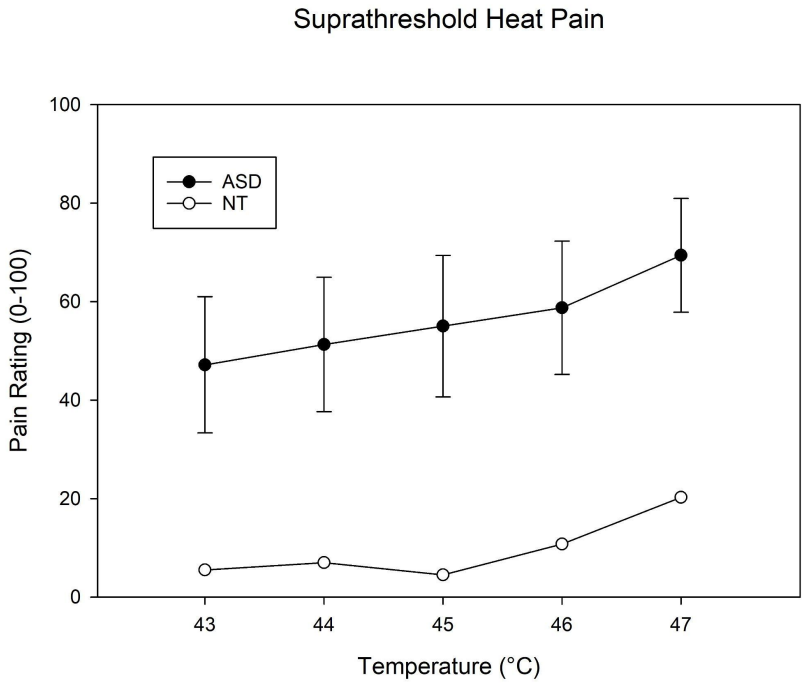

Figure 10: Mean suprathreshold heat pain ratings of ASD group (n = 10) and neurotypical (NT) control participant (n = 1). Error bars represent standard error of the mean.

Thermal Grill Illusion

The mean pain rating in response to the thermal grill illusion was much greater in the ASD group (36.75 ± 40.86) than in the one control participant (8.00) (**Figure 11**). As can be seen from the large standard deviation in the ASD group, pain ratings on this stimulus varied greatly within the group. Therefore, it is difficult to speculate about general trends in sensitivity to this

stimulus in adolescents with ASD versus neurotypical adolescents at this time. However, if data were to be collected from additional control and ASD participants, increased sensitivity to the thermal grill in the ASD group would fall in line with the hypersensitivity observed in response to other thermal pain stimuli.

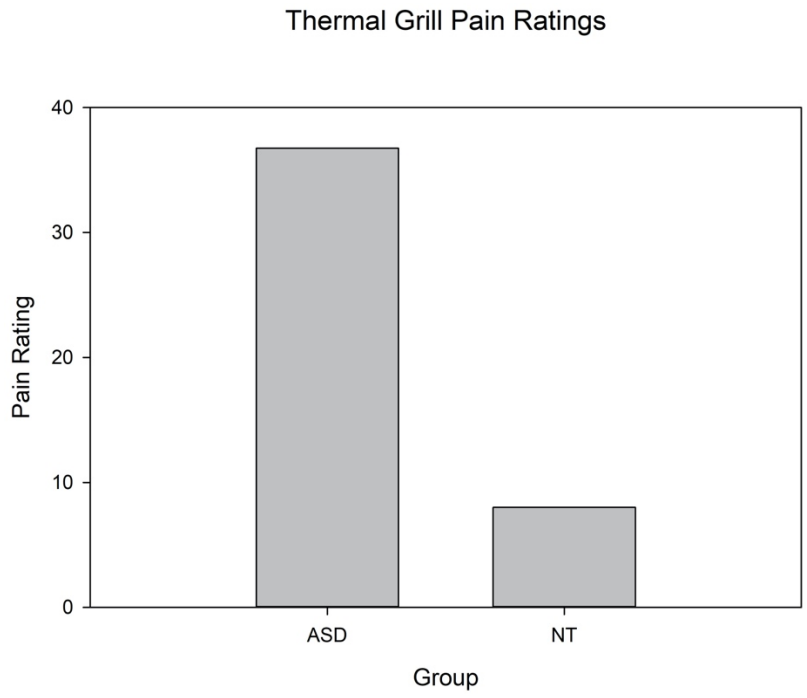

Figure 11: Mean thermal grill pain ratings of ASD group (n = 8) and neurotypical (NT) control participant (n = 1).

Hedonics Testing

In contrast with some of the measures of pain and detection thresholds that yielded relatively unidirectional results in the ASD group to indicate hyper- or hyposensitivity, hedonic ratings of various stimulus types yielded a wide range of results that did not indicate a clear pattern in most cases. Some ASD participants rated cool, warm, noxious cold, noxious heat, TGI, and auditory stimuli as pleasant, while others rated these stimuli as unpleasant (**Figure 12**). The more extreme ratings that were closer to the ends of the scale (i.e., most unpleasant sensation

imaginable, -100 and most pleasant sensation imaginable, +100) were not attributable to one participant. In fact, seven of ten (70%) ASD participants provided extreme ratings (either ≥ +50 or ≤ -50) in response to at least one of the stimuli. Further data collection from neurotypical controls is necessary to determine whether the heterogeneity observed in hedonic ratings is unique to the ASD population. However, taken together with the heterogeneity observed in the standardized QST measures as indicated by calculation of Z-scores, sensory sensitivity appears to manifest in a variety of ways in ASD, with differences observed across modalities and aspects of somatosensation (i.e., pain versus pleasantness).

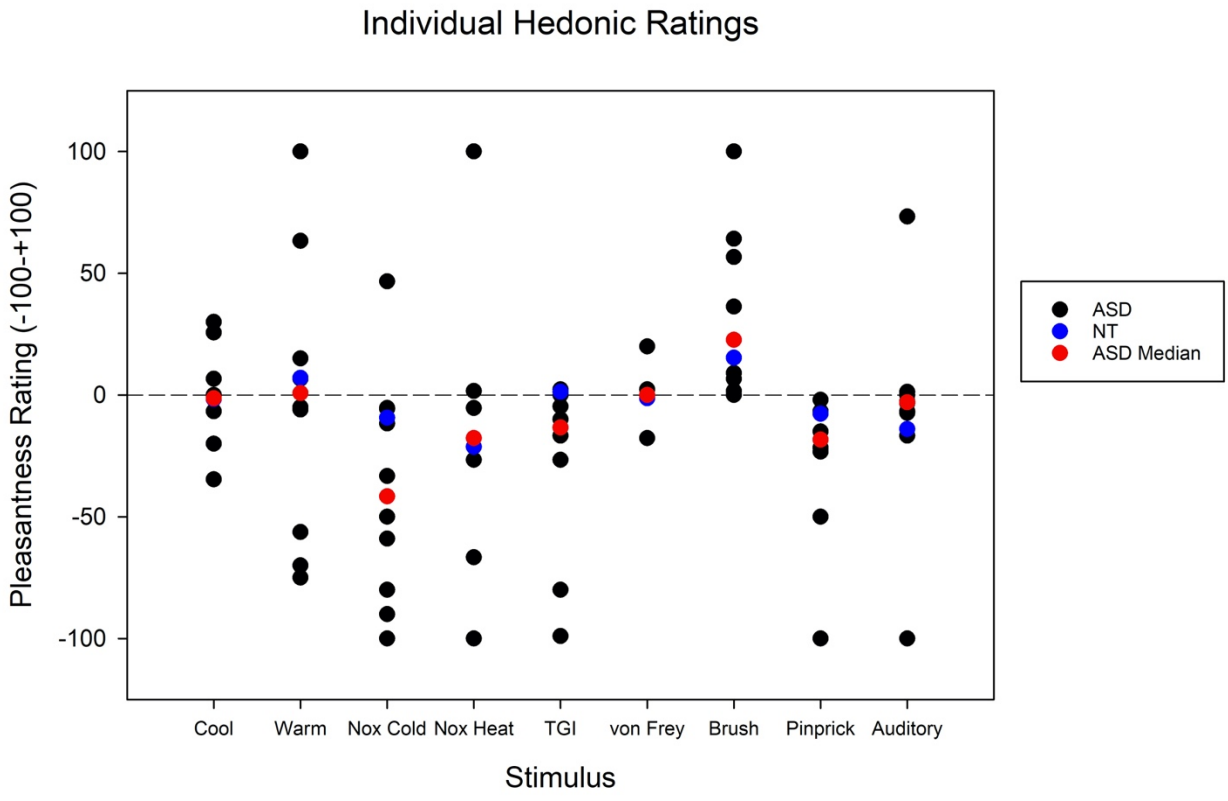

Figure 12: Individual hedonic ratings of ASD group (n = 10) and neurotypical (NT) control participant (n = 1). The median rating for the ASD group is also indicated for each test.

Discussion

This study was conducted primarily for the purpose of collecting data to quantify differences in responses to various noxious and innocuous QST parameters between adolescents with ASD and neurotypical controls. In support of our original hypothesis, adolescents with ASD generally exhibited hypersensitivity to noxious measures as demonstrated by their decreased heat pain and pressure pain thresholds, as well as their increased mechanical pain sensitivity. However, contrary to these findings as well as the original hypothesis, adolescents with ASD demonstrated increased mechanical pain thresholds (i.e., hyposensitivity). Additionally, none of the adolescents with ASD rated the dynamic mechanical stimuli of the brush, cotton wisp, or Q-tip as being painful in both hand dorsum (CT afferent-innervated) and palm (non-CT afferent-innervated) test sites, and these findings directly contradict the hypothesis that CT afferent dysfunction underlies tactile hypersensitivity, especially in the context of social touch, in ASD. Decreased cool detection thresholds and increased warm and mechanical detection thresholds were also observed in the ASD group, suggesting an unexpected hyposensitivity to innocuous stimuli.

While it is difficult to make any definitive conclusions regarding the exploratory measures that do not have published reference values available, on average, the ASD group rated all temperatures tested in the suprathreshold heat pain testing (43-47℃) and the thermal grill illusion as more painful than the control participant did. These results support both our initial hypothesis and the separate finding of hypersensitivity demonstrated by decreased heat pain thresholds in adolescents with ASD. Additionally, the ASD group rated all loudness intensities delivered during the auditory sensitivity test (40-90 dB) as louder than the control participant did, highlighting another area in which adolescents with ASD may be more sensitive than

neurotypical controls. Lastly, as expected, the ASD group exhibited a wide range of ratings when using the hedonic rating scale to rate the pleasantness/unpleasantness of various stimuli.

Contextualizing Current Findings with Those of Previous Studies

First, our findings of increased pressure pain sensitivity align with those of Riquelme et al. (2016), who observed decreased pressure pain thresholds in children ages 4-15 with ASD (n = 27) compared to controls (n = 30) at face, palm, and hand dorsum sites, while they contradict those of Fründt et al. (2017) and Vaughan et al. (2019), who found no significant differences in pressure pain thresholds between adults with ASD and controls. As the reference data used in the current study were separated by age group (i.e. younger children, older children, and adolescents), Blankenburg et al. (2010) reported that age had the greatest effect on reference data, with pressure pain thresholds decreasing in older age groups. Considering these previous studies in ASD populations that also assessed pressure pain thresholds, the current findings support the idea that pressure pain sensitivity may decrease over time in ASD in a manner similar to that observed in non-ASD reference data, although it is unclear whether the time frame for sensitivity decrease in ASD and neurotypical populations would be similar.

Considering previous studies that have assessed heat pain thresholds in ASD and yielded variable results, our findings of hypersensitivity to heat pain support those of Cascio et al. (2008), who observed increased heat pain sensitivity in adults with ASD in both palm and forearm test sites. However, heat pain hypersensitivity directly contradicts observations in the one study that has specifically focused on sensory sensitivity in adolescents with ASD to date (Duerden et al., 2015) as well as in adult studies (Fründt et al., 2017; Vaughan et al., 2019) in which significant differences were not observed between groups. Fründt et al. (2017) did,

however, note multiple ASD participants whose heat pain threshold Z-scores fell outside of the 95% confidence interval of the normal distribution established by the DFNS, indicating that heterogeneity within the ASD group may account for some of the insignificant findings in previous studies. The present findings suggest that adolescents with ASD may in fact exhibit significantly heightened sensitivity to heat pain stimuli.

Our results of insignificant differences in cold pain thresholds between adolescents with ASD and the reference group, however, deviate from those of Cascio et al. (2008), who observed significant hypersensitivity to both cold and heat pain in adults with ASD compared to controls. On the other hand, they support those of Duerden et al. (2015), Fründt et al. (2017), and Vaughan et al. (2019), who observed no significant differences in cold pain thresholds in adolescents and adults with ASD compared to controls.

In another test of response to noxious stimuli, we observed increased mechanical pain sensitivity in the ASD group compared to the reference group. In the few studies that have examined mechanical pain sensitivity in ASD, significant differences between ASD and control groups have not been previously observed (Fründt et al., 2017; Vaughan et al., 2019). Further, mechanical pain sensitivity has not yet been assessed in adolescents with ASD, as these two previous studies were conducted with adult participants. Our study is the first, to our knowledge, to observe increased mechanical pain sensitivity in adolescents with ASD.

Unexpectedly, however, the ASD group demonstrated decreased mechanical pain thresholds, indicating hyposensitivity to mechanical pain stimuli, which directly contrasts the hypersensitivity observed in the test for mechanical pain sensitivity. However, a similar finding arose in the study by Vaughan et al. (2019), who observed significant increases in mechanical pain thresholds in adults with ASD along with a slight gain in mechanical pain sensitivity,

although this gain was insignificant. The only other study to date that has assessed mechanical pain thresholds in ASD reported no significant differences between ASD adults and controls (Fründt et al., 2017).

Further, when tactile stimuli (i.e., brush, cotton wisp, and Q-tip) were presented at both hand dorsum and palm test sites, none of the ASD participants rated these stimuli as being painful. These findings directly contradict those of Fründt et al. (2017) and Vaughan et al. (2019), who each observed dynamic mechanical allodynia in two out of thirteen total ASD adult participants when tested at the hand dorsum site. However, the findings in the present study align with those of Cascio et al. (2008), who observed no significant differences in pleasantness/unpleasantness ratings in response to various textures between forearm and palm test sites in adults with ASD, and thus our results also do not seem to support the hypothesis of CT afferent dysfunction leading to abnormal response to stroking touch in ASD.

Regarding innocuous measures, in contrast to the hypersensitivity observed in response to thermal pain stimuli, significant hyposensitivity was observed in response to innocuous cool and warm stimuli in the ASD group. These findings support those of the one QST study specifically focusing on adolescents with ASD to date, in which ASD adolescents demonstrated a loss of sensory function for thermal detection (Duerden et al., 2015). However, adult studies have yielded different results, with Cascio et al. (2008), Fründt et al. (2017), and Vaughan et al. (2019) finding no significant differences in thermal detection thresholds between ASD adults and controls. Williams et al. (2019) also did not observe significant differences between individuals with ASD (n = 83) and neurotypical controls (n = 42) ages 7-54.

Interestingly, the ASD group also demonstrated increased mechanical detection thresholds (i.e., hyposensitivity) compared to the reference group. This finding was quite

unexpected, as multiple studies on tactile sensitivity in both children (O'Riordan & Passetti, 2006; Riquelme et al., 2016) and adults (Cascio et al., 2008; Fründt et al., 2017) reported no significant differences in mechanical detection between ASD and control groups using similar methods. However, similar to the present study, Vaughan et al. (2019) did find significantly increased mechanical detection thresholds in adults with ASD compared to controls, and mean Z-scores of the ASD group fell outside of the normative range of healthy individuals established by the DFNS.

Mechanisms for Increased Pain Sensitivity in ASD

Given that adolescents with ASD in the present study demonstrated hypersensitivity across multiple pain measures compared to the reference group, dysfunction of Aδ and C nociceptors may be a plausible explanation for the increased pain sensitivity observed. Specifically, the significantly decreased heat pain and pressure pain thresholds and increased mechanical pain sensitivity, all of which are mediated by these nociceptors, in the ASD group could indicate that early, peripheral stages of pain processing may be altered in this population.

However, the puzzling discrepancy between the hyposensitivity to noxious mechanical stimuli indicated by increased mechanical pain thresholds and the hypersensitivity indicated by increased mechanical pain sensitivity puts this theory into question. In these two tests, the same set of weighted pinpricks was applied to the same site (hand dorsum) by the same experimenter, so the same set of fibers should have been activated in both cases, rendering the fiber dysfunction explanation of altered pain perception in ASD inadequate in this context.

These unexpected results may be attributable to the different methods used to assess responses to the same stimulus type across these two tests that may be particularly relevant in

ASD. A key difference between these tests lies in how participants were instructed to indicate their pain. In the test for mechanical pain thresholds, as pinpricks of increasing intensity were applied to the test site, participants were instructed to say "sharp" as soon as one of the stimuli elicited any sort of painful sensation. In the test for mechanical pain sensitivity, however, participants used a numeric rating scale (NRS) to rate their pain, with a rating of 0 meaning no pain and a rating of 100 meaning the most intense pain imaginable. As is evident through the anchor labels, use of this scale requires participants to imagine what the most intense possible pain would feel like and depends on participants' personal experience and knowledge of other people's pain (Stinson et al., 2006). Use of this scale may have been problematic in this particular study population, as imagination and the ability to take another person's perspective can be significantly impaired in ASD (Crespi et al., 2006). These qualities that are inherent in ASD may indicate an explanation behind elevated pain ratings in the test for mechanical pain sensitivity despite increased mechanical pain thresholds, although further research with larger sample sizes should be conducted to investigate the relationship between these two quantities in adolescents with ASD. Additionally, studies employing similar methods but using modified rating scales (e.g., scales with additional tick marks between 0 and 100) could be used to clarify the pain rating process for individuals with ASD.

Another alternative explanation for the observed hypersensitivity to painful stimuli in adolescents with ASD may be increased intolerance of uncertainty as well as pain-related anxiety, and these factors may be especially relevant in framing the divergent results that were observed in tests for mechanical pain thresholds and mechanical pain sensitivity. In the case of mechanical pain sensitivity, while this test used the same metal pinpricks that were used before to determine mechanical pain thresholds, this test was one of the first in which participants did

not have as much direct control over the intensity of the stimulus, except that they were told that if they rated a stimulus as the most intense pain imaginable (100), then this stimulus would not be presented again throughout the rest of the experiments. Prior to this test, measurements for cold pain, heat pain, pressure pain, and mechanical pain thresholds had been assessed, and these tests included very similar instructions stating that participants should press the button (or say "sharp" in the case of mechanical pain threshold assessment) as soon as the sensation changed its quality towards an additional sensation of pain, and that they should not wait until the sensation became unbearably painful to indicate their perception of pain. The test for mechanical pain sensitivity did not follow this pattern.

In children with ASD, intolerance of uncertainty has been shown to contribute to sensory over-responsiveness as measured by parent report questionnaires, and this effect is partially mediated by anxiety levels (Wigham et al., 2015; Neil et al., 2016). The explanation of these effects as seen in these studies is generally outlined as follows: difficulties coping with uncertainty at the neural level may lead to psychological beliefs that uncertainty is negative and should be avoided. Desire to reduce uncertainty may contribute to an increase in anxiety symptoms (e.g., ruminative thoughts about possible negative outcomes, hypervigilance to threats in the environment), and thus in these situations individuals experiencing this anxiety surrounding uncertainty may be more likely to have heightened responses to aversive external sensory stimuli. Following this logic, uncertainty and anxiety surrounding an unfamiliar format of pain assessment may have contributed to increased mechanical pain ratings during the assessment of mechanical pain sensitivity.

It is possible that pain-related anxiety played a role in assessment of other pain thresholds (e.g., heat, pressure) as well, especially the ones measured earlier during the study session while

participants were still becoming acclimated to the unfamiliar testing environment. However, this seems less likely for heat pain considering that during the later suprathreshold heat pain testing, mean pain ratings in the ASD group were higher than those of the one control participant at all temperatures tested (43-47°C). This test was conducted after participants had already completed four tests involving the thermode, and two tests involving use of the NRS, so uncertainty that may have contributed to elevated ratings was minimized in this particular test as participants had already been exposed to its core components multiple times. More data should be collected from neurotypical control participants as well as additional ASD participants to provide further evidence for decreased pain thresholds that are independent of anxiety in ASD.

Mechanisms for Social Touch Aversion in ASD

Also during the test of stimulus/response functions in which mechanical pain sensitivity was assessed, presentations of tactile stimuli (brush, cotton wisp, Q-tip) were interleaved in the presentations of pinprick stimuli, as discussed in the methods section. This test was conducted on both the glabrous skin of the palm and the hairy skin of the hand dorsum to address the hypothesis established in previous studies that CT afferent dysfunction may lead to altered tactile perception and aversion to social touch in ASD (Cascio et al., 2008; Riquelme et al., 2016). Findings in the present study do not support this hypothesis. First, dynamic mechanical allodynia was not identified in any of the ASD participants at palm or hand dorsum sites (i.e., none of the ASD participants rated any of the tactile stimuli as being painful). Further, none of the ASD participants rated the brush stimulus as being unpleasant during the hedonic testing. Two (20%) of the ten participants even rated the brush as being the most pleasant sensation imaginable

(+100) on average across three trials, contradicting previous findings that individuals with ASD do not respond as strongly to gentle, stroking stimuli (Kaiser et al., 2015).

A possible explanation for the lack of perception of this stroking stimulus as painful or unpleasant in this small sample could have been that all participants simply happened to not have an aversion to this kind of social touch; however, three parents of participants specifically reported that their child disliked being touched/squeezed in the CARS-2 Questionnaire for Parents or Caregivers, and a separate participant reported herself during her clinical interview that she disliked being touched. Considering that aversion to social touch was reported in some capacity for four (40%) ASD participants, it is unlikely that the overall lack of pain response to these tactile stimuli came about simply due to sampling bias.

However, these results do not entirely preclude the possibility that there are elements of tactile perception that are altered in ASD such that individuals with ASD are specifically more averse to social touch. For example, in addition to the CT afferent hypothesis, Riquelme et al. (2016) suggested a relationship between pressure pain hypersensitivity and dysfunction in systems of social touch due to reports of aversion to social touch including components of pressure (e.g., hugs) in ASD. The significantly decreased pressure pain thresholds (i.e., hypersensitivity) observed in the present study in conjunction with the aforementioned parent and self-reports of participant aversion to social touch support this mechanistic explanation.

These findings may even have implications for treatment of sensory sensitivity in ASD. In fact, the application of deep pressure stimuli to calm children with ASD has become a technique widely used by occupational therapists since Temple Grandin's description of her "squeeze machine," which she designed to provide self-administered lateral body pressure (Bestbier & Williams, 2017). Prior to designing this device, Grandin (1992) described how

whenever anyone hugged or touched her, “an overwhelming tidal wave of sensation” flowed through her, and she “stiffened, flinched, and pulled away” (p. 66). As she used the squeeze machine repeatedly, her tolerance for deep pressure grew, and “a once overwhelming stimulus” became “a pleasurable experience” (p. 66). When the squeeze machine was used with children with ASD, significant reductions in tension and anxiety were observed, indicating a therapeutic use for targeting altered pressure pain perception in ASD (Edelson et al., 1999). While the results from the small sample in this study should not completely discount theories of CT afferent involvement in aversion to social touch, they also point toward pressure pain perception as another realm of somatosensation that is worthy of investigation in future studies regarding social touch aversion and potential treatments in ASD.

Mechanisms for Hyposensitivity to Innocuous Stimuli in ASD

Much like alterations in heat pain thresholds, the significant alterations in cool and warm detection thresholds in the adolescents with ASD compared to the reference group may be due to differences in peripheral processing. Here, hyposensitivity to cool would suggest Aδ fiber dysfunction, while hyposensitivity to warm would suggest C fiber dysfunction.

Another possible explanation for the observed hyposensitivity to thermal detection, however, would be slowed reaction time in the ASD group. Due to the repetitive nature of the study procedures and the significant amount of time that it took participants to complete them (3-5 hours), results were bound to be subject to effects of distraction, boredom, and fatigue (Chong & Cros, 2004). Completing this set of tests requires a considerable amount of attention over a long period of time, and especially in the ASD group in which three (30%) of the participants also had been previously diagnosed with ADHD, attentional and reaction time differences could

have had a major impact on the results obtained. Thus, in order to draw strong conclusions regarding differences in these thresholds, reaction time-independent methods should be used to minimize effects of this potential confounding factor.

In consideration of potential reaction time effects on threshold measurements, in addition to the method of limits protocol outlined by the reference group, we employed a two-alternative forced-choice task as part of the study procedures to determine participants' warm and cool detection thresholds in a way that does not depend on reaction time. Unfortunately, reference data are not available for these tests, so we are unable to draw strong conclusions regarding thermal detection thresholds determined in adolescents with ASD versus neurotypical controls using this method at this time. One major aim of this project going forward will include collecting additional data from control participants on this measure so that we can examine whether significant hyposensitivity to thermal stimuli remains when a reaction time-independent method is used.

Considerations of Heterogeneity Within the ASD Population

Previous studies on sensory sensitivity in ASD that have found heterogeneity or insignificant differences across QST measures between ASD participants and controls have suggested a central rather than peripheral mechanism for altered pain processing in ASD. While individual QST parameters have been designed to assess the function of specific classes of nerve fibers, it is also important to consider that beyond peripheral detection of somatosensory information, this signal is transformed, propagated along spinal cord interneurons and projection neurons, and further communicated to the brainstem, thalamus, and the primary somatosensory cortex and other brain regions that are relevant in the processing of such stimuli (Orefice, 2020).

In the present study, all of these processing steps occurred before participants provided a pain rating in response to a stimulus, or pressed a button to indicate the specific moment at which they first perceived the sensation of pain, so alterations at any of these points could underlie the observed hypersensitivity or hyposensitivity to specific measures. Further, the fact that in certain ASD participants, hypersensitivity was apparent in response to some noxious stimuli (e.g., heat and pressure pain) but not others (e.g., mechanical pain sensitivity) indicates that alterations in central processing of pain may strongly contribute to the observed selective hypersensitivity to certain types of noxious stimuli in individual adolescents with ASD.

One study by Failla et al. (2018) has attempted to explain a central mechanism underlying the presence of both hypo- and hypersensitivity to different types of pain in individuals with ASD using neuroimaging techniques. While pain ratings and neural pain signature responses were indistinguishable between adults with ASD and controls during acute heat pain, the neural pain signature response was dramatically reduced in ASD during later phases of sustained pain. This diminished late response following intact early responses to painful stimuli was interpreted to potentially reflect altered pain coping or evaluation in ASD, behaviors which could contribute to the coexistence of hypo- or hypersensitivity to different types of painful stimuli in one individual (Moulton et al., 2012). More studies like this one should be conducted to examine potential alterations in mechanisms of central processing of painful stimuli in ASD, especially in adolescent and child populations, as there have been no such studies in these age groups to date.

Beyond the heterogeneity observed in previous studies as well as the present one regarding responses to pain in ASD, results from hedonics testing revealed even greater heterogeneity in the ASD group regarding responses to a wide range of noxious and innocuous

stimuli, including thermal, tactile, and auditory stimuli. For seven of the nine stimuli presented, average pleasantness/unpleasantness ratings of ASD participants spanned both unpleasant (-100-0) and pleasant (0-+100) ranges. One particularly fascinating example comes about in the ratings for the noxious heat (45°C) stimulus, as one ASD participant rated this stimulus -100 (the most unpleasant sensation imaginable) on average, while another rated it as +100 (the most pleasant sensation imaginable) on average. Interestingly, average pain ratings of the 45°C stimulus during the suprathreshold heat pain testing did not appear to differ greatly between these two participants, with the first participant providing an average pain rating of 96.67 and the second participant providing an average pain rating of 90. These results suggest an additional element of heterogeneity in perception of unpleasantness/pleasantness of stimuli in ASD that extends beyond that observed in differential responses to pain, which, to our knowledge, has not been investigated across such a wide variety of stimuli in any sensory sensitivity studies in ASD populations to date.

Also of note, the only stimuli which yielded unidirectional pleasantness/unpleasantness ratings were the brush, as mentioned earlier, which was only rated as pleasant, and the pinprick, which was only rated as unpleasant, although at varying levels. Further data collection in neurotypical controls and more adolescents with ASD should be prioritized to determine whether similar heterogeneity would exist in a group of neurotypical adolescents as well, or whether this heterogeneity is unique to ASD. Considering the current findings, results from these novel tests could reveal interesting new targets for future research on alterations in tactile perception in ASD.

In a broader sense, the heterogeneity observed in pain and unpleasantness/pleasantness ratings in the present study may reflect the heterogeneity in causes and presentations of ASD

itself. Individuals with ASD display diverse levels of social, communication, behavioral, and intellectual development, all of which could impact responses to the tests performed in this study (Wiggins et al., 2011). Importantly, the heterogeneity observed in the ASD population extends even further beyond that seen in this small sample of adolescents with ASD. The fact that the nature of this study design requires verbal responses and the ability to follow detailed instructions for many different tests influenced the decision to exclude participants whose verbal IQ fell below 65. This factor automatically prevented a significant portion of the ASD population from participating in this study, as the CDC estimates that 31-50% of individuals with ASD also meet the criteria for an intellectual disability, many of whom can also be described as minimally verbal (Tager-Flusberg et al., 2016; Christensen et al., 2019; Maenner et al., 2021). Due to this limitation and the generally small size of the sample tested, the results seen here should be interpreted with caution as they cannot necessarily be generalized to the ASD population as a whole.

Conclusions

This study was conducted to identify alterations, or lack thereof, regarding sensitivity to both noxious and innocuous sensory stimuli in adolescents with ASD compared to neurotypical controls. Beyond previous studies that utilized only the standardized QST procedures outlined by the DFNS or even that focused solely on one aspect of somatosensation (e.g., thermal) to assess sensory sensitivity in ASD, this study employed novel methods of testing auditory sensitivity alongside tactile sensitivity, incorporating a test for alterations in sensory integration with the thermal grill illusion, and collecting ratings on the unpleasantness/pleasantness of a variety of stimuli to provide a richer characterization of sensory sensitivity in ASD. Since these novel

measures do not have reference values, data from additional neurotypical controls must be collected in order for stronger conclusions to be drawn from these tests. However, in conjunction with results from the measures that do have reference values, the current findings indicate hypersensitivity in ASD across a number of modalities, which may indicate alterations in peripheral or central processing of such stimuli. Considering the heterogeneity in responses among ASD participants across various modalities with extremely different peripheral processing mechanisms in some cases (e.g., tactile versus auditory), it seems likely that alterations in central processing underlie sensory sensitivity in ASD.

Although only ten adolescents with ASD and one neurotypical control have participated in the study to date, hypersensitivity across multiple pain measures in the ASD group indicate alterations in response to painful stimuli specifically in ASD. In contrast, hyposensitivity was observed across multiple innocuous perception measures, further suggesting that hypersensitivity in adolescents with ASD is specific to perception of noxious stimuli. However, reaction time-independent methods should be employed in the future to confirm these findings.

Data collection from more participants is necessary to solidify these findings, especially from neurotypical adolescents. Also, to allow for participation of individuals with ASD who cannot provide verbal responses and to reduce the subjectivity that is inherent in responses to the tests currently used, methods such as skin conductance or neuroimaging should be used in future studies to further investigate alterations in sensitivity in ASD.

Given that sensory sensitivity is such a common occurrence in ASD that can significantly impact even the simplest daily activities, this topic is extremely worthy of further investigation. This study adds to the small body of literature on sensory sensitivity in ASD in the way that it reveals differential responses in ASD not only to the traditionally studied QST parameters, but

also in novel tests of auditory sensitivity, the thermal grill illusion, and hedonic ratings. Hopefully future studies will expand upon this current work by testing these parameters in larger sample sizes spanning different age groups and in ways that can allow for inclusion of individuals with ASD with limited verbal or intellectual abilities to advance understanding of neurophysiological underpinnings of this core ASD symptom.

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