Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 2024 25(11):996-1008
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Research Article

https://doi.org/10.1631/jzus.B2300462

Pupillometry reveals hyper-arousal in response to auditory stimuli in autistic children

Ci SONG[1,2], Runsheng MA[1,2], Wei NI[1,2], Xinyue PENG[1,2], Xue LI[3,4,5], Ruoxi SHI[6], Yuanping ZHANG[7], Li YI[1,2,8]✉

[1] *School of Psychological and Cognitive Sciences, Peking University, Beijing 100871, China*
[2] *Beijing Key Laboratory of Behavior and Mental Health, Beijing 100871, China*
[3] *Peking University Institute of Mental Health, Beijing 100191, China*
[4] *NHC Key Laboratory of Mental Health, Peking University, Beijing 100191, China*
[5] *National Clinical Research Center for Mental Disorders, Peking University Sixth Hospital, Beijing 100191, China*
[6] *Qingdao Autism Research Institute, Qingdao 266107, China*
[7] *Tianjin YiTong Autism Research Service Center, Tianjin 300392, China*
[8] *IDG/McGovern Institute for Brain Research at PKU, Peking University, Beijing 100871, China*

Abstract: Atypical sensory responsivity is widely reported in autistic individuals and is related to elevated functional difficulties. Dynamically, altered initial responses and/or habituation rates could underlie their atypical averaged responses to repeated sensory stimuli. In this study we aimed to measure the arousal level in response to different types of auditory stimuli and the dynamic change of atypical arousal level using pupillometry in autistic children. In Experiment 1, 43 autistic children and 49 neurotypical (NT) children were asked to passively listen to a mild sound and an aversive sound repeatedly. In Experiment 2, 39 autistic children and 44 NT children who went through Experiment 1 listened to a gradually emerging non-startling sound and a suddenly emerging startling sound in a random order. We found that the autistic group showed hyper-arousal in response to the aversive sound and the startling sound as reflected by their larger change in pupil area. In comparison, these autistic children demonstrated normal arousal in response to the mild sound and the non-startling sound. Dynamically, the autistic group had a larger peak pupil area change than the NT group in the first trial and a normal habituation rate to the aversive sound. In summary, our results suggest hyper-arousal to aversive and startling stimuli and the role of larger initial responses in hyper-arousal in autism. Minimizing aversive and startling sensory stimuli or gradually increasing the volume of aversive auditory stimuli to allow autistic children to adapt using the principle of habituation is recommended to reduce the arousal level and problematic behaviors of autistic children.

Key words: Autism spectrum disorder; Arousal; Habituation; Auditory; Pupillometry

1 Introduction

Autism spectrum disorder is a neurodevelopmental disorder characterized by difficulties in social interaction and repetitive behaviors (American Psychiatric Association, 2013). These core symptoms are often accompanied by atypical sensory responsivity (Ben-Sasson et al., 2009), which has been added to the diagnostic criteria of autism in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (American Psychiatric Association, 2013). As a disorder with high heterogeneity, atypical sensory sensitivity ranges widely from under- to over-responsivity, with its degree ranging from mild to severe, and may be observed when receiving different sensory stimuli (e.g., bright light, strong orders, and touch) among different autistic people (Baranek et al., 2006; Lane et al., 2014; Robertson and Simmons, 2015; Uljarević et al., 2017). Sensory over-responsivity has been considered to relate to elevated functional difficulties in autistic individuals, including social difficulties, maladaptive behaviors, depression, and anxiety (Pfeiffer et al., 2005; Ben-Sasson et al., 2008; Green and Ben-Sasson, 2010). Expressions of sensory

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✉ Li YI, yilipku@pku.edu.cn
Li YI, https://orcid.org/0000-0003-4117-8828
Ci SONG, https://orcid.org/0000-0002-8677-1732

Received June 26, 2023; Revision accepted Oct. 29, 2023;
Crosschecked Oct. 21, 2024

over-responsivity include a wide range of exaggerated and/or prolonged negative emotional and behavioral responses. Given the potential association between sensory over-responsivity and physiological abnormalities (e.g., an increased arousal) (Miller et al., 2007; Chang et al., 2012), it is important to determine if autistic individuals would show a hyper-autonomic response to sensory stimuli. In the present study, we examined the arousal level of autistic children in response to different sounds using pupillometry.

Auditory stimuli often elicit over-responsivity in autism, such as covering ears when listening to a dog bark (Baranek et al., 2006; Haesen et al., 2011). Previous studies examined arousal levels of autistic individuals in response to auditory stimuli by averaging physiological or neural responses to several repeated sounds, and reported normal arousal (Palkovitz and Wiesenfeld, 1980; Stevens and Gruzelier, 1984; Chang et al., 2012; McCormick et al., 2014; Kuiper et al., 2019) or hyper-arousal (van Engeland, 1984; Barry and James, 1988; Chang et al., 2012; Green et al., 2013, 2015; Kuiper et al., 2019; Jung et al., 2021). Dynamically, atypical averaged responses to repeated auditory stimuli could be attributed to atypical initial responses and/or habituation rates. Habituation is often defined as the rate of amplitude decrement of physiological signals (Dawson et al., 2016). Studies revealed either slower habituation rates to auditory stimuli (James and Barry, 1984; Stevens and Gruzelier, 1984; Barry and James, 1988; Gandhi et al., 2021; Jamal et al., 2021) or normal habituation to auditory stimuli in autistic compared to neurotypical (NT) people (Chang et al., 2012; McCormick et al., 2014; Kuiper et al., 2019; Jung et al., 2021). It is important to consider habituation rates and initial responses together to interpret the dynamic trial-by-trial change of the arousal level (McDiarmid et al., 2017). However, only a few studies have specifically looked at the initial responses to auditory stimuli. Those studies revealed either normal (Schoen et al., 2009) or reduced (Stevens and Gruzelier, 1984; van Engeland, 1984) initial responses in autistic individuals. In summary, previous studies have not reached a consensus on whether autistic individuals show hyper-arousal in response to auditory stimuli or the role of initial responses and habituation in their response. This lack of consensus could be accounted for by variation in the stimuli and measurements used in previous studies.

Hyper-arousal of autistic individuals does not occur in response to all sensory stimuli. The characteristics of auditory stimuli play important roles in the arousal levels of autistic individuals. Autobiographies and clinical observations revealed that autistic individuals were sensitive to loud, unexpected, and high-pitched sounds (O'Connor, 2012). Studies that used specially designed auditory stimuli (e.g., traffic noise) to best differentiate autistic individuals from NT people indeed detected hyper-arousal in autistic individuals (Green et al., 2013, 2015). However, normal arousal levels were also reported in autistic individuals, probably because the auditory stimuli presented were not sufficiently intense or aversive. For instance, autistic individuals were more likely to manifest hyper-arousal to an 84-dB tone than to a 78-dB siren in sensory challenge tasks (Chang et al., 2012; Kuiper et al., 2019), probably because the former sound had a higher volume and was more aversive. The rapid onset of startling sounds may also contribute to hyper-arousal in autism, as subjects reported suddenly emerging sounds hurting their ears (Jones et al., 2003) and demonstrated a larger acoustic startle response as measured by eyeblinks (Takahashi et al., 2014) and cardiac activity (Ezell et al., 2022). In the present study, we tested if aversive sounds and startling sounds could lead to hyper-arousal in autism.

As for the measurements, most studies used reliable electrodermal activity (EDA) data to evaluate the arousal level modulated by the sympathetic nervous system in young autistic children. Pupillometry is another measurement of the autonomic nervous system that reflects arousal in response to auditory stimuli. Pupil diameter is modulated by the locus coeruleus-norepinephrine system as well as other neurotransmitters such as acetylcholine (Joshi et al., 2016), and has been verified to measure autonomic arousal of a combination of both the sympathetic and parasympathetic nervous systems (Bradley et al., 2008). Contactless pupillometry is more friendly to autistic children who may have tactile hypersensitivity than EDA, which requires attachment of an electrode (Marco et al., 2011). The placement of an electrode on autistic children with tactile hypersensitivity may make them hyper-aroused (Nuske et al., 2014), which may confound the response to the auditory stimuli. Moreover, excessive motion during experiments is common in autistic children who have difficulties sitting still, which may cause artefacts in EDA data but is less of a problem with advanced pupillometry systems (Nuske et al., 2014). Recent research has applied pupillometry to measure the arousal level in response to auditory stimuli of autistic adults (Top et al., 2019) and infants who later received an autism diagnosis (Rudling et al., 2022). For example, Top et al. (2019) reported normal averaged phasic pupillary responses, initial responses, and habituation rates in autistic adults. To the best of our knowledge, few studies have used pupillometry to measure the arousal level of autistic children in response to auditory stimuli.

In the present study we aimed to investigate the arousal levels of autistic children in response to non-social auditory stimuli using pupillometry. We also investigated whether atypical initial responses and/or habituation rates were underlying dynamic factors of an atypical arousal level. To this end, we asked autistic children and age- and intelligence quotient (IQ)-matched NT children to listen to sounds passively. In Experiment 1, children listened to sounds with different aversiveness, and we hypothesized that autistic children would show hyper-arousal to aversive auditory stimuli and demonstrate larger initial responses and/or slower habituation to this sound. In Experiment 2, children listened to non-startling and startling sounds, and we hypothesized that autistic children would demonstrate hyper-arousal to the startling sound.

2 Experiment 1

2.1 Methods

2.1.1 Participants

Forty-three autistic children and 49 NT children completed a passive listening task. Their ages ranged from 4.68 to 6.77 years. Six autistic children and three NT children were excluded from the analysis due to poor eye data quality (Section 2.1.4 Data analysis). Therefore, the final sample included 37 autistic children and 46 NT children. Parents and teachers reported no evidence of hearing impairments among the children. Full-scale IQ was measured using the Wechsler Preschool and Primary Scale of Intelligence—Fourth Edition (Wechsler, 2014). All autistic children were previously diagnosed by professional pediatricians based on DSM-5. In addition, we used the Childhood Autism Rating Scale (CARS) (Schopler et al., 1980) to assess the severity of symptoms in the autistic group. The NT children were recruited from typical kindergartens and primary schools. The two groups of children were matched on age and full-scale IQ. The detailed characteristics of participants are presented in Table 1.

Table 1 Participant characteristics in each group

Group	Number (boy)	Age (years)	Full-scale IQ	CARS
Experiment 1				
Autistic	37 (34)	5.72±0.55	109.03±18.67	33.36±3.22
NT	46 (43)	5.87±0.61	115.09±13.19	
t		−1.12	−1.73	
P		0.27	0.09	
Experiment 2				
Autistic	35 (32)	5.75±0.57	110.91±16.11	33.63±3.40
NT	41 (38)	5.86±0.57	114.15±12.88	
t		−0.78	−0.97	
P		0.44	0.33	

Data are expressed as mean±standard deviation (SD), except number (boy). Full-scale IQ was measured using the Wechsler Preschool and Primary Scale of Intelligence—Fourth Edition (Wechsler, 2014). CARS: Childhood Autism Rating Scale (Schopler et al., 1980); NT: neurotypical; IQ: intelligence quotient.

2.1.2 Stimuli and apparatus

We modified the two sound stimuli from Top et al. (2019) by Audacity (https://www.audacityteam.org). After modification, one sound stimulus was a rather mild sound consisting of a 2000-Hz sinewave tone presented at 75 dB, and the other was an aversive sound consisting of a 2000-Hz sawtooth tone (scratchier than the sinewave tone) presented at 85 dB. These sounds lasted for 2 s and gradually increased from silence to peak volume in the first 0.75 s to minimize startle effects. We adopted these sounds to test our hypotheses because they were designed to be non-social, novel to children, and different in aversiveness. Compared to Top et al. (2019), we modified these sounds in two respects. The first aim was to reduce the startle effects which otherwise could have confounded the effect of aversiveness on arousal. Second, we increased the volume of the original stimuli to increase the signal-to-noise ratio of pupil data, as well as to make the original aversive sound more aversive. Top et al. (2019) found normal arousal levels in autistic adults with the original aversive sound, so we increased the volume of the aversive sound to increase the effect of sound on arousal levels.

Pupil and gaze data were recorded using a Tobii Pro Spectrum eye tracker (Tobiitech Technology, Stockholm, Sweden) with a sampling rate of 300 Hz. The sound stimuli were presented through speakers placed on both sides of a monitor. Custom script written in Matlab was used to run the experiment, in which visual and sound stimuli were presented by Psychtoolbox (http://psychtoolbox.org), and the eye tracker was controlled by Tobii Matlab SDK.

2.1.3 Procedure

The experiment was conducted in a quiet room with lighting maintained at around 50 lx (measured around the eyes of the participants). Children sat about 60 cm away from the monitor. After completing a successful 5-point calibration, children were asked to look at the gray central fixation on the black monitor and listen to the sound throughout the task (Fig. 1a). They were told that some sounds might be annoying and loud and they were free to cancel their participation if they wished at any time. The first part of the experiment was a silent period of 20 s designed to measure the tonic pupil diameter. Then each of the two sound stimuli was played ten times in separate blocks (Top et al., 2019). This block-wise design was suitable for measuring habituation rates. Children were told that a new sound would be presented before each block started. The first block contained the mild sound and the second block the aversive sound (Top et al., 2019). In each trial, the sound was presented for 2 s and was followed by a compulsory resting time of 5.5 s for pupil recovery. After this resting time, the next sound was still not presented until the child stably looked at the predefined invisible 4° square at the center of the monitor. The inter-trial interval was paired between the autistic and the NT groups (Table S1).

2.1.4 Data analysis

Pupil data preprocessing (Supplementary information) was conducted before further analysis. We excluded invalid trials that contained less than 60% valid pupil data during the sound presentation or participants with less than six valid trials in any of the conditions. As a result, six autistic children and three NT children were excluded from the analysis. In the final sample, the NT group had significantly more valid trials than the autistic group in both sound conditions (Table S1). The pupil data validity was lower in the autistic group than in the NT group and did not systematically change over trials in both groups (Supplementary information: Trial-by-trial analysis of pupil data validity).

2.1.4.1 Tonic pupil diameter

The tonic pupil diameter was determined by averaging pupil diameters from the 20-s-silent period at the beginning of the experiment. An independent *t* test was applied to explore the difference of this index between the groups.

2.1.4.2 Time-course analysis of phasic changes in pupil area

To illustrate the time-course of phasic pupillary responses, we first averaged pupil diameters from 1 s before to 2 s after the sound onset across trials for each participant and condition. A centered 25-point moving average filter was applied to pupil diameter before further analysis. For each participant, each sound condition, and each time point (from 1 s before to 2 s after the sound onset), we calculated the 1-s baseline-corrected pupil area change to represent the amplitude of pupil dilation relative to baseline pupil area and indicated the arousal level raised by sounds. Each pupil was modeled as a circle, so pupil area equals $\pi\times(D/2)^2$, where D is

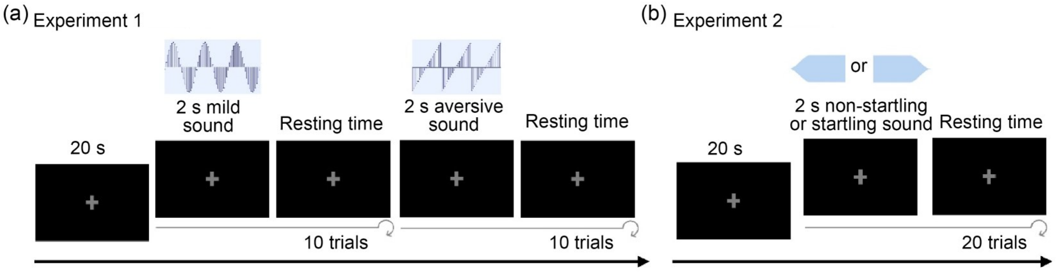

Fig. 1 Experimental procedures of Experiment 1 and Experiment 2. Both experiments started with a 20-s-silent period where tonic pupil size was recorded. Then in each trial, the sound was presented for 2 s, followed by a silent resting period. (a) In Experiment 1, 10 mild sounds were presented in the first block and 10 aversive sounds in the second block. (b) In Experiment 2, 10 non-startling and 10 startling sounds were presented in a random order.

the pupil diameter. Thus, the change in pupil area was calculated by taking the pupil area at a certain time point and subtracting the 1-s baseline pupil area (mm^2) using the formula $\pi/4\times(D_t^2-D_0^2)$, where D_0 is the average baseline pupil diameter 1 s before sound onset, and D_t is the pupil diameter at this time point. For each sound, we performed *t* tests to compare group differences in the pupil area changes at each time point and cluster-based permutation tests to do multiple comparisons.

We extracted two characteristics from the time-course. The first was the peak pupil area change (PPAC) between 0.5 s after the sound onset and the sound offset. This time window was set to ensure that the PPAC was related to the sound but not signal noise, as the pupil takes time to dilate (Hoeks and Levelt, 1993). The PPAC equals the maximum pupil area in this time window minus the 1-s baseline pupil area before the sound was present. The PPAC indicated the peak amplitude change of the pupillary response and characterized the peak level of arousal in response to sounds. We extracted the peak amplitude of pupillary response because pupillary response to sounds clearly shows a rise-and-fall pattern over time (Zhao et al., 2019), and this is in line with previous studies (Wang et al., 2017; Top et al., 2019). The second characteristic we extracted was the PPAC latency, that is, the time elapsed from the sound onset till the PPAC was reached. We examined these indices using 2 (group: autistic and NT)×2 (sound condition: mild and aversive) repeated-measure analysis of variance (ANOVA). Simple effect analysis of each independent variable was performed if a significant interaction effect was found in ANOVA.

2.1.4.3 Trial-by-trial analysis of PPAC

In view of the trial-by-trial change of the PPAC, we examined the initial response and habituation rate in each sound condition to determine which dynamic factor(s) was associated with atypical arousal level in autistic children. A centered 25-point moving average filter was applied to pupil diameter in each trial before further analysis. We operationalized initial responses as the PPAC in the first trial and used *t* tests to reveal its difference between groups in each sound condition. To evaluate the habituation rate, in each sound condition we applied a generalized estimation equation (GEE) model (Halekoh et al., 2006) to the PPAC with the main effects of group and trial, and the interaction between them as predictors. This model could master missing data due to invalid trials in the study. An autoregression of order 1 was used as the working correlation structure. We also applied a trial-by-trial analysis of the 1-s averaged baseline pupil size (Supplementary information) before sound onset.

2.2 Results and discussion

2.2.1 Tonic pupil diameter

The tonic pupil diameter of one NT child was not recorded. The tonic pupil diameter did not differ between the autistic ((4.69±0.49) mm) and NT groups ((4.72±0.62) mm) ($t(80)=-0.19$, $P=0.85$, Cohen's $d=0.04$), reflecting a normal resting-state arousal level in autistic children.

2.2.2 Time-course analysis of phasic changes in pupil area

Time-course analysis revealed no difference in the change in pupil area between the autistic and NT groups at all time points when listening to the mild sound (Fig. 2a). When listening to the aversive sound, the autistic children showed a larger change in pupil area than NT children between 0.21 s and 1.93 s after sound onset (Fig. 2b), suggesting that the autistic group experienced hyper-arousal when listening to the aversive sound.

The ANOVA for PPAC revealed significant main effects of group ($F(1, 81)=9.86$, $P=0.002$, $\eta_p^2=0.11$) and sound condition ($F(1, 81)=91.40$, $P<0.001$, $\eta_p^2=0.53$), as well as a significant group×condition interaction ($F(1, 81)=11.93$, $P<0.001$, $\eta_p^2=0.13$). Simple effect analysis showed that this interaction derived from the larger PPAC responding to the aversive sound in the autistic group than in the NT group ($F(1, 81)=15.63$, $P<0.001$, $\eta_p^2=0.16$); however, the PPAC was the same between the groups in the mild sound condition ($F(1, 81)=1.51$, $P=0.22$, $\eta_p^2=0.02$) (Fig. 2c). Simple effect analysis of the sound condition revealed that both the autistic group and the NT group had a larger PPAC when listening to the aversive sound than to the mild sound ($F(1, 81)=76.41$, $P<0.001$, $\eta_p^2=0.49$; $F(1, 81)=20.91$, $P<0.001$, $\eta_p^2=0.21$). Excluding the largest outlier in the autistic group in the aversive sound condition did not change the results. These results were in accordance with the results from the time-course analysis. The PPAC was not correlated with the 1-s baseline pupil diameter in each sound

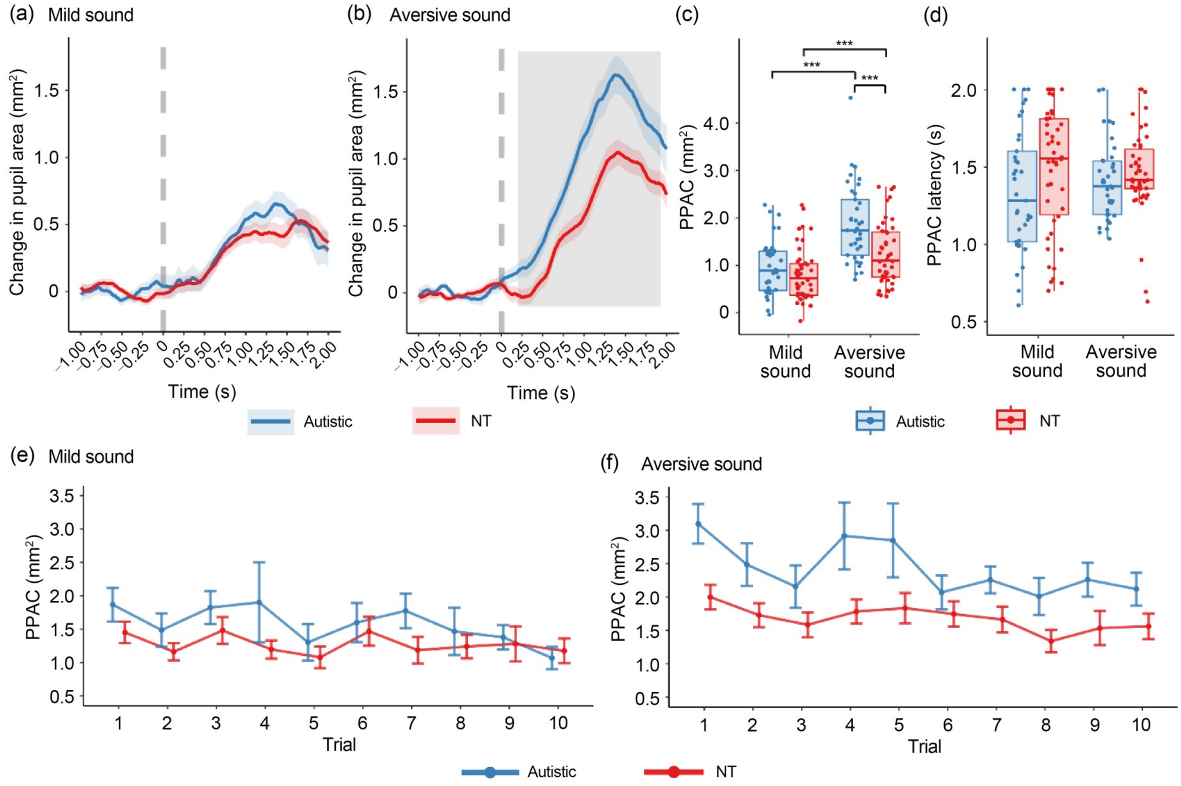

Fig. 2 Pupillary response of the autistic and neurotypical (NT) groups in Experiment 1. (a, b) Pupil area changes from baseline over time to the mild (a) and the aversive (b) sounds. The shallow area represents standard errors. The gray shaded area illustrates the time epoch when the autistic group had a significantly larger change in pupil area than the NT group. The two groups did not differ in pupil area change when listening to the mild sound. The autistic group had a larger change in pupil area than the NT group between 0.21 s and 1.93 s after the onset of the aversive sound. (c) Box plot of the peak pupil area change (PPAC) from baseline. Each dot represents one individual data point. The PPAC was larger in the autistic group than in the NT group only in the aversive sound condition. Both groups had a larger PPAC when listening to the aversive sound than the mild sound. (d) Box plot of PPAC latency. Each dot represents one individual data point. The PPAC latency did not differ between sound conditions or groups. (e, f) PPAC to the mild (e) and aversive (f) sounds across all trials. Error bars denote standard errors. The participants involved in each trial were not the same, since invalid trials were excluded. The autistic group had a larger initial response to the aversive but not to the mild sound. The two groups did not differ in habituation rate in both sound conditions. *** *P*<0.001.

condition (*P*>0.92). The ANOVA of PPAC latency showed no significant main effect of group or sound condition, or their interaction (all *P*>0.059; Fig. 2d). The PPAC or the PPAC latency was not correlated with the valid trial number in each group or sound condition (all *P*>0.14).

2.2.3 Trial-by-trial analysis of PPAC

In the mild sound condition, the initial response was not available for five autistic children and one NT child, as their first trial was invalid. As shown in Fig. 2e, the initial pupillary responses of autistic children were similar to those of NT children ($t(75)=1.46$, $P=0.15$, Cohen's $d=0.34$). The GEE model for PPAC revealed a significant main effect of trial, showing children habituated to the mild sound (Wald $\chi^2=4.23$, $P=0.04$). The main effect of group was not significant (Wald $\chi^2=2.44$, $P=0.12$). The interaction between trial and group was not significant (Wald $\chi^2=2.12$, $P=0.15$), indicating normal habituation rates in the autistic group.

In the aversive sound condition, the initial response was not valid for eight autistic children and five NT children. As shown in Fig. 2f, the autistic group responded more to the aversive sound in the first trial than the NT group as reflected by their larger PPAC ($t(68)=3.32$, $P=0.001$, Cohen's $d=0.81$). The GEE model revealed a significant main effect of trial (Wald $\chi^2=17.83$, $P<0.001$), demonstrating habituation to the aversive sound. The main effect of group was also significant (Wald χ^2=18.39, P<0.001), indicating a larger PPAC in the autistic group than in the NT group. The interaction effect between group and trial was not significant (Wald χ^2=2.88, P=0.09), indicating normal habituation rates in the autistic group.

The 1-s averaged baseline pupil diameter before the sound onset increased over trials at similar rates and stayed comparable between the autistic group and the NT group (Supplementary information: Trial-by-trial analysis of baseline pupil size).

In summary, Experiment 1 showed that autistic children demonstrated a normal arousal level to the mild sound, but a hyper-arousal level to the aversive sound. This hyper-arousal state was related to a hyper initial response rather than a slower habituation rate.

3 Experiment 2

3.1 Methods

3.1.1 Participants

Among all the participants who completed Experiment 1, 39 autistic children and 44 NT children participated in Experiment 2 a few minutes after Experiment 1. Four autistic children and three NT children were excluded from analysis based on the same criteria as in Experiment 1. Therefore, the final sample included 35 autistic children and 41 NT children. Detailed characteristics of participants are presented in Table 1.

3.1.2 Stimuli and apparatus

We modified the 2-s 2000 Hz sawtooth tone that was presented at 85 dB to create a non-startling sound and a startling sound. The non-startling sound gradually increased from silence to peak volume in the first 0.75 s. The startling sound suddenly reached peak volume and then gradually decreased to silence in the last 0.75 s to match the average intensity with the non-startling sound. Note that Experiment 1 also adopted this 2000-Hz sawtooth waveform. Therefore, participants had been familiarized with the waveform of these sounds, and the non-startling sound was exactly the same as the aversive sound in Experiment 1. This familiarization to the non-startling sound before Experiment 2 was in accordance with the acclimation to the background noise before testing acoustic startle response (Takahashi et al., 2014).

3.1.3 Procedure

Experiment 2 followed the same procedure as Experiment 1, except that the non-startling and startling sounds were presented 10 times in a random order rather than in separate blocks (Fig. 1b). The inter-trial interval was paired between the autistic and NT groups (Table S1). The NT group had significantly more valid trials than the autistic group in both sound conditions (Table S1). The pupil data validity was lower in the autistic group than in the NT group and did not systematically change over trials in either group (Supplementary information: Trial-by-trial analysis of pupil data validity).

3.1.4 Data analysis

We analyzed the tonic pupil diameter and the time-course of phasic changes in pupil area using the identical procedure as in Experiment 1. We did not perform the trial-by-trial analysis of arousal level as we presented two sound stimuli in a random order, which may interfere with habituation (Ghani et al., 2020).

3.2 Results and discussion

3.2.1 Tonic pupil diameter

The tonic pupil diameter of one NT child was not recorded. As in Experiment 1, the tonic pupil diameter did not differ between the autistic group ((4.74±0.56) mm) and the NT group ((4.82±0.58) mm) (t(73)=−0.61, P=0.54, Cohen's d=0.14).

3.2.2 Time-course analysis of phasic changes in pupil area

Compared to NT children, time-course analysis indicated that autistic children had a normal change in pupil area in response to the non-startling sound at all time points (Fig. 3a) and a larger change in pupil area between 0.75 s and 1.55 s after the onset of the startling sound (Fig. 3b). This suggests that the autistic group experienced hyper-arousal only when listening to the startling sound.

We also conducted 2 (group: autistic and NT)×2 (sound condition: non-startling and startling) repeated measure ANOVA on PPAC and PPAC latency. The

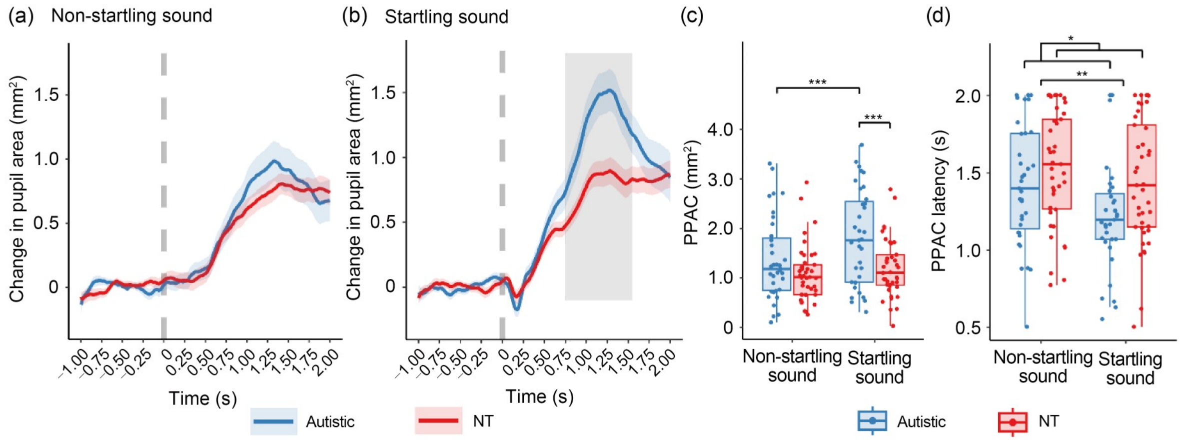

Fig. 3 Pupillary response of the autistic and neurotypical (NT) groups in Experiment 2. (a, b) Pupil area change from baseline over time to the non-startling (a) and startling (b) sounds. The shallow area represents standard errors. The gray shaded area illustrates the time epoch when the autistic group had a significantly larger change in pupil area than the NT group. The two groups did not differ in pupil area change when listening to the non-startling sound. The autistic group had a larger change in pupil area than the NT group between 0.75 s and 1.55 s after the onset of the startling sound. (c) Box plot of PPAC from baseline. Each dot represents one individual data point. The peak pupil area change (PPAC) was larger in the autistic group than in the NT group only in the startling sound condition. The PPAC was larger in response to the startling sound than to the non-startling sound only in autistic children. (d) Box plot of PPAC latency. Each dot represents one individual data point. The PPAC latency was shorter in the autistic group than in the NT group, and it was also shorter in the startling sound condition than in the non-startling sound condition. * *P*<0.05, ** *P*<0.01, *** *P*<0.001.

ANOVA for PPAC revealed significant main effects of group ($F(1, 74)=8.46$, $P=0.005$, $\eta_p^2=0.10$) and sound condition ($F(1, 74)=13.45$, $P<0.001$, $\eta_p^2=0.15$), as well as a significant interaction effect ($F(1, 74)=4.83$, $P=0.03$, $\eta_p^2=0.13$). For the group×condition interaction, a simple effect analysis of group revealed the same PPAC between the groups in the non-startling sound condition ($F(1, 74)=3.14$, $P=0.08$, $\eta_p^2=0.04$), but a larger PPAC to the startling sound in the autistic than in the NT group ($F(1, 74)=11.51$, $P=0.001$, $\eta_p^2=0.14$) (Fig. 3c). These results are in accordance with the results from the time-course analysis. Simple effect analysis of the sound condition revealed that the startling sound triggered a larger PPAC than the non-startling sound only in the autistic group ($F(1, 74)=15.93$, $P<0.001$, $\eta_p^2=0.18$), but not in the NT group ($F(1, 74)=1.18$, $P=0.28$, $\eta_p^2=0.21$). The PPAC was not correlated with the 1-s baseline pupil diameter in either sound condition ($P>0.14$). As shown in Fig. 3d, the ANOVA of PPAC latency showed a significant main effect of group ($F(1, 74)=5.39$, $P=0.02$, $\eta_p^2=0.07$), indicating that autistic children had a shorter PPAC latency than NT children. The main effect of sound condition was also significant ($F(1, 74)=7.60$, $P=0.007$, $\eta_p^2=0.09$), indicating that PPAC latency was shorter when children listened to the startling sound compared to the non-startling sound. The group×condition interaction was not significant ($F(1, 74)=1.05$, $P=0.31$, $\eta_p^2=0.01$). The PPAC and the PPAC latency were not correlated with the valid trial number in each group or sound condition (all $P>0.12$).

To conclude, autistic children showed hyper-arousal to the startling sound relative to NT children, even though these autistic children had been familiarized and showed normal arousal response to the non-startling sound of the same averaged intensity, frequency, and pitch as the startling sound. Notably, unlike Experiment 1, which found hyper-arousal to the aversive/non-startling sound, Experiment 2 found normal arousal to the non-startling sound in autistic children. This was probably because children were continually habituated to the sawtooth waveform through Experiments 1 and 2, and there was a trend of faster habituation to the aversive sound in the autistic group in Experiment 1 ($P=0.09$).

4 General discussion

In this study, we investigated the arousal level in response to auditory stimuli in autistic children using pupillometry. Consistent with our hypothesis, we found that: (1) autistic children demonstrated hyper-arousal to the aversive and the startling sounds; (2) in comparison, these children demonstrated normal arousal to the mild sound and the habituated non-startling sound; (3) moreover, hyper-arousal to the aversive sound in autism was accounted for by a larger initial response rather than a slower habituation rate.

First, when listening to the aversive and startling sounds, we observed hyper-arousal revealed by larger pupillary responses in the autistic group than in the NT group, which was in accordance with most previous findings (Barry and James, 1988; Chang et al., 2012; Jung et al., 2021). This hyper-arousal state of autism was also found in other scenes (Espinosa et al., 2020; Stuart et al., 2023) and supports theories that use hyper-reactivity to explain symptoms of autism, such as the intense world theory (Markram and Markram, 2010). This theory proposes that the microcircuits in the autistic brain are hyper-reactive and hyper-functional, which causes hyper-sensitivity, hyper-fear, and hyper-emotionality in cognitive processing. This cognitive processing pattern renders the world intense and aversive to autistic individuals, which accounts for their routine repetition and social withdrawal (Markram and Markram, 2010). However, some other studies such as Top et al. (2019), with similar auditory stimuli and paradigm as in Experiment 1, did not find this hyper-arousal pattern in response to auditory stimuli in autistic individuals (McCormick et al., 2014). Note that we increased the volume of the sound compared to Top et al. (2019), which may explain the inconsistent results. Another explanation is that Top et al. (2019) recruited much older participants than we did, and maturation of the sensory processing system and coping strategies to regulate sensory input in autistic individuals causes their auditory sensitivity to decrease with age (O'Connor, 2012).

Second, the hyper-arousal to sounds in the autistic group should be discussed together with their normal pupillary response to the mild sound and the habituated non-startling sound. As the stimuli became more intense, autistic children had steeper increases in arousal levels than NT children, suggesting their altered gain control. This is consistent with previous findings such as that autistic youth showed sensorilimbic hyper-activation to a greater extent when mildly aversive stimuli were presented in two modalities simultaneously compared to a single modality (Green et al., 2015), as well as higher subjective loudness perception to loud rather than soft sounds in autistic individuals compared to controls (Khalfa et al., 2004). More specifically, our results suggest that aversiveness and the rapid onset of sound contribute to hyper-arousal in autism. This echoes previous findings showing that receiving unpleasant other than pleasant and neutral textures causes greater affective somatosensory processing (Cascio et al., 2012) and a hyper-acoustic startle response (Takahashi et al., 2014) in autistic individuals, and broadly speaking may explain intolerance to unexpected events in autism (Hodgson et al., 2017).

Third, we found that a larger initial response, but not a slower habituation rate, was an explanatory factor of hyper-arousal to repetitive aversive auditory stimuli in autism in Experiment 1. The lack of difference in habituation rate between the autistic group and the NT group could not be explained by baseline pupil size and data quality that may potentially have changed over trials, as these factors did not change or changed at the same rate between groups over the course of the experiment (Supplementary information). Our results showing a larger initial response and normal habituation in autism seem to be in accordance with studies that found overall-hyper-arousal in all trials in autism (Jung et al., 2021), yet contradict those that found normal or smaller initial responses and/or slower habituation rates in autism (Stevens and Gruzelier, 1984; van Engeland, 1984; Schoen et al., 2009). This controversy may be accounted for by differences in participant characteristics and measurements. For instance, slower habituation is probably more common in the low-functioning rather than in the high-functioning autistic children of the present study (Kuiper et al., 2019). It is also possible that slower habituation in autism is less likely to occur in EDA measures and pupillometry (McCormick et al., 2014; Top et al., 2019) relative to brain activity measures, as evidence for slower neural habituation in autism has increased in recent years (Gandhi et al., 2021; Jamal et al., 2021).

Our results on sensory hyper-arousal in autism are potentially linked with comorbid symptoms in autism. First, autonomic hyper-arousal in an aversive scene may indicate and/or cause anxiety. For example, Espinosa et al. (2020) found stronger electrodermal activities in response to cues with aversive outcomes after observing a model’s distress to this aversive stimulus in autistic individuals, indicating that their enhanced vicarious fear learning contributes to the development of anxiety. Hyper-arousal to sensory stimuli may elicit anxiety in autism as this overresponse could generalize through contextual conditioning (Green and Ben-Sasson, 2010; Green et al., 2012). On the contrary, it is also possible that anxiety elicits hyper-arousal to sensory stimuli in autism, as anxiety may make autistic people hypervigilant to the aversive stimuli and prevent emotional regulation (Green and Ben-Sasson, 2010). Second, hyper-arousal to sensory stimuli in autism may relate to elevated emotional and behavioral problems, as this over-responsiveness may accompany negative emotion, make children act defensively to sensory stimuli, and consequently hinder the development of skills (Miller et al., 2007; Chang et al., 2012). However, a blunted arousal level in response to sounds has also been found to relate to elevated externalizing problem behaviors (Kleberg et al., 2023). Future studies should further explore how atypical arousal levels are related to other symptoms in autism.

Pupillometry has been widely used in autism research in recent years. de Vries et al. (2021) carried out a systematic review and meta-analysis of pupillometry in autism, and found a normal baseline pupil size, normal amplitude of pupillary response, and longer latency of pupillary response in autistic compared to NT individuals. Our findings on baseline pupil diameter before a sound was presented were in accordance with this meta-analysis. However, we found a larger pupillary response to the aversive sound and the startling sound as well as a shorter latency to peak pupillary response in Experiment 2 in the autistic group than in the NT group, which is inconsistent with de Vries et al. (2021). de Vries et al. (2021) reviewed studies on pupillary response to light and complex visual stimuli but not sounds, which may explain this contradiction. In addition, the inconsistency on latency may be accounted for by differences in the definition of this parameter in the meta-analysis (for example, in pupillary light reflex, latency is defined as the time elapsed from light being present to the start of pupil constriction) from the present study. Future studies could optimize research design and participant enrollment to deepen our understanding of pupillometry in autism.

There were several limitations in the current study. First, we did not include low-functioning autistic children since our method placed a relatively high demand on visual attention to the screen. However, low-functioning autistic children may display different types of physiological responses to sounds compared to high-functioning autistic children (Orekhova et al., 2008). Future studies should involve low-functioning autistic individuals with proper paradigms and apparatus. Second, autism has high heterogeneity. A subgroup of autistic individuals has been documented to show normal or below normal brain or electrodermal responses to sensory stimuli (Schoen et al., 2008; Green et al., 2015). However, limited by the small sample size, we were not able to detect this subgroup with physiological data through the clustering method. Future research could recruit more autistic participants or distinguish this subgroup using other measures such as parent-report questionnaires (Green et al., 2015, 2019). Third, we did not collect comorbidity information such as anxiety level or include it in our analyses. Comorbidities of autism, such as attention-deficit/hyperactivity disorder (ADHD) and anxiety disorders, have been linked to atypical arousal levels (Chiu et al., 2016; Kleberg et al., 2021). For example, hypo-arousal has been considered a potential pathogenetic factor of ADHD in recent decades (Geissler et al., 2014), and both hypo- and hyper-arousal have been documented when processing emotional stimuli in this population (Conzelmann et al., 2014; Kleberg et al., 2021). Anxiety could cause activation of the sympathetic nervous system (Hoehn-Saric and McLeod, 2000), which raises the arousal level. Future research could consider comorbidities when examining arousal response to auditory stimuli in autism.

5 Conclusions

In summary, we found that compared to NT children, autistic children demonstrated hyper-arousal to an aversive sound and a startling sound, but showed a similar arousal level to a mild sound and a habituated non-startling sound. The hyper-arousal response to the aversive stimuli in autistic children was related to larger initial responses but not slower habituation rates. Practically, minimizing aversive and startling sensory stimuli in daily life and building top-down coping strategies to manage sensory stimuli should be helpful in reducing the arousal level, overwhelming feelings, and problematic behaviors in autistic children. When facing an inevitable aversive sound, gradually increasing the volume of the sound could help autistic children to adapt through the principle of habituation.

Future research could explore how atypical responses to sensory stimuli are related to other functional difficulties such as social information processing barriers in autism.

Data availability statement

The data are available from the corresponding author on reasonable request.

Acknowledgments

This study was supported by the Key-Area Research and Development Program of Guangdong Province (No. 2019B030335001) and the National Natural Science Foundation of China (No. 32271116). The authors are grateful to the staff at the Qingdao Elim School, the Peking University Sixth Hospital, Tianjin YiTong Autism Research Service Center, and the children and parents who participated in our study.

Author contributions

Li YI, Ci SONG, and Xue LI contributed to the conception of the study. Ci SONG and Wei NI designed the experiments. Ruoxi SHI, Yuanping ZHANG, and Xue LI recruited the participants. Runsheng MA, Xinyue PENG, Ci SONG, and Wei NI performed the experiments. Ci SONG, Runsheng MA, and Xinyue PENG performed the data analyses. Ci SONG and Li YI wrote the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Ci SONG, Runsheng MA, Wei NI, Xinyue PENG, Xue LI, Ruoxi SHI, Yuanping ZHANG, and Li YI declare that they have no conflicts of interest.

The authors complied with the ethical standards laid down in the 1964 Declaration of Helsinki and this work was approved by the Committee for Protecting Human and Animal Subjects at School of Psychological and Cognitive Sciences at Peking University, China (No. 2019-01-02). Informed consent was obtained in writing from all individual caregivers included in this study. Children's oral consents were obtained before the experiment. Children were told that they were going to listen to sounds and some might be annoying and loud before the experiment. Parents and children were informed that they were free to cancel their participation at any time if they wished to and would not be punished.

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Supplementary information

Table S1; Pupil data preprocessing; Trial-by-trial analysis of pupil data validity; Trial-by-trial analysis of baseline pupil size