RESEARCH ARTICLE

Trial-to-Trial Variability in Electrodermal Activity to Odor in Autism

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Abnormal trial-to-trial variability (TTV) has been identified as a key feature of neural processing that is related to increased symptom severity in autism. The majority of studies evaluating TTV have focused on cortical processing. However, identifying whether similar atypicalities are evident in the peripheral nervous system will help isolate perturbed mechanisms in autism. The current study focuses on TTV in responses from the peripheral nervous system, specifically from electrodermal activity (EDA). We analyzed previously collected EDA data from 17 adults with autism and 19 neurotypical controls who viewed faces while being simultaneously exposed to fear (fear-induced sweat) and neutral odors. Average EDA peaks were significantly smaller and TTV was reduced in the autism group compared to controls, particularly during the fear odor condition. Amplitude and TTV were positively correlated in both groups, but the relationship was stronger in the control group. In addition, TTV was reduced in those with higher Autism Quotient scores but only for the individuals with autism. These findings confirm the existing results that atypical TTV is a key feature of autism and that it reflects symptom severity, although the smaller TTV in EDA contrasts with the previous findings of greater TTV in cortical responses. Identifying the relationship between cortical and peripheral TTV in autism is key for furthering our understanding of autism physiology. *Autism Res 2020, 00: 1–11.* © 2020 International Society for Autism Research and Wiley Periodicals LLC

Lay Summary: We compared the changes in electrodermal activity (EDA) to emotional faces over the course of repeated faces in adults with autism and their matched controls. The faces were accompanied by smelling fear-inducing odors. We found smaller and less variable responses to the faces in autism when smelling fear odors, suggesting that the peripheral nervous system may be more rigid. These findings were exaggerated in those who had more severe autism-related symptoms.

Keywords: trial-to-trial variability; electrodermal activity; autism; faces; fear

Introduction

Autism is characterized by a host of atypical cognitive, social, and sensory behaviors (DSM 5) suggesting widespread perturbations in neural processing. One finding that has been reported in many of these domains is that of abnormal trial-to-trial variability (TTV), which has led to the suggestion that this abnormal variability may be a potential biomarker of autism [Bazelmans et al., 2019; Dinstein, Heeger, & Behrmann, 2015; Haigh, 2018; Haigh et al., 2016]. TTV refers to the variability in responses *within the same individual* from one trial or session to the next (rather than variability between individuals, which may also be greater in autism; Hahamy, Behrmann, & Malach, 2015; Hasson et al., 2009). For example, one of the first papers to document greater TTV in autism showed that the latency and amplitude of the P1 event-related potential to deviant (oddball) visual stimuli varied from one presentation to the next, more so than in neurotypical

controls [Milne, 2011]. While it is evident that even a "typical" brain exhibits a degree of variability over time, there is debate as to how much variability is ideal [Dinstein et al., 2015]. Some variability in neural responses counter-intuitively encourages stability in sensory percepts by adding flexibility in the system to an ever-changing external environment [Dinstein et al., 2015; Ermentrout, Galán, & Urban, 2008; Faisal, Selen, & Wolpert, 2008; Mandelblat-Cerf, Paz, & Vaadia, 2009; Stein, Gossen, & Jones, 2005]. However, if the system is too unstable, then this variability might make it difficult to establish statistical regularities in the sensory environment.

The evidence that autism is associated with abnormal TTV that might reflect an unstable neural system is mounting. Sensory responses to visual, auditory, and tactile stimuli recorded using functional magnetic resonance imaging (fMRI) showed greater TTV in all three sensory modalities, relative to controls, despite the absence of significant differences in average peak amplitude [Dinstein

et al., 2012]. This finding was replicated in a second group of individuals with autism [Haigh, Heeger, Dinstein, Minshew, & Behrmann, 2014] and found to be unique to autism (and not evident in schizophrenia; Haigh et al., 2016). The greater TTV has also been associated with greater sensory sensitivity (subjective reports of increased roughness; Haigh, Minshew, Heeger, Dinstein, & Behrmann, 2015), with impaired thermal detection [Williams et al., 2019], and with greater symptom severity [Park, Schauder, Zhang, Bennetto, & Tadin, 2017].

The reports of abnormal TTV in autism, however, are not limited to sensory systems. For example, greater TTV was reported in theta oscillations from prefrontal cortex during decision-making tasks in individuals with autism relative to controls [van Noordt et al., 2017], which highlights the possibility that the variability could have multiplicative effects on more complex processing. Abnormal TTV has also been found in behavioral responses, suggesting functional implications of a variable system. Geurts et al. [2008] were among the first to identify greater TTV in reaction times in children with autism compared with controls when identifying the location of an object on a screen. Psychophysically, greater TTV in autism has been reported in measures of visual contrast detection, identification of facial emotional intensity, and perception of number summation [Vilidaite, Yu, & Baker, 2017], measures of tactile roughness [Haigh et al., 2015], and thermal detection [Williams et al., 2019]. Neuromotor assessment of hand movements and finger tapping were also slower and more variable in autism [Morrison et al., 2018]. Park et al. [2017] used visual psychophysical measures of internal and external noise (using the equivalent noise model; for a summary of the method see Lu & Dosher, 2008), which simulates the instability of the visual system (internal noise) and the instability of the external environment (added noise to a stimulus), respectively. They found that individuals with autism were only significantly impaired on the tasks that relied on stable internal noise, highlighting the lack of stability in the visual system. Together, these findings of variability across many systems indicate that the enhanced variability may serve as a potential biomarker of an unstable neural system.

However, not all studies have found abnormal TTV in autism. Butler, Molholm, Andrade, and Foxe [2017] extensively assessed intertrial coherence and spectral perturbations in EEG signals to visual and tactile stimuli and found no evidence of differences in TTV—their individuals with autism produced similarly stable responses as neurotypical controls. Similarly, Coskun et al. [2009] found that tactile-evoked magnetoencephalography (MEG) responses were equally stable in autism and in control participants. TTV in heart rate in autism is also currently under debate. Greater TTV was reported in heart rate in children with autism at rest and during tasks [Billeci et al., 2018] and was related to improved language ability [Bazelmans et al., 2019]; however, in adults with autism, variability in heart rate was lower compared to controls when at rest [Thapa et al., 2019], and when reacting to a stressful situation [Dijkhuis, Ziermans, van Rijn, Staal, & Swaab, 2019].

It is currently unclear why there are discrepancies in the presence of TTV across studies. As always, there is the concern with the heterogeneity of the samples tested but one further possibility is that some of the findings of abnormal TTV in autism are due to additional confounds. Masquelier [2013] highlighted the point that fluctuations in a wide range of variables, from attention to room conditions could differentially impact individuals' responses, giving rise to abnormal TTV that is unrelated to the stability of the system. While these are valid comments and, ideally, these environmental fluctuations and measures must be controlled for, the TTV results were found to be consistent, even when measures of attention were accounted for [Dinstein et al., 2012; Haigh et al., 2014] and when autism and control individuals were tested under identical experimental conditions.

As evident, there remains some ongoing discussion about the generality of TTV as a characteristic of autism and further investigation of this issue is warranted. Another aspect of TTV in autism that has received relatively less consideration is whether the source of the TTV is cortical in nature. Consistent with this, Dinstein et al. [2012] reported that in the functional MRI results, the abnormal TTV in autism was only present in primary sensory cortices and not in thalamic activity (MGN or LGN). These findings indicate that some neural systems (cortical versus not cortical) might exhibit more TTV than others. To address this issue specifically, we examined in detail previously collected electrodermal activity (EDA) data in individuals with autism and controls. The EDA response is partly monitored by subcortical structures such as the hypothalamus and brainstem [Critchley, 2002] and, therefore, its study will help establish whether abnormal TTV in autism is solely a cortical perturbation or, alternatively, is present in other physiological responses as well. Endevelt-Shapira et al. [2018] collected sensory-evoked EDA data in response to faces while two different odors were presented—one odor was fear inducing (fear-induced sweat) and the other was more neutral, not evoking an emotion. Their results showed that the neurotypical controls produced a larger EDA response to the fear than the nonfear odor whereas the autism group produced similar trough-to-peak amplitudes response regardless of odor.

Here, we reanalyzed these EDA data with two goals. First, we aimed to establish whether adults with autism showed enhanced TTV in EDA and we were able to evaluate this using a pre-existing dataset in a very different sensory domain, notably that of olfaction (social

chemosignaling). Using this dataset is advantageous too as it was not designed to evaluate TTV and, therefore, the likelihood that the design of the study was biased toward generating variable results is reduced. Note too that the ability to analyze existing data highlights the ease with which to assess TTV in healthy and clinical populations. Second, we wished to determine how the TTV relates to other parameters of the EDA response. There are mixed findings as to whether TTV is related to the amplitude of the response, with some finding a correlation between the two dependent measures [Williams et al., 2019; Butler et al., 2017] and others not observing this pattern [Dinstein et al., 2012; Haigh et al., 2014; Haigh et al., 2015]. If the TTV is not correlated with amplitude, then this would suggest that the TTV may offer unique information on the sensitivity of the system independent of peak response. For the EDA response, one prediction is that the TTV might be greater in the autism individuals to the fear odor (which previously evoked the greatest group difference) but not in the neutral odor condition. Alternatively, the enhanced TTV might be present independent of EDA peak amplitude.

One previous study has reported on EDA variability in (children with) autism and found that increased TTV in EDA signal during cognitive tasks correlated with increased symptom severity [Fenning et al., 2017]. Therefore, in the reanalysis of the EDA data from Endevelt-Shapira et al. [2018], we anticipated that the adults with autism would also evince greater TTV compared to neurotypical controls, and that the TTV would be correlated with symptom severity. Identifying which physiological mechanisms contain abnormal TTV in autism is key for assessing which systems are perturbed and the findings have implications for understanding autism physiology.

Methods
Participants

Twenty male adults with autism (mean age 27 years; range 20–40 years) and 20 neurotypical (NT) male controls (mean age 28 years; range 22–35 years) participated (Experiment 2 in the study by Endevelt-Shapira et al., 2018). All of the individuals in the autism group met the DSM criteria for Autism (4th and 5th edition) [American Psychiatric Association, 2013] and were diagnosed by a trained clinician. ADOS-2 (module 4) general scores (summed from the social affect and communication scores) for the autism group ranged from 2 to 16 (mean 9.9, SD 3.55). All NTs scored below 30 on the Autism Quotient (mean 17.05; range 8–23). In comparison, the adults with autism obtained an average AQ score of 25.25 (range 14–36) that was significantly greater than the control group ($t(32.3) = 4.35$, $P < 0.001$). All procedures were approved by the Weizmann Institute IRB and the Asaf Harofe Medical Center Helsinki Committee.

Stimuli

Two odor conditions were used to generate different emotional states: a fear odor condition and a neutral odor condition. The fear odor was created from absorbent pads that were placed in the underarms of skydivers 2 hr before their sky dive and were removed once they had landed. Cortisol swabs collected before and after the sky dive verified increased stress levels. The neutral odor was a clean absorbent pad. To verify that the fear-induced sweat swabs did smell more fearful, participants were asked to rate swabs on how pleasant, intense, and fear-smelling they were. The fear swabs were rated as being significantly less pleasant, more intense, and more fear inducing than the neutral swabs. This finding was consistent across autism and NT groups. The ratings of the swabs occurred around 8 months before the EDA study. After participating in the EDA study, participants were asked if they noticed any difference between the two sessions of the experiment. Only three participants in each group noticed a change in the odors. Therefore, the ratings study was unlikely to have influenced participants' EDA responses (see Endevelt-Shapira et al., 2018, for more information).

During the presentation of the fear or neutral odor, face stimuli were presented on a screen for 250 ms. EDA was time locked to the onset of the face stimuli to ascertain differences in responses during the odor conditions. The face stimuli were from the NimStim face database [Tottenham et al., 2009]. Nine neutral emotion faces, nine happy, and nine fearful faces were presented. Importantly, both odor types occurred in association with the three types of faces in a crossed factorial design.

Procedure

Two electrodermal electrodes were placed on the participant's nondominant hand. One electrode was placed on the second phalanx of the index finger and the other electrode on the third finger. EDA responses were measured by applying a 0.5-μA/cm^2 current from a low voltage (~40-mV AC excitation at 75 Hz) GSR Amp. FE116 (ADInstruments). Data were recorded using a PowerLab 16SP instrumentation amplifier that used LabChart7 software (ADInstruments, New South Wales, Australia) at 400 Hz.

To collect baseline data in an emotionally calm state, participants watched a nature video for 2 min. They then saw 27 faces (presented for 250 ms each) on a computer screen and were asked to rate them on how fearful they appeared using an on-screen rating scale. The subjects were asked to rate "how fearful the face" using a visual analogue scale, and were also asked to respond as

accurately and as quickly as possible, yet they were not limited in time. There was an inter-stimulus-interval of around 30 sec between each of the faces, resulting in each odor being presented for around 14 min. The same faces were presented during the fearful odor condition and during the neutral odor condition. The order of odor condition presentation was counterbalanced across participants and a 5-min nature video was presented between the two odor conditions to avoid cross-contamination. The odor was administered using a nasal mask and was presented at the same time as the faces. Participants were told that the mask was used to measure respiration and were not told about the presence of the odors.

Data Analysis

The raw EDA data were first filtered using a bandpass filter at 0.5–35 Hz. Consistent with the analysis reported by Endevelt-Shapira et al. [2018], one individual with autism was excluded due to excessive motion, and two additional individuals with autism and one NT were removed from analysis due to no EDA response (under 0.02 mS). EDA signal was then selected from 1 sec before stimulus-onset (faces and odors were presented at the same time) to 14 sec after stimulus-onset. Each epoch was baseline corrected (–1 to 0 sec). The data were not normalized (unlike the data reported by Endevelt-Shapira et al., 2018) as, by definition, this impacts the standard deviation. The mean EDA response was then calculated over the peak of the response (the mean response over the 4–8 sec time period after face onset) and this mean response was averaged across trials for each participant. The SD of the response (which was the TTV measure) was calculated by taking the average
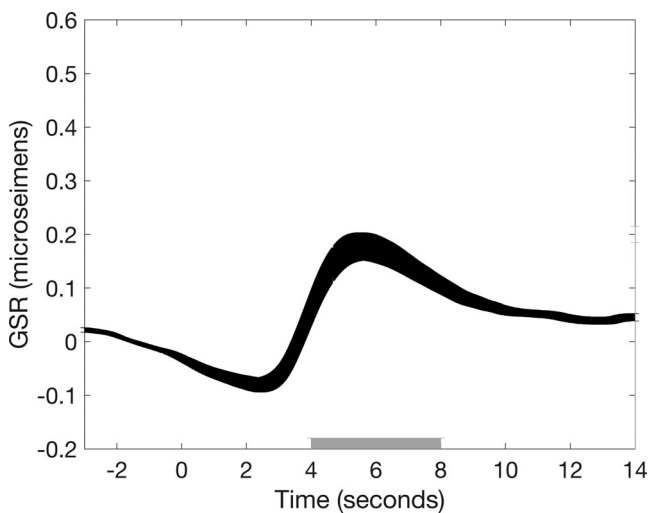
peak response (mean response over the 4–8 sec time period after face onset) and then measuring the standard deviation in the average peaks across trials for each participant. The time window for analysis was chosen as it captured the peak response for both the fear and the neutral conditions for both autism and NT groups, and so the analyses were not biased for one group or one condition (see Fig. 1). To investigate if any group differences in average peak EDA response created differences in signal-to-noise ratios (SNR), the mean EDA response for each participant was divided by their squared standard deviation. Reponses to fear and neutral odors were separated and analyzed using a mixed-measures ANOVA with group (autism and NT) as the between-subject variable and odor condition (fear and neutral) as the within-subject variable. Follow-up comparisons between groups for each odor condition were analyzed using independent-sample t-tests, Bayes Factors, and Cohen's *d* effect sizes. Bayes Factors (BF) were calculated using the R package "BayesFactor" and computed using Monte Carlo sampling over 50,000 iterations. The resulting BFs were then inverted to provide estimates of the probability of the effect occurring under the alternative hypothesis (H_1) compared to the null hypothesis (H_0). This was conducted as part of the "effectsize" R package and "interpret_bf" function following Jeffreys [1961] interpretation. Correlations between the average peak amplitude, TTV, age, and symptom scores (AQ and ADOS) were calculated using Spearman's correlations. All statistics were calculated in R and any violations of assumptions were corrected for by adjusting the degrees of freedom.

To assist comparison between the current study and the original study, we first briefly describe the analyses reported by Endevelt-Shapira et al. [2018]. Data were analyzed using the same filter parameters as reported for the current analysis. There were two key differences between the current and the original study. The first was that, in the original study, the data were normalized, by dividing each participants' EDA data by their absolute maximum value. Normalizing responses would eliminate the TTV and so we did not use this analysis method. The second difference was how the response amplitude was measured: Endevelt-Shapira et al. [2018] measured the difference in amplitude between the trough of the response immediately after stimulus-onset, whereas we measured the response over a period of time that corresponded with the maximum peak. Because previous studies have focused on the variability in the peak of the response as their measure of TTV [Dinstein et al., 2012; Haigh et al., 2014, 2016], we used this same measure too.

Figure 1. The average EDA response for all participants for both the fear and the neutral conditions. The average peak of the response (4–8 sec after face onset) is highlighted in gray. The mean and SD of this peak were used for analysis.

Results

The individuals with autism exhibited similar average peak EDA responses (the mean of the responses) in both fear
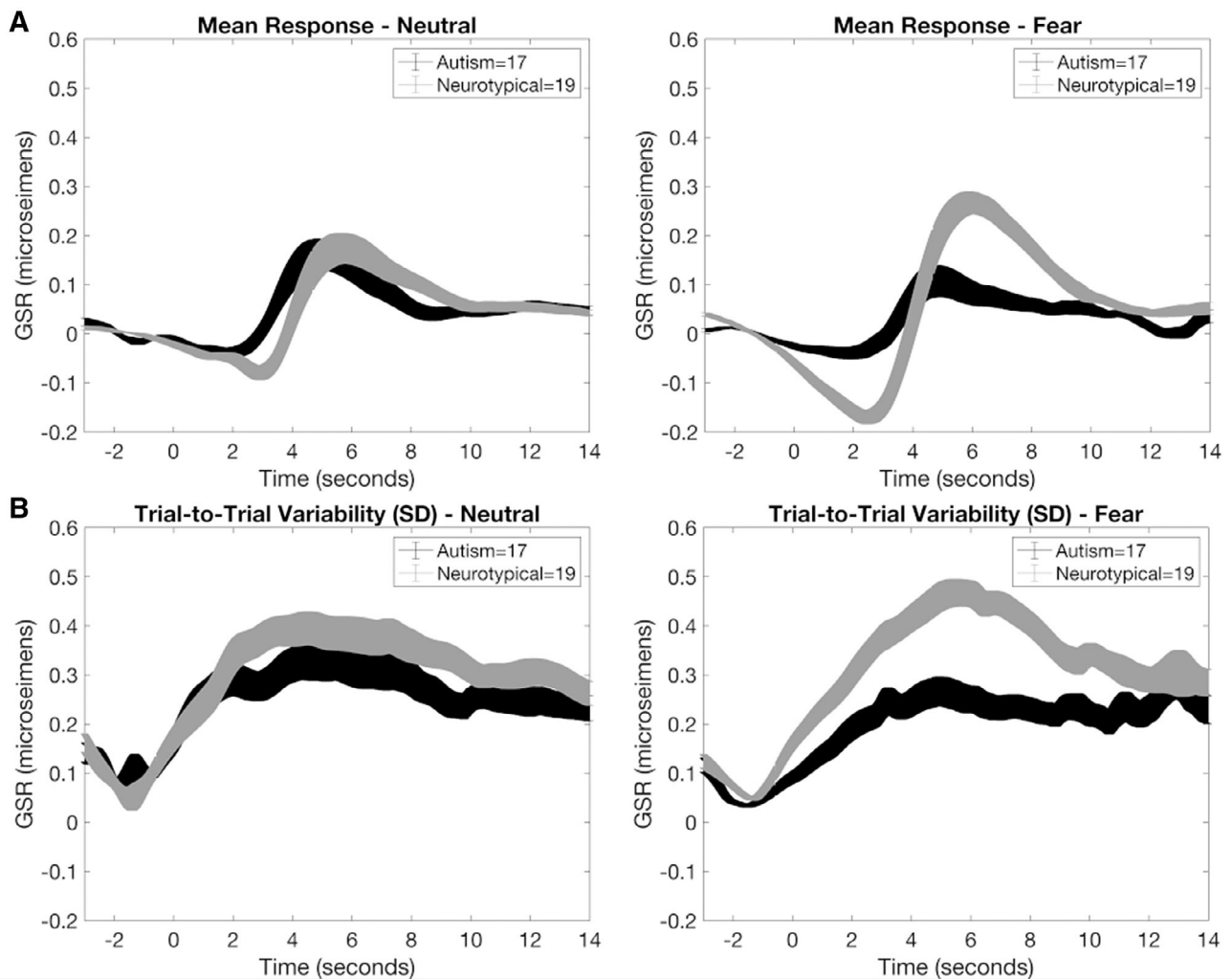
Figure 2. (A) Mean EDA activity to neutral (left) and fear (right) odor conditions, shown separately for autism and neurotypical controls (NT). (B) Standard deviation of EDA activity over trials to neutral (left) and fear (right) odor conditions, shown separately for autism and NTs. Error bars representing one standard error for each time point across individuals are shown.

and neutral odor conditions, whereas the NT controls produced a larger response in the fear compared to the neutral condition (group × odor interaction: $F(1,34) = 6.46$, $P = 0.016$; BF = 7.3 times more likely under H_1; error = 0.018), although there was no significant effect of odor condition ($F(1,34) = 1.01$, $P = 0.321$; BF = 0.94; anecdotal evidence under H_0; error = 0.006) or group ($F(1,34) = 1.13$, $P = 0.295$; BF = 2.8; anecdotal evidence under H_1; error = 0.013). Cohen's d effect sizes showed that group difference in the fear odor condition produced a medium effect size ($d = 0.51$) that was larger than the group differences in the neutral odor condition ($d = 0.11$; Fig. 2A). This result is similar to the findings reported by Endevelt-Shapira et al. [2018] where they reported that NTs participants exhibited greater trough-to-peak amplitudes in their normalized EDA responses to the fear odor compared to the neutral odor, whereas the autism group did not show a significant difference between the odor conditions.

Analysis of the standard deviation across trials at the peak of the EDA response (the measure of TTV) showed a similar trend. The autism group exhibited nominally less variability in their responses compared to NTs ($F(1,34) = 2.35$, $P = 0.135$; BF = 14.5 times more likely under H_1; error = 0.013), particularly in the fear condition, but the interaction was not significant ($F(1,34) = 2.22$, $P = 0.146$; BF = 0.56; anecdotal evidence for H_0; error = 0.013), nor was the main effect of condition ($F(1,34) = 1.01$, $P = 0.231$; BF = 0.56; anecdotal evidence for H_0; error = 0.006). Due to the significant group x odor interaction in the mean amplitude of the response (and the large BF suggesting the autism group differed from controls), independent t-tests were used to directly compare groups in the neutral and fear conditions. The individuals with autism exhibited significantly less variability to the fear odor ($t(29.4) = 2.14$, $P = 0.041$; $d = 0.69$) compared to NT, but there was no significant

difference to the neutral odor ($t(33.6) = 0.77$, $P = 0.446$; $d = 0.25$; Fig. 2B).

For the SNR in the responses, there was a trend interaction between condition and group ($F(1,34) = 3.64$, $P = 0.065$; $d(\text{fear}) = 0.31$; $d(\text{neutral}) = 0.18$), no effect of the fear compared to the neutral odor condition ($F(1,34) = 0.02$, $P = 0.894$), and no significant difference between groups ($F(1,34) = 0.06$, $P = 0.801$).

The relationship between average peak amplitude and standard deviation in the signal across trials was positive for both autism and NT groups in the fear and neutral odor conditions: the larger the amplitude, the greater the variability in the signal across trials. The NT group showed a significant correlation between amplitude and standard deviation in both the fear odor ($r_s(17) = 0.73$,

$P < 0.001$) and neutral condition ($r_s(17) = 0.74$, $P < 0.001$). Similarly, the autism group showed a significant correlation in the neutral condition ($r_s(15) = 0.66$, $P = 0.003$) and a weaker but still significant correlation in the fear condition ($r_s(15) = 0.49$, $P = 0.046$; Fig. 3).

Next, we assessed the relationship between the EDA response and symptoms. Overall, the average peak EDA response decreased with higher AQ score, but this was only significant for the autism group ($r_s(32) = -0.52$, $P = 0.002$) and not the NT group ($r_s(36) < 0.01$, $P = 0.990$). Specifically, the fear odor condition evoked the strongest correlation (fear: $r_s(15) = -0.60$, $P = 0.011$; neutral: $r_s(15) = -0.44$, $P = 0.077$; Fig. 4). Average peak EDA also decreased with age which was driven by the NT group ($r_s(36) = -0.65$, $P < 0.001$) and not the autism
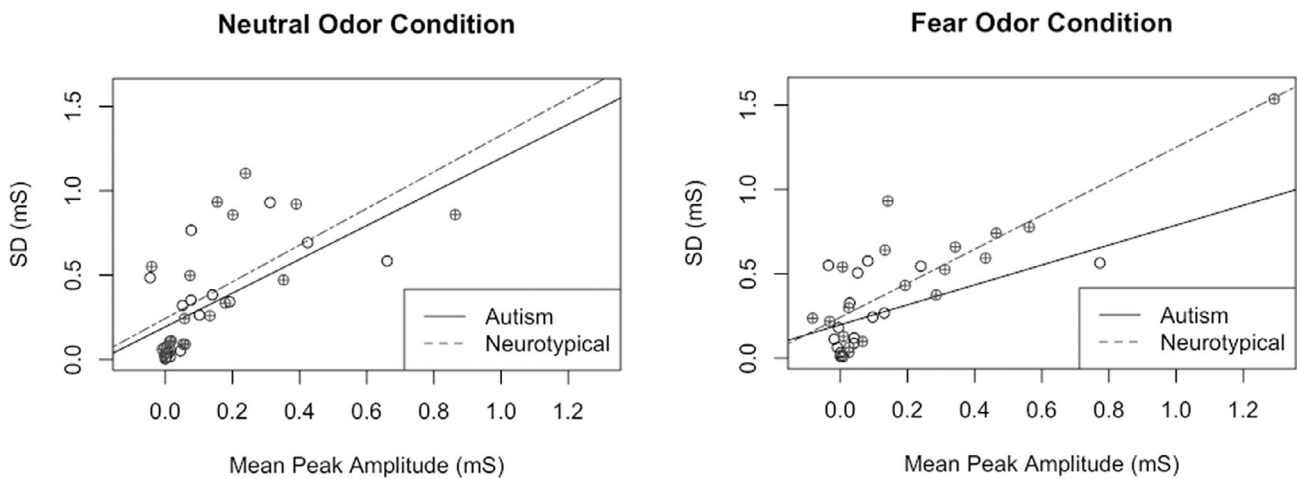

Figure 3. Relationship between mean and SD in the peak amplitude in the neutral odor condition (left) and the fear odor condition (right) shown separately for the autism and for the neurotypical control (NT) groups.
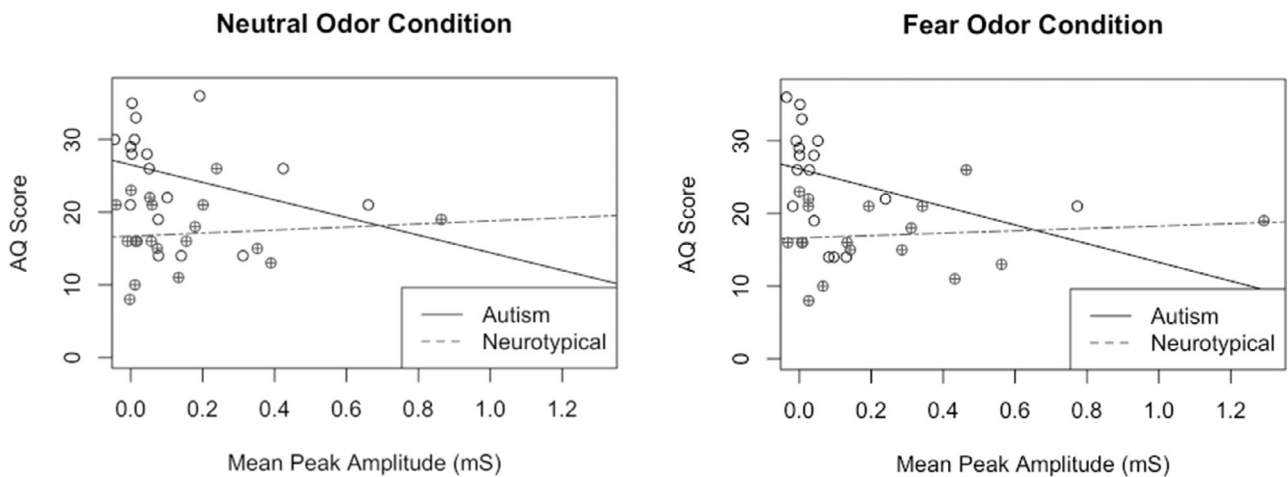

Figure 4. Relationship between AQ score and average peak amplitude in the neutral odor condition (left) and in the fear odor condition (right) in autism (black) and neurotypical (NT) controls (gray). Overall, individuals with autism with higher AQ scores showed smaller responses.
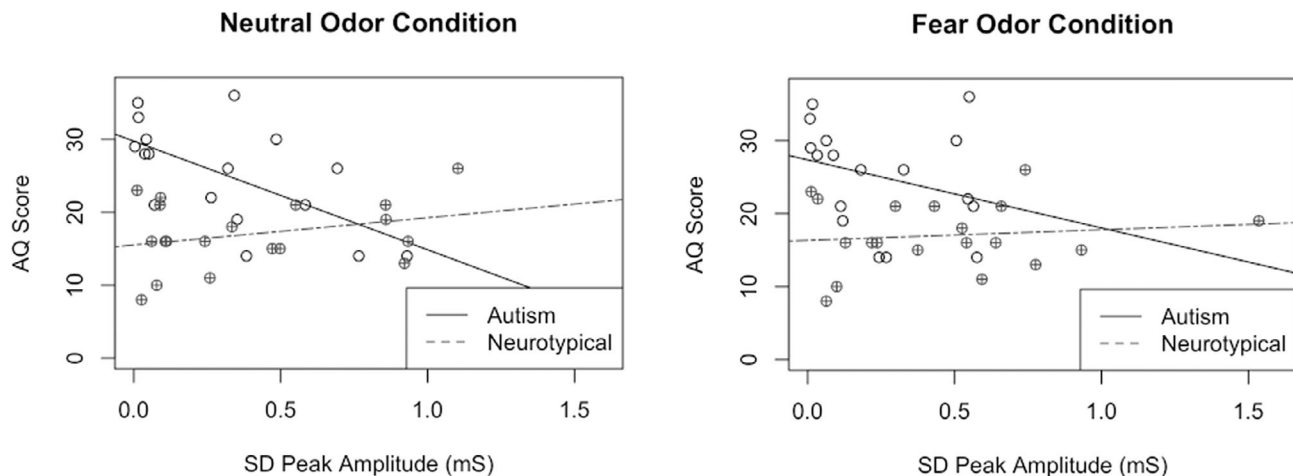
Figure 5. Relationship between AQ score and variability (SD) in peak amplitude in the neutral odor condition (left) and in the fear odor condition (right) in autism (black) and neurotypical (NT) controls (gray). Individuals with autism with higher AQ scores showed less variable responses.

group ($r_s(32) = -0.11$, $P = 0.524$). There were no other significant correlations with the average peak response.

For the variability in the response, variability decreased with higher AQ score, but again, this was only significant in the autism group ($r_s(32) = -0.51$, $P = 0.002$) and not the NT group ($r_s(36) = 0.07$, $P = 0.698$). However, this time, the relationship was stronger between AQ and the variability in the response in the neutral odor ($r_s(15) = -0.64$, $P = 0.005$) than the fear odor condition ($r_s(15) = -0.43$, $P = 0.082$; Fig. 5). Variability in the EDA response also decreased with age and was again driven by the NT group ($r_s(36) = -0.61$, $P < 0.001$) and not the autism group ($r_s(32) < -0.01$, $P = 0.964$). There were no other significant correlations with variability in the response.

Discussion

The goal of this study was to characterize the nature of the EDA during fear and neutral odor conditions in autism compared with neurotypical controls. Evaluating the dynamics of these data can offer insights into the parameters of the neural response that are abnormal in autism and how they relate to other properties of the signal (such as amplitude). These analyses can help delineate those conditions under which this perturbation manifests.

In light of recent findings of abnormal TTV in neural functioning in autism, investigation was warranted into the TTV in the EDA response. To date, abnormal cortical TTV in adults with autism has been identified under a variety of sensory conditions, but TTV in the EDA response has not been investigated, raising questions about how generalized the abnormal TTV is. In addition, EDA is monitored partly by subcortical structures

[Critchley, 2002] and so examination of the TTV in EDA can address whether the abnormal TTV in autism is cortically driven (which has been suggested by Dinstein et al., 2012) or is also evident when other systems are involved.

As documented previously, individuals with autism sometimes have smaller average EDA peak amplitudes than their controls, indicating atypical autonomic functioning [Panju, Brian, Dupuis, Anagnostou, & Kushki, 2015; Bujnakova et al., 2016]. Here, we adopted a new approach to examine the signal variability in an existing dataset of EDA recordings from ASD individuals and controls [Endevelt-Shapira et al., 2018]. The key result is that participants with autism exhibit *smaller* average EDA peaks and *smaller* TTV in response to faces under fear odor conditions. This pattern is surprising and is inconsistent with the previous reports of greater cortical TTV in autism. One possible explanation that might reconcile the seemingly disparate results is that a significant reduction in TTV is a signature of abnormal sensitivity in the peripheral nervous system, whereas a significant increase in TTV is a signature of abnormal cortical processing in autism. This provocative hypothesis lends itself to future investigation.

Of note too is that both the amplitude and TTV of the average peak response were reduced in those with autism who had higher AQ scores. This was not the case in the neurotypical group. This suggests that smaller and less variable EDA responses may be related to behaviors that are characteristic of autism, further supporting the group differences. These findings mirror the results of Fenning et al. [2017] who reported significant correlations between EDA TTV and symptom severity in children with autism. However, their findings revealed that greater EDA variability was associated with greater symptom severity, which is the opposite of the current results. It is

important to note that there may be a developmental effect moderating this relationship. Interestingly, in the current study, only AQ scores but not ADOS general scores were correlated with either EDA measure in the autism group, which is consistent with previous findings [Dinstein et al., 2012; Haigh et al., 2014]. Together, these findings clearly demonstrate that measures of variability are sensitive to individual differences in underlying symptomatology. When combined with the mean amplitude, TTV could provide a more detailed profile of physiological responses in autism.

One issue to consider is that the reduced TTV in EDA responses in autism in the fear odor condition could be a function of the smaller EDA changes in the same condition. While this is certainly possible, the correlation between amplitude and TTV in the autism group to the fear condition was the weakest out of all of the comparisons suggesting some decoupling of the two measures under certain conditions. In addition, TTV correlated with AQ scores in the autism group, again indicating that TTV is not simply related to average peak amplitude. A potential cause of the smaller EDA response in autism could be due to heightened EDA activity at baseline compared to the neurotypical controls, which could explain the smaller signal change in the autism group. The odors were presented with each face but likely had a lingering effect across the whole session. If the odor alone evoked a greater EDA response in autism, then the repeated exposure paired with the faces may have had less effect on the evoked EDA response than in the neurotypical controls.

There are a few limitations to this study that need to be addressed. The first is that the sample size is relatively small (17 autism and 19 neurotypical), although previous studies of TTV have reported on findings with samples as small as 15 participants and have shown similarly consistent effects of group differences in variability [Dinstein et al., 2012; replicated by Haigh et al., 2014]. For TTV to be a useful marker of abnormal physiology in autism, it should be easy to detect in small samples [Haigh et al., 2016]. Second, while we found that the autism group showed less SD in the fear condition compared to NT, but not in the neutral condition, the interaction was not significant in the ANOVA, and the Bayes Factor suggested a null effect. However, (1) the interaction was significant when assessing the peak of the EDA response; (2) the Bayes Factor suggested overall that there was a difference between groups in the SD; and (3) Figure 2B shows that the difference in SD between the two groups was bigger in the fear condition than in the neutral condition. Therefore, while the analysis using t-test is valid, we recommend some caution in interpreting the strength of these effects in SD. Third, all of the participants were male, which may limit the generalizability. While typically, autism diagnosis is more male prevalent [Werling, Parikshak, & Geschwind, 2016], there is some debate as to whether environmental and social factors are impacting the gender bias [Hiller, Young, & Weber, 2015]. Therefore, care must be taken when generalizing these findings to females who may manifest different chemosignaling responses. Fourth, as mentioned previously, it is difficult to ascertain whether the EDA response was already heightened in autism during the fear odor condition. EDA measurements work by detecting relative changes in the response. Therefore, it is difficult to know how the absolute EDA response differs in autism compared to neurotypical controls. Fifth, the behavioral responses to ascertain whether the fear odor did smell fearful was conducted to verify the stimuli. It was not designed to detect subtle group differences in fear odor identification. When measuring EDA responses, the odors were presented subliminally and so the study was not specifically designed to investigate group differences in chemosignal detection per se. Sixth, the study was designed to measure the responses to odors while faces were presented. The small number of face presentations for each emotion (nine happy, fearful, and neutral emotion) means that any potential interactions between odor and facial emotion cannot be explored. However, it should be noted that the very same faces were presented in both the fear and neutral odor conditions, meaning that the differences in EDA responses were due to odor and not the faces.

Neural Basis of Reduced TTV in Autism

In the context of previous findings of TTV, this is one of the first studies known to report reduced TTV in autism. This finding could be due to abnormalities in the peripheral nervous system that differ from the variability in the central nervous system (which has been the focus of the majority of studies to date). The EDA response is partially controlled by subcortical structures [Critchley, 2002], and subcortical structures such as the thalamus have been shown to produce normal TTV compared to neurotypical controls, despite the same individuals with autism showing greater TTV in cortical (sensory) areas [Dinstein et al., 2012]. Similarly, heart rate variability in adults with autism has also been reported to be reduced compared to controls [Dijkhuis et al., 2019; Thapa et al., 2019], suggesting a distinction between central and peripheral nervous system TTV. This suggests a disconnect between cortical TTV and TTV from subcortically driven systems.

It is possible that the two measures of stability (from cortical and EDA responses) are related in autism: if the central nervous system is unstable, then perhaps signals to the peripheral nervous system become dampened. It is also possible that there is a Yerkes-Dodson law effect where individuals with autism can exhibit too much variability (suggesting an unstable system) or too little variability (the system is too rigid) and neither option is

helpful for efficiently navigating complex environments. Measuring cortical and EDA TTV in the same individuals would go some way to understanding this relationship. A detailed profile of TTV and its relationship to other physiological responses can help identify abnormal neural mechanisms in autism that can be used as biomarkers and targets for treatment.

Previous findings of EDA responses in autism are mixed. Several studies have shown reduced amplitude of EDA responses to cognitive tasks [Panju et al., 2015] and reduced resting EDA activity (in boys only; Bujnakova et al., 2016), similar to the current results. However, other studies have reported larger EDA responses in, for example, boys with autism compared to typically developing boys in response to images, including faces [Cohen, Masyn, Mastergeorge, & Hessl, 2015], and larger EDA responses correlated with greater restrictive and repetitive behaviors (in toddlers; Prince et al., 2017). In addition, one study reported no significant differences in EDA in children during play [McCormick et al., 2014]. Sometimes these findings are context dependent. For example, Kushki et al. [2013] found larger EDA responses in baseline conditions and reduced responses in anxiety-inducing conditions in children with autism compared to controls [Kushki et al., 2013]. It should be highlighted that all of these studies were based on children of different ages, and so some of the discrepancy may be a function of altered developmental trajectories as well as due to the differing social and cognitive tasks. Including measures of TTV may also add further context to the response profile of EDA in autism.

Assessing variability in physiological measures reveals additional information on the pathophysiology of autism. From our reanalysis of the EDA response, adults with autism produce less variability in their responses under certain conditions. In addition, the variability is sensitive to individual differences in behavioral traits, as seen in the correlations with AQ score. Together, these findings lend support to an increased focus on abnormal TTV as a key characteristic of perturbed processing in autism. This is one of the first studies to report reduced TTV in autism, and so the next step is to ascertain how cortical variability is related to responses in the peripheral nervous system. Understanding how perturbations in different physiological responses are related to one another will help identify their impact on behavior and symptoms.

Conflict of interest

The authors have no conflict of interest to declare.

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