



**HAL**  
open science

## **Autonomic disequilibrium at rest in autistic children and adults**

Maria Rosa Bufo, Marco Guidotti, Mathieu Lemaire, Joëlle Malvy, Emmanuelle Houy-Durand, Frederique Bonnet-Brilhault, Frédéric Briend, Nadia Aguillon-Hernandez, Claire Wardak

► **To cite this version:**

| Maria Rosa Bufo, Marco Guidotti, Mathieu Lemaire, Joëlle Malvy, Emmanuelle Houy-Durand, et al..  
| Autonomic disequilibrium at rest in autistic children and adults. 2023. hal-04231045

**HAL Id: hal-04231045**

**<https://hal.science/hal-04231045>**

Preprint submitted on 6 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Autonomic disequilibrium at rest in autistic children and adults

Maria Rosa Bufo<sup>a,b</sup>, Marco Guidotti<sup>a,b,c,d</sup>, Mathieu Lemaire<sup>a,b,c,§</sup>, Joëlle Malvy<sup>a,b,c</sup>, Emmanuelle Houy-Durand<sup>a,b,c</sup>, Frédérique Bonnet-Brilhault<sup>a,b,c</sup>, Frédéric Briend<sup>a,b</sup>, Nadia Aguilon-Hernandez<sup>a,b,#</sup>, Claire Wardak<sup>a,b,#\*</sup>

a : UMR 1253, iBrain, Université de Tours, Inserm, France

b : Exac.t (EXcellence Center for Autism – Tours), FHU HUGO, CHRU de Tours, Tours, France

c : Centre Universitaire de Pédopsychiatrie, CHRU de Tours, Tours, France

d : Centre Hospitalier du Chinonais, Saint-Benoît-la-Forêt, France

§ Present adress: Medical office, 11 bis place Jean Jaurès, Tours, France

# co-last authors

\* Corresponding author: Claire Wardak, [claire.wardak@univ-tours.fr](mailto:claire.wardak@univ-tours.fr)

## Abstract

Autism Spectrum Disorders (ASD) symptoms have been proposed to be linked to Autonomic Nervous System (ANS) dysfunctions, in particular sympathetic hyper-arousal and parasympathetic under-activation. The objective of this study was to characterize these possible ANS dysfunctions at rest in children and adults.

To characterize several aspects of autonomic functioning, we recorded simultaneously pupil diameter, heart rate and electrodermal activity during 5 minutes of rest in 44 children (6-12 years old, 22 autistic) and 42 adults (19-52 years old, 21 autistic). Several parameters allowed to characterize tonic and phasic indices of sympathetic and parasympathetic systems at rest.

Autistic children exhibited the expected pattern of parasympathetic under-activation at rest compared to their typically developing (TD) peers, and with a tendency for a higher phasic

sympathetic activity. Adults exhibited a reverse autonomic pattern, with ASD individuals showing higher sympathetic tonus and lower sympathetic phasic activity than their TD peers.

In conclusion, we observed dysautonomia at rest both in autistic children and adults, but with opposite patterns that could reflect adaptive compensation mechanisms during maturation. The autonomic disequilibrium observed in autistic children would switch from excessive phasic components to excessive tonic components in adults, possibly subtended by an atypical locus coeruleus functioning.

**Keywords:** Autonomic homeostasis; pupil; skin conductance; heart rate; heart rate variability; tonic parameter; phasic parameter; parasympathetic nervous system; sympathetic nervous system; Autism Spectrum Disorder.

## **Abbreviations**

ADI-R: autism diagnostic interview-revised;

ADOS: autism diagnostic observation schedule;

ANS: autonomic nervous system

AQ: autism quotient

ASD: autism spectrum disorder

AUC: area under the curve

BMI: body mass index

CDA: continuous decomposition analysis

CIM-10: international classification of diseases and related health problems -10th revision

DSM-5: diagnostic and statistical manual of mental illnesses - 5th revision

ECG: electrocardiogram

EDA: electrodermal activity

GLM: general linear model

HF: high frequency

HRV: heart rate variability

IQ: intellectual quotient

LC: locus coeruleus

LF: low frequency

PNS: parasympathetic nervous system

RMSSD: root mean square of successive differences between normal heartbeats

RSA: respiratory sinus arrhythmia

SNS: sympathetic nervous system

TD: typically developing

## 1. Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder. With a prevalence estimated around 1% in the world (Maenner et al. 2021; Matson et Neal 2009), it is defined by qualitative impairments in social communication, repetitive behaviors and restrictive interests, and sensory alterations (DSM-5, 2013). Several clinical symptoms have been interpreted as possibly reflecting Autonomic Nervous System (ANS) dysfunction in ASD, like social engagement deficits (Porges 2001), atypical sleep patterns (Liu et al., 2006) or gastrointestinal disorders (Ferguson et al., 2017).

The ANS maintains homeostasis (Cannon, 1929), mainly through a complex interaction between its two sympathetic and parasympathetic branches, involved respectively in high and low arousal states (McCorry, 2007), in response to a stimulus (autonomic reactivity) but also at rest (autonomic tone). Extending Cannon's "Fight or Flight" theory (Cannon, 1929), Porges' Polyvagal theory (1995; 2009) focused on adaptive mobilization of parasympathetic (PNS) and sympathetic (SNS) nervous systems in response to the context. In particular, the influence of the PNS has been evaluated by the measure of the cardiac vagal tone. These studies have revealed that, on top of its role in cardiac balance restoration, the PNS may play a crucial role in freezing (Friedman et al., 1993) but also in empathy and social engagement (Fabes et al., 1994; Hastings et al., 2008; Suess et al., 1994).

The cardiac vagal tone has been described as downregulated in ASD (Porges et al., 2013), possibly related to social communication maladjustment in these individuals. Cardiac studies mainly suggest a hyper-arousal state in children with ASD, with an elevated basal heart rate (Kushki et al., 2014) and a reduced cardiac parasympathetic activity (Ming et al., 2016). In their meta-analysis, Arora et al. (2021) reported that 83% of studies using cardiac autonomic measures point towards a hyper-arousal in ASD. This hyper-arousal at rest could interfere with the generation of optimal ANS responses to external stimuli (Porges, 1995; Dalton et al., 2005; Schultz et al., 2006; Arora et al., 2021; Dell'Osso et al., 2022). Moreover, it has been proposed that repetitive behaviors and social avoidance may be adaptive strategies to regulate arousal in ASD individuals (Hirstein, Iversen, et Ramachandran 2001; Hutt et al. 1964)

Most of the cardiac indices studied reflect PNS regulation (e.g. Respiratory Sinus Arrhythmia -RSA, or Low and High Frequencies in Heart Rate Variability -HRV analyses), while heartbeat is influenced by both SNS and PNS. Other autonomic measures could thus help to better understand the respective SNS and PNS modulations of autonomic functions in ASD. Indeed,

in their review, Arora et al., (2021) showed that studies using electrodermal activity (EDA), under strict SNS control mostly found hypo-arousal (in 56% of the studies) in ASD, while studies using pupil diameter (influenced by both SNS and PNS) are too few to properly conclude about arousal in ASD. It is important to note that SNS and PNS are not necessarily antagonistic, with possibilities of co-activation, co-inhibition, and uncoupled modes of functioning that could depend on the organ considered (Berntson et al., 1991; 2007).

The inconsistency of arousal differences between ASD and typically developing (TD) peers could thus arise from the precise autonomic indices and effectors chosen. This suggests that simultaneous measures of several ANS parameters could help resolve these inconsistencies. Moreover, ANS studies in ASD have targeted different age groups. While Arora et al. (2021) evidenced that differences (or absence of difference) between TD and ASD populations could be found whatever the age, only one study directly tested in the same paradigm several age groups (Tessier et al., 2018) and showed hypo-PNS modulation in ASD, only in adults. Other factors could influence the overall pattern of results, like the experimental state (activation or rest), and Arora et al., (2021) pointed towards a larger difference between ASD and TD populations during rest. At rest, most studies have focused on tonic values of ANS effectors, i.e. stable components characterizing the tonic ANS discharge at rest, while other phasic parameters can also be observed, like spontaneous EDA peaks. These phasic parameters are not usually studied but could reflect, in a pathological context as in our study, the lability of resting state. Another factor influencing the difference between ASD and TD populations at rest is the duration of the recordings, with higher differences when the recordings lasted longer than 3 minutes (Arora et al., 2021). More spontaneous fluctuations of ANS state in ASD could indeed lead to more variable measurements if the recording interval is short, raising the question of ANS lability in ASD. This lability could be an exacerbation of the natural ANS physiological noise observed in TD populations (Iacovella and Hasson, 2011), and possibly linked to intra-individual physiological unreliability (Dinstein et al., 2012; Latinus et al., 2019).

The goal of this study was to characterize ANS functioning at rest in ASD populations. To this end, we tested both children and adults, comparing ASD and TD age-matched participants. As previously used to study ANS functioning at rest and its maturation process in TD populations (Bufo et al., 2022), we tested a 5 minutes rest paradigm, recording simultaneously electrodermal activity, heart rate and its variability, and pupillary diameter. These three effectors being differently controlled by sympathetic and parasympathetic branches, this approach could help

to better understand the hypo- and hyper-arousal suspected in ASD. Considering the literature, we hypothesized that, if ANS dysregulation was present, we should observe mainly hypo-PNS and hyper-SNS modulations of our parameters (Arora et al., 2021). Moreover, within each individual, possible temporal ANS physiological fluctuations could be observed in ASD participants compared to their TD peers (Iacovella and Hasson 2011; Dinstein et al., 2012; Latinus et al., 2019).

## **2. Material and method**

### **2.1. Participants**

Twenty-two children with ASD (aged 6 to 12 years, mean age: 8.1 years  $\pm$  1.7, 2 females) and twenty-one adults with ASD (aged from 19 to 49, mean age: 29.4 years  $\pm$  7.0, 11 females) were recruited through the University Hospital of Tours, EXcellence in Autism Center-Tours (see Table 1). Inclusion criteria were ASD diagnosis according to DSM-5 (DSM-5, 2013) or CIM-10 criteria, and age between 6 and 12 years old for children and above 18 years old for adults. The ASD diagnosis was made by a child or an adult psychiatrist by using ADI-R (Lord et al. 2016) and/or ADOS-2 (Lord, Rutter, et Le Couteur 1994) assessments, after a multidisciplinary evaluation including detailed developmental history, psychiatric assessment and pediatric, psychological and neurological examinations. Non-inclusion criteria were the use of medical treatments affecting neural activity. Age-matched TD participants were recruited according to the following criteria: children aged between 6 and 12, no psychiatric disorders or neurologic diseases previously diagnosed, no learning disabilities or difficulties. The TD groups were constituted of twenty-two children (aged 6 to 12 years, mean age: 9.5 years  $\pm$  1.7, 16 females) and twenty-one adults (aged from 23 to 52, mean age: 34.7 years  $\pm$  6.5, 17 females). All the TD recruitments were done locally in the University, University Hospital, and local schools. The TD children were already part of the data described in a previous paper (Bufo et al., 2022).

The children gave verbal consent. Their parents and the adult participants provided written informed consent according to institutional guidelines. The experiment conformed to the Code of Ethics of the World Medical Association (World Medical Association, 2013). The protocol was ethically approved by the Comité de Protection des Personnes (CPP; protocol PROSCEA 2017-A00756-47). All the participants were recorded in the same conditions, in the University Hospital of Tours, France.

For ASD participants, IQ and CARS scores were evaluated during their clinical evaluation outside of the research protocol. For TD participants, IQ scores were evaluated with 4 subtests of the WISC-IV in children (cube, matrix, vocabulary, and similarities) and 6 subtests of the WAIS-IV in adults (cube, matrix, puzzles, vocabulary, similarities, and information), before or after the experimental protocol for children and in a separate session for adults. For adults, AQ and questionnaires about weight, size, coffee drinking and smoking habits were filled before the protocol. Indeed, ANS basal state can also be sensitive to several parameters affecting directly the metabolism and that have been shown to differ between ASD and TD populations (e.g. Body Mass Index BMI; Molino et al., 2009). The effect of BMI, smoking and coffee drinking are presented in Supplementary Information (Table S1).

	Children		Children subgroups		Adults	
	TD <sub>C</sub>	ASD <sub>C</sub>	TD <sub>C-PUP</sub>	ASD <sub>C-PUP</sub>	TD <sub>A</sub>	ASD <sub>A</sub>
<i>N</i>	22	22	8	8	21	21
<i>Age (± SD)</i>	9 ± 1	8 ± 1	8 ± 1	8 ± 1	34 ± 6	29 ± 7
<i>Sex F:M</i>	10:12	2:20	6:2	7:1	11:10	11:10
<i>Verbal IQ [range]</i>	124 [88-155]	68 [20-95]***	116 [88-135]	58 [20-86]***	111 [79-129]	117 [92-139]
<i>Non verbal IQ [range]</i>	117 [90-146]	82 [43-129]***	106 [90-114]	66 [43-90]***	106 [74-124]	105 [74-144]
<i>CARS [range]</i>		30 [24-40]		31 [26-40]		
<i>ADOS - 2 [range]</i>						
- <i>Communication + Social Interaction</i>						9 [1-17]
- <i>Imagination</i>						1 [0-3]
- <i>Stereotyped behaviors and Restricted interests</i>						2 [0-5]
<i>ADI - R [range]</i>						
- <i>Age of onset</i>						2 [0-5]
- <i>Social</i>						20 [11-30]
- <i>Communication</i>						13 [4-25]
- <i>Repetitive restricted</i>						6 [2-11]
<i>AQ [range]</i>					9 [1-17]	34 [22-47]***

**Table 1: Characteristics of TD and ASD children (C) and adult (A) participants.** For children, pupil data were obtained only in a subgroup of 8 ASD children, and we compared it with an age-matched subgroup of TD children, noted C-PUP. Age is expressed in mean ± standard deviation, all the other information is expressed in range from minimum to the maximum value. ADI-R: Autism Diagnostic Interview Revised, ADOS-2: Autism Diagnostic Observation Schedule Second Edition, AQ: Autism Quotient, CARS: Childhood Autism Rating Scale, IQ: Intellectual Quotient. \*\*\*:  $p < 0.001$  for the comparison between TD and ASD groups.



## 2.2. Material

Cardiac and electrodermal tones were recorded using BIOPAC MP36® (BIOPAC Systems Inc. Goleta, CA, USA), with a constant voltage of 0.5 V. The AcqKnowledge® 4.1 software was used to monitor these signals during the experiment. EDA was recorded using two 8 mm Ag/AgCl cup electrodes (EL258, BIOPAC Systems IN., Goleta CA, USA) and 0.5%-NaCl electrode paste (GEL101; Biopac Systems), positioned on the second phalange of the index and medium finger of the right hand for the children, and of the non-dominant hand for the adults (i.e. left hand in all adults except 3 ASD and 1 TD participants). The frequency of acquisition for EDA was 1 kHz (range band: 0 - 5 Hz). The electrocardiogram (ECG) was recorded using two disposable vinyl electrodes (EL503, BIOPAC Systems Inc., Goleta CA, USA) placed on the sternum and on the right shoulder. The frequency of acquisition for ECG was fixed at 1 kHz (range band: 0 - 35 Hz). The pupil diameter was acquired with a frequency of 500Hz by using the eye tracking system SMI RED500® synchronized with BIOPAC MP36®.

## 2.3. Procedure

The recordings were all carried out in the same room, with constant conditions of luminosity (10 Lux for children and 19 Lux for adults), hygrometry (27% rh) and temperature (23°C/ 76.4°F). After EDA and ECG captors were installed, the participants were seated in an armchair opposite a computer screen (resolution 1920 x 1080 pixels, visual angle around 45x25°) on which the eye tracking illuminator ( $\lambda = 870$  nm, norm compliance: CE, EMC, Eye Safety) and camera were fixed. Optimal distance and height were set up by using the IView-X software. Children's position could be adjusted with a booster seat when necessary. The first experimental step was the eye tracking calibration, either in five points when the participants could concentrate on the white spot displayed in five sequential locations on the gray screen, or in a unique central point, in particular for ASD children. During the experiment, only a central black fixation cross on a gray background appeared on the screen. All participants were asked to stay still and to look at the screen during the experiment. The experimenter and participant were separated by a panel. After placement of the electrodes, the participants adjusted themselves to be comfortably seated in the armchair, and a period of 5 minutes was respected before starting the recording to stabilize the physiological constants. We then recorded simultaneously pupillary, cardiac and electrodermal tone during 5 minutes of rest.

## 2.4. Signal processing: tonic and phasic parameters extraction

Overall, the same methodological approach was used as in Bufo et al., (2022).

### 2.4.1. Pupil tone

The pupil raw signal was preprocessed with MATLAB® (r2016a; MathWorks). The first step of preprocessing eliminated artifacts, such as blinking and brief signal losses, thanks to a velocity-based algorithm (Kret and Sjak-Shie, 2019; Nyström and Holmqvist, 2010). Afterwards, the resulting signal was smoothed using a median filter and a band pass filter (0.0004 – 0.0150 Hz). For each participant, we extracted three tonic parameters calculated on the 5 minutes of recording: median pupil diameter (in mm), hippus frequency (in Hz), and hippus amplitude (in mm), evaluated thanks to a Fourier transform method. We also performed a temporal analysis on the median pupil diameter evaluated on three successive 1-1.5 minutes time windows.

### 2.4.2. Cardiac tone

The heart rate and heart rate variability were analyzed in Kubios (Tarvainen et al., 2014). On the 5 minutes of the recording, we first calculated the interbeat interval (RR interval) between each QRS complex expressed in milliseconds (ms). Artifacts were then detected and corrected based on the deviation of the RR interval value from a local average interval. The HRV analysis was performed on the whole 5 minutes window, with equidistant sampling interpolation at 4 Hz. The temporal domain was explored by calculating the RMSSD (Root Mean Square of Successive Differences between normal heartbeats), index of parasympathetic activity (Shaffer et Ginsberg 2017; Shaffer, McCraty, et Zerr 2014; Shaffer et Meehan 2020; Cheng et al., 2020). The frequency of the oscillations was evaluated by using a Fourier transform. We focused on high frequency (HF; 0.15-0.4 Hz), reflecting cardiac parasympathetic activity (Pomeranz et al., 1985), and low frequency (LF; 0.04-0.15 Hz), reflecting baroreflex activity at rest, index of vagal tone (Malliani et al., 1991; Pagani et al., 1986; Posada-Quintero et al., 2016; Shaffer et al., 2014; Shaffer and Ginsberg, 2017). For each participant, we thus obtained seven tonic parameters: RR interval (in ms), RMSSD (in ms), LF and HF peak frequency (in Hz), LF and HF absolute power (in  $ms^2$ ), and LF/HF ratio (absolute density LF/ absolute density HF minus very low frequency; Berntson et al., 1997). Only the RR interval and the RMSSD were analyzed in the temporal analysis considering three successive 1-1.5 minutes time windows.

### 2.4.3. EDA tone and phasic parameters

Preprocessing of the electrodermal activity was performed in Ledalab (Benedek and Kaernbach, 2010), an open-source software for MATLAB® (r2016a; MathWorks). The data were down-sampled to 10 Hz and bandpass-filtered with a first order Butterworth filter and cut-off frequencies of 5 Hz (Bach et al., 2009). Artifacts due to noise were corrected by using the spline interpolation. To ensure a conservative estimate of residual variance, we did not exclude non-responses (Staib et al., 2015). We used a Continuous Decomposition Analysis (CDA) to analyze four EDA parameters: tonic component of EDA (in  $\mu\text{S}$ ), area under the curve (EDA AUC; in  $\mu\text{S}\cdot\text{s}$ ) for the whole five minutes, number of EDA peaks (the only phasic parameter), and amplitude of EDA peaks (in  $\mu\text{S}$ ), based on Standard Deconvolution method (Benedek and Kaernbach, 2010; Boucsein, 2012). The tonic component was also analyzed temporally in three successive 1-1.5 min time windows.

## 2.5. Statistical analyses

Considering the differences in acquisition (luminosity, location for EDA electrodes) and in clinical profiles between children and adults, we explored only the effect of group (ASD vs. TD) on our measures, separately for children and adults. Other factors, like age or sex, were not taken into consideration.

All the statistical analyses were performed in JAMOVI® (version 2.2.1.0) and XLSTAT® (version 2020.1.2). The normality of the distribution of the data was verified by using the Shapiro-Wilk test and the homogeneity of the variance was verified by using the Levene test.

We evaluated the statistical difference between TD and ASD groups on pupil, cardiac and electrodermal parameters by using several one-way ANOVAs. The influence of BMI, coffee drinking and smoking was also evaluated as described in the Supplementary Information.

To assess temporal stability of ANS, we performed for each autonomic effector a time course analysis (time windows 1, 2 and 3) by considering only tonic parameters (median pupil diameter, tonic value of EDA, RMSSD and RR interval). We tested fluctuations in time of the ANS tonus across the three different effectors by using a repeated measure ANOVA.

Finally, we performed non-parametric Spearman correlations to verify the covariation of the different autonomic parameters with clinical data (autism severity: CARS in children and AQ in adults; verbal IQ) only in ASD children and adults.

As LH and HF absolute power, amplitude of EDA peaks and EDA AUC differed greatly among individuals, to meet the assumptions of inferential statistics they were logarithmically transformed (Benedek and Kaernbach, 2010; Sinnreich et al., 1998; Young and Leicht, 2011). All results are expressed in mean with standard error (S.E.), with effect size expressed in eta squared ( $\eta^2$ ) and partial eta squared when necessary ( $\eta^2_p$ ). Repeated measures ANOVAs were corrected with Greenhouse-Geisser correction. P-values were corrected according to the number of correlations performed when necessary.

### 3. Results

#### 3.1. Autonomic parameters in TD and ASD children

##### 3.1.1. Pupil tone

Only eight ASD children (ASD<sub>C-PUP</sub>) looked long enough at the screen to properly evaluate pupillary parameters. An age-matched TD group (TD<sub>C-PUP</sub>) was created to perform the statistical comparisons between the two groups.

As can be observed in Figure 1A, none of the three pupillary parameters differed as a function of the group (median pupil diameter: ASD<sub>C-PUP</sub>  $5.26 \pm 1.27$  mm vs. TD<sub>C-PUP</sub>  $5.30 \pm 0.34$  mm,  $F_{15} = 0.006$ ,  $p = 0.93$ ; hippus frequency: ASD<sub>C-PUP</sub>  $0.22 \pm 0.08$  Hz vs. TD<sub>C-PUP</sub>  $0.21 \pm 0.04$  Hz,  $F_{15} = 0.10$ ,  $p = 0.76$ ; hippus amplitude: ASD<sub>C-PUP</sub>  $0.30 \pm 0.08$  mm vs. TD<sub>C-PUP</sub>  $0.30 \pm 0.06$  mm,  $F_{15} = 0.002$ ,  $p = 0.96$ ).

The temporal analysis on successive time windows, performed on median pupil diameter, did not reveal a significant interaction time x group ( $F_{1, 12} = 0.89$ ,  $p = 0.41$ ) but a main effect of time ( $F_{2, 12} = 18.62$ ,  $p < 0.001$ ,  $\eta^2 = 0.60$ ). Post hoc test revealed that the median pupil diameter decreased with time (time window 1 larger than time windows 2 and 3,  $p = 0.008$  and  $p < 0.001$  respectively; time window 2 larger than time window 3,  $p = 0.004$ ).

##### 3.1.2. Cardiac tone

Cardiac parameters could be extracted for the whole group of 22 ASD children (ASD<sub>C</sub>) and TD children (TD<sub>C</sub>).

RR interval was smaller (i.e., the heartbeat faster; Figure 1B) for ASD<sub>C</sub> ( $630 \pm 71.2$  ms) than for TD<sub>C</sub> ( $706 \pm 90.7$  ms;  $F_{42} = 9.63$ ,  $p < 0.01$ ,  $\eta^2 = 0.18$ ). For HRV parameters (Figure 1B), a group effect was reported for RMSSD, LF peak frequency, HF absolute power. RMSSD was lower for ASD<sub>C</sub> ( $2.89 \pm 0.07$  ms<sup>2</sup>) than for TD<sub>C</sub> ( $3.08 \pm 0.07$  ms<sup>2</sup>;  $F_{42} = 7.45$ ,  $p = 0.01$ ,  $\eta^2 = 0.15$ ). LF peak frequency was lower for ASD<sub>C</sub> ( $0.08 \pm 0.006$  Hz) than for TD<sub>C</sub> ( $0.11 \pm 0.004$  Hz;  $F_{42} = 6.82$ ,  $p = 0.01$ ,  $\eta^2 = 0.14$ ). HF absolute power was lower for ASD<sub>C</sub> (in log scale,  $0.08 \pm 0.006$  Hz) than for TD<sub>C</sub> ( $0.11 \pm 0.004$  Hz;  $F_{42} = 6.50$ ,  $p = 0.01$ ,  $\eta^2 = 0.13$ ). A tendency was observed for LF absolute power (in log scale, ASD<sub>C</sub>  $2.68 \pm 0.10$  ms<sup>2</sup> vs. TD<sub>C</sub>  $2.92 \pm 0.07$  ms<sup>2</sup>;  $F_{42} = 3.49$ ,  $p = 0.06$ ;  $\eta^2 = 0.07$ ).

No significant difference between groups was observed for HF peak frequency (ASD<sub>C</sub>  $0.26 \pm 0.06$  Hz vs. TD<sub>C</sub>  $0.26 \pm 0.07$  Hz;  $F_{42} = 0.007$ ,  $p = 0.93$ ).

The temporal analysis on successive time windows, performed on RR interval and RMSSD, confirmed the main effect of group already described but did not reveal any main effect of time (RR interval:  $F_{2,82} = 2.63$ ,  $p = 0.67$ ; RMSSD:  $F_{2,72} = 0.90$ ,  $p = 0.39$ ) or significant time x group interaction (RR interval:  $F_{2,82} = 2.63$ ,  $p = 0.07$ ,  $\eta^2_p = 0.05$ ; RMSSD:  $F_{2,72} = 0.83$ ,  $p = 0.42$ ).

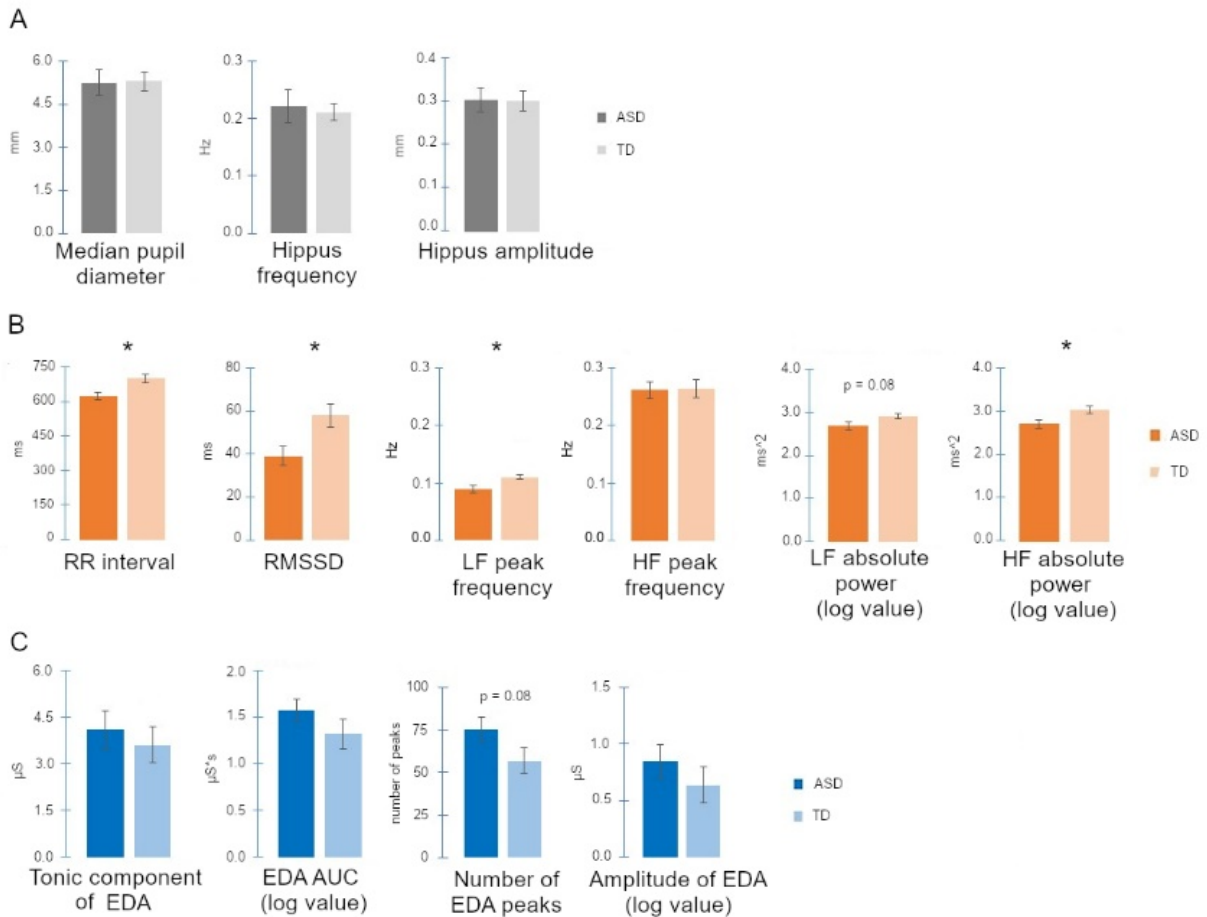
### 3.1.3. EDA tone and phasic parameters

EDA parameters could be extracted for the whole group of 22 TD children (TD<sub>C</sub>), but only in 19 ASD children (ASD<sub>C</sub>) due to agitation for 3 children.

No significant difference between groups (Figure 1C) was observed for three extracted EDA parameters (tonic component of EDA: ASD<sub>C</sub>  $4.12 \pm 2.74$   $\mu$ S vs. TD<sub>C</sub>  $3.64 \pm 2.68$   $\mu$ S,  $F_{39} = 0.32$ ,  $p = 0.57$ ; EDA AUC in log scale: ASD<sub>C</sub>  $1.58 \pm 0.53$   $\mu$ S $\cdot$ s vs. TD<sub>C</sub>  $1.32 \pm 0.75$   $\mu$ S $\cdot$ s,  $F_{39} = 1.54$ ,  $p = 0.22$ ; amplitude of EDA peaks: ASD<sub>C</sub>  $0.84 \pm 0.63$   $\mu$ S vs. TD<sub>C</sub>  $0.63 \pm 0.74$   $\mu$ S,  $F_{39} = 0.91$ ,  $p = 0.34$ ). There was a tendency for the number of EDA peaks, with a higher number of peaks in ASD children (ASD<sub>C</sub>  $75 \pm 31$  vs. TD<sub>C</sub>  $57 \pm 35$ ,  $F_{39} = 3.10$ ,  $p = 0.08$ ).

The temporal analysis on successive time windows, performed on tonic values, did not reveal a main effect of time ( $F_{2,56} = 0.60$ ,  $p = 0.49$ ) nor a significant interaction time x group ( $F_{2,56} = 1.55$ ,  $p = 0.22$ ).

## Autonomic parameters in children



**Figure 1: Autonomic parameters in ASD and TD children.** A. Pupil parameters. Histograms represent the mean values ( $\pm$  standard error) of median pupil diameter (in mm), pupillary hippus frequency (in Hz), and hippus amplitude (in mm), extracted for both ASD children (dark grey/left columns) and TD children (light grey/right columns) for the subgroups  $ASD_{C-PUP}$  and  $TD_{C-PUP}$ . B. Cardiac parameters. Histograms represent the mean values ( $\pm$  standard error) of RR interval (in ms), RMSSD (in ms), LF peak frequency (in Hz), HF peak frequency (in Hz), LF absolute power (log scale, in  $ms^2$ ), and HF absolute power (log scale, in  $ms^2$ ), extracted for both  $ASD_C$  (dark orange/left columns) and  $TD_C$  (light orange/right columns) groups. C. EDA parameters. Histograms represent the mean values ( $\pm$  standard error) of the tonic component of EDA (in  $\mu S$ ), EDA AUC (log scale, in  $\mu S*s$ ), number of EDA peaks, and amplitude of EDA peaks (log scale, in  $\mu S$ ), extracted for both  $ASD_C$  (dark blue/left columns) and  $TD_C$  (light blue/right columns) groups. \*  $p < 0.05$ .

## 3.2. Autonomic parameters in TD and ASD adults

### 3.2.1. Pupil tone

Pupillary parameters could be evaluated in the 21 TD adults (TD<sub>A</sub>) and 19 out of the 21 ASD adults (ASD<sub>A</sub>). Pupil acquisition failed in two participants due to technical problems.

As illustrated in Figure 2A, we observed a significant difference for the median pupil diameter between the two groups ( $F_{38} = 7.05$ ,  $p = 0.01$ ,  $\eta^2 = 0.15$ ), with a larger pupil diameter in ASD ( $3.56 \pm 0.11$  mm) than in TD participants ( $3.21 \pm 0.07$  mm). There was also a significant difference in hippus amplitude (ASD<sub>A</sub>  $0.21 \pm 0.01$  mm vs. TD<sub>A</sub>  $0.14 \pm 0.0008$  mm;  $F_{38} = 10.4$ ,  $p = 0.002$ ,  $\eta^2 = 0.21$ ), but not in hippus frequency (ASD<sub>A</sub>  $0.22 \pm 0.02$  Hz vs. TD<sub>A</sub>  $0.22 \pm 0.02$  Hz;  $F_{38} = 0.007$ ,  $p = 0.93$ ). The difference observed on hippus amplitude has to be interpreted cautiously as we cannot exclude that it reflected a difference in BMI between the two groups (Supplementary Information).

The temporal analysis on successive time windows, performed on median pupil diameter, did not reveal a significant interaction time x group ( $F_{2,70} = 0.22$ ,  $p = 0.80$ ) but, as in children, a main effect of time ( $F_{2,70} = 5.30$ ,  $p = 0.009$ ,  $\eta^2 = 0.13$ ). Post hoc test revealed that the median pupil diameter decreased with time (time window 1 larger than time windows 3,  $p = 0.003$ , but not time window 2  $p > 0.2$ ; time window 2 larger than time window 3,  $p = 0.02$ ).

### 3.2.2. Cardiac tone

Cardiac parameters could be evaluated in 19 out of the 21 TD adults (TD<sub>A</sub>) and the 21 ASD adults (ASD<sub>A</sub>). Cardiac acquisition failed in two participants due to technical problems.

Tendencies for group differences were observed for three parameters (Figure 2B): RR interval, RMSSD and HF absolute power. RR interval tended to be lower (i.e., the heartbeat faster) for ASD adults ( $754 \pm 23$  ms) than for TD adults ( $812 \pm 22$  ms;  $F_{38} = 3.20$ ,  $p = 0.08$ ,  $\eta^2 = 0.08$ ) (but see the significant effect of BMI on RR interval). RMSSD was also lower for ASD participants ( $29.2 \pm 3.21$  ms) than for TD participants ( $40.1 \pm 5.31$ ;  $F_{38} = 3.18$ ,  $p = 0.08$ ,  $\eta^2 = 0.07$ ). Finally, HF absolute power was lower for ASD adults ( $2.45 \pm 0.10$  Hz) than for TD adults ( $2.72 \pm 0.10$  Hz;  $F_{38} = 3.41$ ,  $p = 0.07$ ,  $\eta^2 = 0.08$ ).

LF peak frequency exhibited no difference between TD ( $0.09 \pm 0.005$  Hz) and ASD ( $0.09 \pm 0.005$  Hz) participants ( $F_{38} = 0.58$ ,  $p = 0.44$ ), nor did HF peak frequency (ASD<sub>A</sub>  $0.25 \pm 0.01$  Hz

vs. TD<sub>A</sub>  $0.24 \pm 0.01$  Hz;  $F_{38} = 0.54$ ,  $p = 0.46$ ), or LF absolute power (ASD<sub>A</sub>  $2.70 \pm 0.07$  ms<sup>2</sup> vs. TD<sub>A</sub>  $2.76 \pm 0.09$  ms<sup>2</sup>;  $F_{38} = 0.25$ ,  $p = 0.61$ ).

The temporal analysis on successive time windows, performed on RR interval and RMSSD, revealed no main effect of time (RR interval:  $F_{2,64} = 0.36$ ,  $p = 0.69$ ; RMSSD:  $F_{2,64} = 2.40$ ,  $p = 0.11$ ), nor any significant time x group interaction (RR interval:  $F_{2,64} = 1.82$ ,  $p = 0.17$ ; RMSSD:  $F_{2,64} = 0.05$ ,  $p = 0.94$ ).

### 3.2.3. EDA tone and phasic parameters

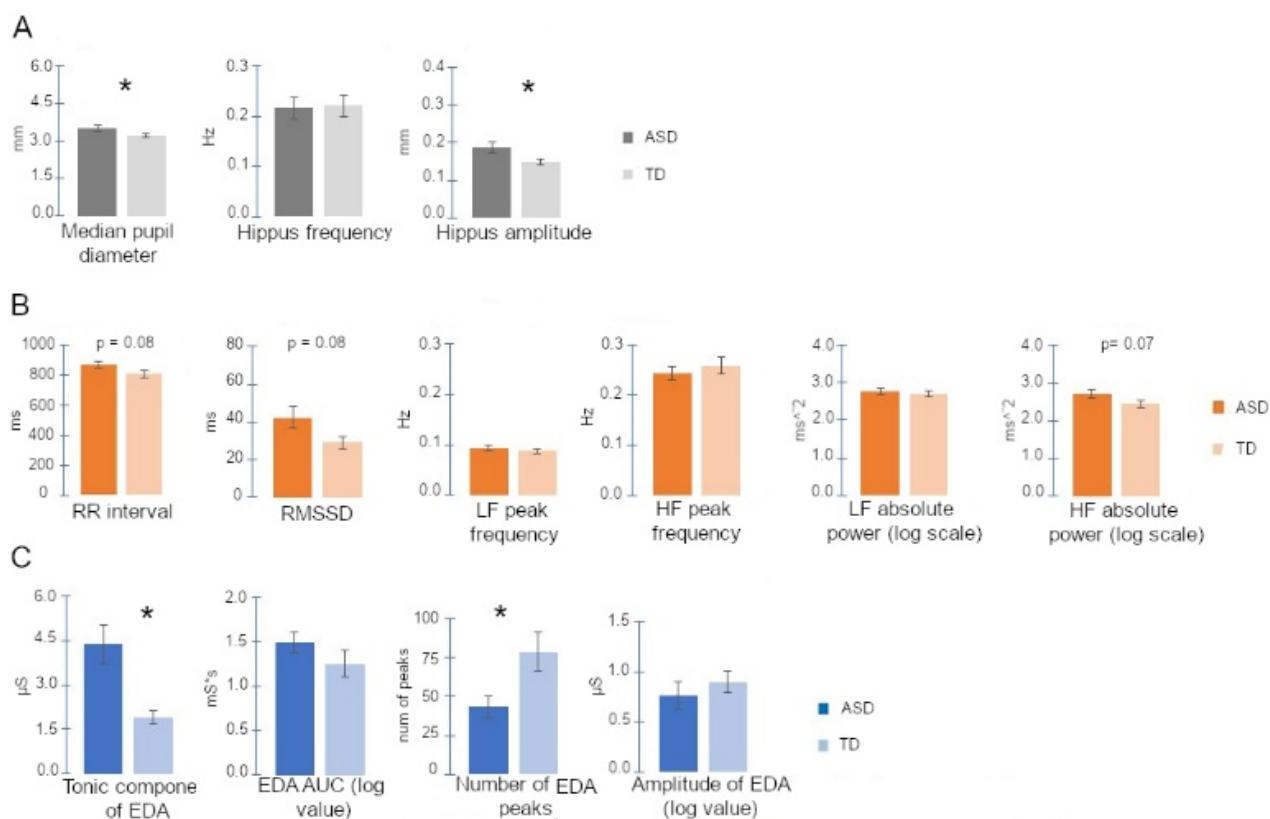
Skin conductance parameters could be evaluated in 19 out of the 21 TD adults (TD<sub>A</sub>) and the 20 out of the 21 ASD adults (ASD<sub>A</sub>). Moreover, 1 TD adult was a complete non-responder, so no number of EDA peaks and no amplitude of EDA peaks could be evaluated.

We found a group effect on tonic component of EDA (ASD<sub>A</sub>  $4.39 \pm 0.64$   $\mu$ S vs. TD<sub>A</sub>  $1.91 \pm 0.21$   $\mu$ S;  $F_{37} = 12.82$ ,  $p < 0.001$ ,  $\eta^2 = 0.25$ ), with ASD adults exhibiting a higher tonus than TD adults (Figure 2C). Moreover, the number of EDA peaks was significantly higher for TD adults ( $81 \pm 12$ ) than ASD adults ( $45 \pm 7$ ;  $F_{36} = 6.66$ ,  $p = 0.01$ ,  $\eta^2 = 0.15$ ). There was no significant effect of the group for EDA AUC (log scale: ASD<sub>A</sub>  $1.49 \pm 0.11$   $\mu$ S·s vs. TD<sub>A</sub>  $1.25 \pm 0.14$   $\mu$ S·s;  $F_{37} = 1.56$ ,  $p = 0.21$ ), nor for the amplitude of EDA peaks (log scale: ASD<sub>A</sub>  $0.76 \pm 0.13$   $\mu$ S vs. TD<sub>A</sub>  $0.89 \pm 0.10$   $\mu$ S;  $F_{36} = 0.55$ ,  $p = 0.46$ ).

The temporal analysis on successive time windows, performed on the tonic component of EDA, revealed no main effect of time ( $F_{2,72} = 0.92$ ,  $p = 0.36$ ) nor any time x group interaction ( $F_{2,72} = 1.32$ ,  $p = 0.26$ ).



## Autonomic parameters in adults



**Figure 2: Autonomic parameters in ASD and TD adults.** A. Pupil parameters. Histograms represent the mean values ( $\pm$  standard error) of median pupil diameter (in mm), pupillary hippus frequency (in Hz), and hippus amplitude (in mm), extracted for both ASD<sub>A</sub> (dark grey/left columns) and TD<sub>A</sub> (light grey/right columns) groups. B. Cardiac parameters. Histograms represent the mean values ( $\pm$  standard error) of RR interval (in ms), RMSSD (in ms), LF peak frequency (in Hz), HF peak frequency (in Hz), LF absolute power (log scale, in ms<sup>2</sup>), and HF absolute power (log scale, in ms<sup>2</sup>), extracted for both ASD<sub>A</sub> (dark orange/left columns) and TD<sub>A</sub> (light orange/right columns) groups. C. EDA parameters. Histograms represent the mean values ( $\pm$  standard error) of the tonic component of EDA (in  $\mu$ S), EDA AUC (log scale, in  $\mu$ S\*s), number of EDA peaks, and amplitude of EDA peaks (log scale, in  $\mu$ S), extracted for both ASD<sub>A</sub> (dark blue/left columns) and TD<sub>A</sub> (light blue/right columns) groups. \*  $p < 0.05$ .

### 3.3. Correlations of ANS parameters with clinical data in ASD participants

In ASD children, no autonomic parameters correlated with CARS scores ( $p > 0.2$ ). In ASD adults, there was a tendency for a positive correlation between AQ scores and two autonomic parameters: the median pupil diameter ( $r = 0.44$ ,  $p = 0.062$ ) and the LF peak frequency ( $r = 0.40$ ,  $p = 0.074$ ), but no correlation for the other parameters ( $p > 0.11$ ).

In ASD children, no autonomic parameters correlated with verbal IQ scores ( $p > 0.2$ ). In ASD adults, there was a positive correlation between pupil hippus amplitude and verbal IQ ( $r = 0.52$ ,  $p = 0.03$ ), but no correlation for the other parameters ( $p > 0.14$ ).

## 4. Discussion

### 4.1. Reduced parasympathetic tonus in ASD children

In children, the four cardiac parameters significantly lower in ASD compared to TD individuals were principally under PNS influence (RMSSD, LF peak frequency, HF absolute power, while RR interval is influenced both by PNS and SNS). LF peak frequency measured at rest is mainly considered as an index of parasympathetic activity (Billman 2013; Reyes del Paso et al., 2013; Shaffer et Ginsberg 2017) and its lower value in ASD children may reflect a reduced PNS regulation on cardiac dynamics. This hypothesis is supported by the lower HF absolute power (index of tonic cardiac outflow) and the lower RMSSD (index of vagal control) in this group. ASD children would thus present a reduced parasympathetic regulation than TD children, as proposed in several studies (Cheng et al., 2020; McCormick et al., 2018; Thapa et al., 2019) and summed up in a recent review (Arora et al., 2021) when based on cardiac indices. However, authors usually conclude that this PNS under-activation would reflect a SNS hyper-arousal, with the simplistic hypothesis of a SNS/PNS antagonistic balance. Results from other autonomic effectors allow us to refute this hypothesis in our children population. Indeed, we observed a tendency of a higher number of EDA peaks in ASD children compared to TD children, reflecting a higher spontaneous phasic SNS activity, but no significant difference in SNS tonus (contrary to Bujnakova et al., 2016), indexed by EDA tonic component and median pupil diameter. Pupil diameter at rest in ASD children is inconsistently reported as higher or unchanged compared to their TD peers (e.g. Anderson, Colombo, et Unruh 2013; Arora et al. 2021; Zhao, Liu, et Wei 2022). The autonomic pattern we describe would thus suggest rather a SNS/PNS co-inhibition functioning (Berntson et Cacioppo 2007; Berntson, Cacioppo, et Quigley 1991) in our ASD children group. It could also point towards a disharmony in the autonomic mobilization of different effectors in autism.

#### 4.2. High sympathetic tonus in ASD adults

In adults, a different ANS pattern was observed. First, cardiac parameters were not significantly different between adults with and without ASD, suggesting a rather similar PNS regulation in these populations. However, we observed significant differences for SNS parameters extracted from EDA and pupil recordings. For EDA, the tonus was higher in ASD adults than in their TD peers, while the spontaneous events (phasic) were less numerous. The difference in EDA tonus was confirmed by a significant difference in pupil tonus between these two groups, with globally a higher SNS tonus in ASD adults. As this elevated SNS tonus was accompanied by a tendency of an increased PNS regulation (indexed by RMSSD), our results suggest a SNS/PNS co-activation in ASD adults (Berntson et al., 1997, 1991; Berntson et Cacioppo 2007). Our results in ASD adults thus do not correspond to the majority of results described in the literature (Arora et al., 2021), even if ANS dysregulation in ASD adults is less consensual (Ben Shalom et al., 2006; Joseph et al., 2008). These results have to be mitigated by the influence of some other factors, not frequently reported. Indeed, we observed in adults an effect of BMI and smoking on several autonomic parameters (Supplementary Information), that might explain our adult groups' differences. This is the reason why we did not emphasize the differences on RR interval and pupil hippus amplitude in adults.

#### 4.3. Temporal stability of autonomic tonus

We had hypothesized a possible temporal instability of autonomic tonus in ASD that could account for the non-consensual results about autonomic markers in autism. Indeed, measures done during longer recordings seemed to lead to more reproducible differences between ASD and TD groups (Arora et al. 2021). We explored this question by evaluating the stability of SNS and PNS tonus in three successive time windows. We found an effect of time only on pupil tonus, independent of the groups. The diminution of pupil size along time would thus rather reflect a general variation in wakefulness/arousal (Reimer et al., 2014). If physiological signals are more variable in ASD participants (e.g. Dinstein et al., 2012; Latinus et al., 2019), autonomic measures do not seem to have the proper temporal resolution to capture this signature. Shorter time-windows, and possibly BOLD signal (Wu et Marinazzo 2016), would be more appropriate to study this question.

#### 4.4. Atypical autonomic maturation in ASD

While in the literature (see Arora et al., 2021 for review) there is no substantial influence of age (children studies vs. adolescents/adults studies) on the overall autonomic pattern in autism, our study is one of the few that tested both age groups in rather similar conditions. In a previous paper (Bufo et al., 2022), we showed a higher PNS tone in TD children than in TD adults. This maturation of PNS tonus is less clear in the ASD population, as ASD children exhibited already a lower PNS tone than their peers. On the contrary, we observed a clear maturation effect for SNS parameters. The comparison between adults and children (Figures 1 and 2) suggests that EDA tonic component would decrease in TD population while it would stay constant in ASD population through maturation. While we cannot deny the difference between the two adult groups, we have to be careful about the interpretation of the maturation of EDA values. Indeed, for TD adults, we observed a lower tonic component of EDA (and a higher number of EDA peaks) than in our previous study (Bufo et al., 2022). The slight experimental differences between the two studies were the level of luminance (not expected to influence EDA, but having an impact on median pupil diameter), and the site of EDA recordings (non-dominant hand in the present study but only for adults, right hand - so mainly dominant hand - in Bufo et al., 2022 and in the present children's data). Picard et al., (2015) have shown that EDA signal is higher when recorded on the right hand, which is coherent with the difference we observe between the present study and Bufo et al., (2022). In TD populations, the tonic component of EDA was comparable in children and adults (Bufo et al., 2022). This would suggest that the difference between ASD and TD adults result from an increased EDA tonus in ASD adults. We would expect to observe an overall higher EDA tonus in adults with EDA recordings on the right hand. For pupil parameters, the only maturation effect we can describe considering the experimental differences between children and adults, is the divergence from TD population with age, going towards a higher SNS tonus in ASD adults. In autism, there would thus be an evolution from a low parasympathetic tonus in children to a high sympathetic tonus in adults.

#### 4.5. Compensatory autonomic plasticity in autism: the tonic and phasic (dis)equilibrium hypothesis

Autonomic functioning at rest is mainly characterized by its tonus. However, spontaneous phasic responses can also occur, indicating an autonomic lability of the individual. This lability has already been described in children compared to adults in neurotypical populations (Bufo et

al., 2022). A higher number of spontaneous EDA peaks in TD individuals has been correlated with better allocation of attention towards environmental events in adults (Schell et al. 1988) and better social skills in children (Neuhaus, Bernier, et Beauchaine 2015).

The present study is one of the first to assess phasic autonomic responses during rest in autism (Neuhaus et al. 2015). Interestingly, there seems to have a balance between EDA tonus and EDA spontaneous phasic activity at the group level. This is particularly visible in adults, with a higher tonus associated with a lower number of EDA peaks in ASD adults, and the reverse pattern in TD adults. This observation suggests an exacerbated autonomic maturation in autism, as TD adults already present a higher EDA tonus and smaller number of EDA peaks than TD children (Bufo et al., 2022). Moreover, in children, there is a tendency for a larger number of EDA peaks in ASD than TD individuals. To sum up with what has been observed on the tonic parameters, there would thus be a maturation in autism from an exaggeratedly low autonomic tonus / high phasic reactivity in children, to an exaggeratedly high autonomic tonus / low phasic reactivity in adults.

This autonomic functional pattern, even outside of the context of autism, raises the question of balance between tonic and phasic SNS mobilization. This pattern could be linked to what has been described of the functioning of the locus coeruleus (LC), the main source of norepinephrine in the brain (Usher et al., 1999), whose neuronal discharge has been correlated to SNS regulation (Mathew 1995). Indeed, Aston-Jones and Cohen (2005) suggest that optimal behaviour in response to external stimuli would be correlated to phasic LC neuronal discharge, possible only when the LC tonus is intermediate. If the LC tonus is too low or too high, the phasic discharge would disappear (Aston-Jones, Rajkowski, et Cohen 1999). ASD adults would thus exhibit an inappropriately high SNS tonus, that may be linked to non-optimal behaviours, for example hypo- or hyper-responsiveness to external sensory stimulations (Janitzky 2020). It also questions the possible SNS maturation from autistic children to adults: if autistic children exhibit low SNS tonus but high spontaneous SNS phasic activity, and autistic adults high SNS tonus, could we consider this maturation as a maladaptive neuronal plasticity or an exacerbated compensatory trajectory? Autonomic homeostasis is supposed to be adjusted to our environment and its requirements. Hyperarousal during childhood is costly in energy but fosters developmental acquisitions by seizing environmental opportunities (Mayes, 2000), in order to build an adjusted physiological, cognitive, emotional and social behavior. With maturation, autonomic system converges towards a low-cost functioning (lower ANS tonus) associated with more integrated responses to the environment (higher ANS phasic discharges). The pattern we

describe both in autistic children and adults suggest that the natural equilibrium observed in the typical population is dysfunctional. In ASD children, the tendency for higher spontaneous phasic discharge could reflect inadequate reactions to the environment. Maturation processes are the result of a complex interaction between genetics and environmental factors including life experience (Bonnet-Brilhault et al., 2018), associated with more frequent stressful events during ASD developmental trajectory. It has been shown that contextual factors like stress could influence the switch between a phasic and a tonic mode of functioning of the LC (Valentino et Van Bockstaele 2008), influencing neuroinflammation and potentially neurodegenerative disorders (Janitzky 2020). This maturational process could thus result in a compensatory atypical autonomic equilibrium in ASD adults. The question of disturbed tonic LC activity in autism should thus be studied (Zhao et al., 2022), and its potential link to sensory responsiveness (Janitzky 2020).

#### 4.6. Potential links with clinical symptoms

We observed very few links between our autonomic and clinical parameters reflecting autism severity, possibly due to the heterogeneity of the autism spectrum. One aspect that should be further investigated is the presence of comorbidities that could be linked to autonomic functioning. In particular, autonomic dysregulation in autism has been previously studied in the context of comorbidity with anxiety (e.g. Chiu et al. 2016; Panju et al. 2015; Parma et al. 2021)), with autonomic SNS reactivity lower in anxious ASD children. Some authors even proposed that autonomic dysregulation in autism would only be the result of comorbid anxiety (Barbier et al., 2022). To our knowledge, only one study focused on autonomic tonus at rest and anxiety in ASD. By comparing EDA and cardiac measures in groups of children with ASD and anxiety, ASD without anxiety, anxiety without ASD, and TD children, Parma et al. (2021) suggested that a reduced PNS regulation at rest would characterize autism, while SNS regulation differences would characterize anxiety. Our absence of difference in EDA tonus between ASD and TD children would thus suggest that our results are not explained by a difference in anxiety physiological level. To conclude, more and more studies focus on autonomic dysregulation and its possible links with mental disorders, such as major depressive disorder, generalized anxiety disorder, substance use, schizophrenia, etc. (Alvares et al., 2016; Cheng et al., 2019; Huang et al., 2020; Koch et al., 2019; Thapa et al. 2021). All these studies point towards the necessity of systematic analysis of comorbidities in future studies.

#### 4.7. Conclusion

Our study revealed autonomic dysregulation and atypical maturation in autism observable at rest. The distinctive profiles in autistic children and adults, both for their SNS and PNS tonic components but also their sympathetic phasic component, could illustrate the two endpoints of an atypical maturation trajectory. During maturation in ASD individuals, compensatory mechanisms would induce autonomic disequilibrium to switch from excessive phasic components to excessive tonic components, possibly subtended by an atypical locus coeruleus functioning. Studying autonomic regulation in neurodevelopmental disorders like autism is thus crucial to understand these disorders, but should always consider the developmental trajectory and possible compensatory mechanisms.

**CRedit author statement:** **Maria Rosa Bufo:** Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft & review and editing, Visualization; **Marco Guidotti:** Resources, Writing – review and editing; **Mathieu Lemaire:** Conceptualization, Methodology, Investigation, Resources, Project administration, Funding acquisition; **Joëlle Malvy:** Resources; **Emmanuelle Houy-Durand:** Resources, Writing – review and editing; **Frédérique Bonnet-Brilhault:** Conceptualization, Resources, Writing – review and editing; **Frédéric Briend:** Investigation, Data curation, Writing – review and editing, Project administration; **Nadia Aguilon-Hernandez:** Conceptualization, Validation, Writing – original draft & review and editing, Supervision, Project administration, Funding acquisition; **Claire Wardak:** Conceptualization, Validation, Writing – original draft & review and editing, Supervision, Project administration, Funding acquisition.

**Funding:** MRB was funded by the FEANS (Fondation Européenne pour l'Avancement des Neurosciences). Acquisitions in the adult groups were part of a large project funded by the Fondation de France (2013-00041906) and the Fédération pour la Recherche sur le Cerveau (FRC AOE 8 « Espoir en tête » 2013).

## 5. Bibliography

- Alvares, Gail A., Keely Bebbington, Dominique Cleary, Kiah Evans, Emma J. Glasson, Murray T. Maybery, Sarah Pillar, Mirko Uljarević, Kandice Varcin, John Wray, et Andrew JO Whitehouse. 2020. « The Misnomer of ‘High Functioning Autism’: Intelligence Is an Imprecise Predictor of Functional Abilities at Diagnosis ». *Autism* 24(1):221-32. doi: 10.1177/1362361319852831.
- Anderson, Christa J., John Colombo, et Kathryn E. Unruh. 2013. « Pupil and Salivary Indicators of Autonomic Dysfunction in Autism Spectrum Disorder ». *Developmental Psychobiology* 55(5):465-82. doi: 10.1002/dev.21051.
- Arora, Iti, Alessio Bellato, Danielle Ropar, Chris Hollis, et Madeleine J. Groom. 2021. « Is Autonomic Function during Resting-State Atypical in Autism: A Systematic Review of Evidence ». *Neuroscience & Biobehavioral Reviews* 125:417-41. doi: 10.1016/j.neubiorev.2021.02.041.
- Aston-Jones, Gary, et Jonathan D. Cohen. 2005. « Adaptive Gain and the Role of the Locus Coeruleus-Norepinephrine System in Optimal Performance ». *The Journal of Comparative Neurology* 493(1):99-110. doi: 10.1002/cne.20723.
- Aston-Jones, Gary, Janusz Rajkowski, et Jonathan Cohen. 1999. « Role of Locus Coeruleus in Attention and Behavioral Flexibility ». *Biological Psychiatry* 46(9):1309-20. doi: 10.1016/S0006-3223(99)00140-7.
- Bach, Dominik R., Guillaume Flandin, Karl J. Friston, et Raymond J. Dolan. 2009. « Time-Series Analysis for Rapid Event-Related Skin Conductance Responses ». *Journal of Neuroscience Methods* 184(2):224-34. doi: 10.1016/j.jneumeth.2009.08.005.
- Barbier, Ashley, Ji-Hong Chen, et Jan D. Huizinga. 2022. « Autism Spectrum Disorder in Children Is Not Associated With Abnormal Autonomic Nervous System Function: Hypothesis and Theory ». *Frontiers in Psychiatry* 13:830234. doi: 10.3389/fpsy.2022.830234.
- Ben Shalom, D., S. H. Mostofsky, R. L. Hazlett, M. C. Goldberg, R. J. Landa, Y. Faraon, D. R. McLeod, et R. Hoehn-Saric. 2006. « Normal Physiological Emotions but Differences in Expression of Conscious Feelings in Children with High-Functioning Autism ». *Journal of Autism and Developmental Disorders* 36(3):395-400. doi: 10.1007/s10803-006-0077-2.
- Benedek, Mathias, et Christian Kaernbach. 2010. « A Continuous Measure of Phasic Electrodermal Activity ». *Journal of Neuroscience Methods* 190(1):80-91. doi: 10.1016/j.jneumeth.2010.04.028.
- Benevides, Teal W., et Shelly J. Lane. 2015. « A Review of Cardiac Autonomic Measures: Considerations for Examination of Physiological Response in Children with Autism Spectrum Disorder ». *Journal of Autism and Developmental Disorders* 45(2):560-75. doi: 10.1007/s10803-013-1971-z.
- Berntson, Gary G., et John T. Cacioppo. 2007. « Integrative Physiology: Homeostasis, Allostasis, and the Orchestration of Systemic Physiology ». P. 433-52 in *Handbook of*



*Psychophysiology*, édité par J. T. Cacioppo, L. G. Tassinary, et G. Berntson. Cambridge: Cambridge University Press.

- Berntson, Gary G., John T. Cacioppo, et Karen S. Quigley. 1991. « Autonomic Determinism: The Modes of Autonomic Control, the Doctrine of Autonomic Space, and the Laws of Autonomic Constraint. » *Psychological Review* 98(4):459-87. doi: 10.1037/0033-295X.98.4.459.
- Berntson, Gary G., J. Thomas Bigger, Dwain L. Eckberg, Paul Grossman, Peter G. Kaufmann, Marek Malik, Haikady N. Nagaraja, Stephen W. Porges, J. Philip Saul, Peter H. Stone, et Maurots W. Van Der Molen. 1997. « Heart Rate Variability: Origins, Methods, and Interpretive Caveats ». *Psychophysiology* 34(6):623-48. doi: 10.1111/j.1469-8986.1997.tb02140.x.
- Billman, George E. 2013. « The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance ». *Frontiers in Physiology* 4. doi: 10.3389/fphys.2013.00026.
- Bonnet-Brilhault, Frédérique, Laurice Tuller, Philippe Prévost, Joëlle Malvy, Rasha Zebib, Sandrine Ferré, Christophe dos Santos, Sylvie Roux, Emmanuelle Houy-Durand, Rémy Magné, Yassine Mofid, Marianne Latinus, Claire Wardak, Nadia Aguillon-Hernandez, Magali Batty, et Marie Gomot. 2018. « A Strategic Plan to Identify Key Neurophysiological Mechanisms and Brain Circuits in Autism ». *Journal of Chemical Neuroanatomy* 89:69-72. doi: 10.1016/j.jchemneu.2017.11.007.
- Boucsein, W. 2012. *Electrodermal activity*. Springer Science & Business Media.
- Bufo, Maria Rosa, Marco Guidotti, Cindie De Faria, Yassine Mofid, Frédérique Bonnet-Brilhault, Claire Wardak, et Nadia Aguillon-Hernandez. 2022. « Autonomic Tone in Children and Adults: Pupillary, Electrodermal and Cardiac Activity at Rest ». *International Journal of Psychophysiology* 180:68-78. doi: 10.1016/j.ijpsycho.2022.07.009.
- Bujnakova, I., I. Ondrejka, M. Mestanik, Z. Visnovcova, A. Mestanikova, I. Hrtanek, D. Fleskova, A. Calkovska, et I. Tonhajzerova. 2016. « Autism Spectrum Disorder Is Associated With Autonomic Underarousal ». *Physiological Research* S673-82. doi: 10.33549/physiolres.933528.
- Cannon, Walter B. 1929. « ORGANIZATION FOR PHYSIOLOGICAL HOMEOSTASIS ». *Physiological Reviews* 9(3):399-431. doi: 10.1152/physrev.1929.9.3.399.
- Cheng, Ying-Chih, Yu-Chen Huang, et Wei-Lieh Huang. 2020. « Heart Rate Variability in Individuals with Autism Spectrum Disorders: A Meta-Analysis ». *Neuroscience & Biobehavioral Reviews* 118:463-71. doi: 10.1016/j.neubiorev.2020.08.007.
- Chiu, Tabitha A., Evdokia Anagnostou, Jessica Brian, Tom Chau, et Azadeh Kushki. 2016. « Specificity of Autonomic Arousal to Anxiety in Children with Autism Spectrum Disorder: Specificity of Autonomic Arousal to Anxiety ». *Autism Research* 9(4):491-501. doi: 10.1002/aur.1528.
- Dalton, Kim M., Brendon M. Nacewicz, Tom Johnstone, Hillary S. Schaefer, Morton Ann Gernsbacher, H. H. Goldsmith, Andrew L. Alexander, et Richard J. Davidson. 2005.

- « Gaze Fixation and the Neural Circuitry of Face Processing in Autism ». *Nature Neuroscience* 8(4):519-26. doi: 10.1038/nn1421.
- Dell’Osso, Liliana, Leonardo Massoni, Simone Battaglini, Ivan Mirko Cremonese, Claudia Carmassi, et Barbara Carpita. 2022. « Biological Correlates of Altered Circadian Rhythms, Autonomic Functions and Sleep Problems in Autism Spectrum Disorder ». *Annals of General Psychiatry* 21(1):13. doi: 10.1186/s12991-022-00390-6.
- Dinstein, Ilan, David J. Heeger, Lauren Lorenzi, Nancy J. Minshew, Rafael Malach, et Marlene Behrmann. 2012. « Unreliable Evoked Responses in Autism ». *Neuron* 75(6):981-91. doi: 10.1016/j.neuron.2012.07.026.
- Fabes, Richard A., Nancy Eisenberg, Marissa Karbon, Debra Troyer, et Galen Switzer. 1994. « The Relations of Children’s Emotion Regulation to Their Vicarious Emotional Responses and Comforting Behaviors ». *Child Development* 65(6):1678. doi: 10.2307/1131287.
- Ferguson, Bradley J., Sarah Marler, Lily L. Altstein, Evon Batey Lee, Jill Akers, Kristin Sohl, Aaron McLaughlin, Kaitlyn Hartnett, Briana Kille, Micah Mazurek, Eric A. Macklin, Erin McDonnell, Mariah Barstow, Margaret L. Bauman, Kara Gross Margolis, Jeremy Veenstra-VanderWeele, et David Q. Beversdorf. 2017b. « Psychophysiological Associations with Gastrointestinal Symptomatology in Autism Spectrum Disorder: ASD Psychophysiology & Gastrointestinal Issues ». *Autism Research* 10(2):276-88. doi: 10.1002/aur.1646.
- Friedman, Bruce H., Julian F. Thayer, Thomas D. Borkovec, Richard A. Tyrell, Bjorn-Helge Johnson, et Roberto Columbo. 1993. « Autonomic Characteristics of Nonclinical Panic and Blood Phobia ». *Biological Psychiatry* 34(5):298-310. doi: 10.1016/0006-3223(93)90087-T.
- Hastings, Mark E., June P. Tangney, et Jeff Stuewig. 2008. « Psychopathy and Identification of Facial Expressions of Emotion ». *Personality and Individual Differences* 44(7):1474-83. doi: 10.1016/j.paid.2008.01.004.
- Hirstein, William, Portia Iversen, et V. S. Ramachandran. 2001. « Autonomic Responses of Autistic Children to People and Objects ». *Proceedings of the Royal Society of London. Series B: Biological Sciences* 268(1479):1883-88. doi: 10.1098/rspb.2001.1724.
- Huang, Yunhe, Samuel RC Arnold, Kitty-Rose Foley, et Julian N. Trollor. 2020. « Diagnosis of Autism in Adulthood: A Scoping Review ». *Autism* 24(6):1311-27. doi: 10.1177/1362361320903128.
- Hutt, Corinne, S. J. Hutt, Douglas Lee, et Christopher Ounsted. 1964. « Arousal and Childhood Autism ». *Nature* 204(4961):908-9. doi: 10.1038/204908a0.
- Hyun, Uisu, et Jong-Woo Sohn. 2022. « Autonomic Control of Energy Balance and Glucose Homeostasis ». *Experimental & Molecular Medicine* 54(4):370-76. doi: 10.1038/s12276-021-00705-9.
- Iacoboni, Vittorio, et Uri Hasson. 2011. « The Relationship between BOLD Signal and Autonomic Nervous System Functions: Implications for Processing of “Physiological

- Noise» ». *Magnetic Resonance Imaging* 29(10):1338-45. doi: 10.1016/j.mri.2011.03.006.
- Janitzky, Kathrin. 2020. « Impaired Phasic Discharge of Locus Coeruleus Neurons Based on Persistent High Tonic Discharge—A New Hypothesis With Potential Implications for Neurodegenerative Diseases ». *Frontiers in Neurology* 11:371. doi: 10.3389/fneur.2020.00371.
- Joseph, Robert M., Kelly Ehrman, Rebecca McNally, et Brandon Keehn. 2008b. « Affective Response to Eye Contact and Face Recognition Ability in Children with ASD ». *Journal of the International Neuropsychological Society* 14(6):947-55. doi: 10.1017/S1355617708081344.
- Koch, Julia, Kira Willemsen, Imis Dogan, Roman Rolke, Jörg B. Schulz, Johannes Schiefer, Kathrin Reetz, et Andrea Maier. 2022. « Quantitative Sensory Testing and Norepinephrine Levels in REM Sleep Behaviour Disorder – a Clue to Early Peripheral Autonomic and Sensory Dysfunction? ». *Journal of Neurology* 269(2):923-32. doi: 10.1007/s00415-021-10675-7.
- Kret, Mariska E., et Elio E. Sjak-Shie. 2019. « Preprocessing Pupil Size Data: Guidelines and Code ». *Behavior Research Methods* 51(3):1336-42. doi: 10.3758/s13428-018-1075-y.
- Kushki, Azadeh, Jessica Brian, Annie Dupuis, et Evdokia Anagnostou. 2014. « Functional Autonomic Nervous System Profile in Children with Autism Spectrum Disorder ». *Molecular Autism* 5(1):39. doi: 10.1186/2040-2392-5-39.
- Latinus, Marianne, Yassine Mofid, Klara Kovarski, Judith Charpentier, Magali Batty, et Frédérique Bonnet-Brilhault. 2019. « Atypical Sound Perception in ASD Explained by Inter-Trial (In)consistency in EEG ». *Frontiers in Psychology* 10:1177. doi: 10.3389/fpsyg.2019.01177.
- Liu, Xianchen, Julie A. Hubbard, Richard A. Fabes, et James B. Adam. 2006. « Sleep Disturbances and Correlates of Children with Autism Spectrum Disorders ». *Child Psychiatry and Human Development* 37(2):179-91. doi: 10.1007/s10578-006-0028-3.
- Lord, C., M. Rutter, P. C. DiLavore, et s. Risi. 2016. « Autism Diagnostic Observation Schedule--Generic ».
- Lord, Catherine, Michael Rutter, et Ann Le Couteur. 1994. « Autism Diagnostic Interview-Revised: A Revised Version of a Diagnostic Interview for Caregivers of Individuals with Possible Pervasive Developmental Disorders ». *Journal of Autism and Developmental Disorders* 24(5):659-85. doi: 10.1007/BF02172145.
- Maenner, Matthew J., Kelly A. Shaw, Amanda V. Bakian, Deborah A. Bilder, Maureen S. Durkin, Amy Esler, Sarah M. Furnier, Libby Hallas, Jennifer Hall-Lande, Allison Hudson, Michelle M. Hughes, Mary Patrick, Karen Pierce, Jenny N. Poynter, Angelica Salinas, Josephine Shenouda, Alison Vehorn, Zachary Warren, John N. Constantino, Monica DiRienzo, Robert T. Fitzgerald, Andrea Grzybowski, Margaret H. Spivey, Sydney Pettygrove, Walter Zahorodny, Akilah Ali, Jennifer G. Andrews, Thayer Baroud, Johanna Gutierrez, Amy Hewitt, Li-Ching Lee, Maya Lopez, Kristen Clancy Mancilla, Dedria McArthur, Yvette D. Schwenk, Anita Washington, Susan Williams, et Mary E. Cogswell. 2021. « Prevalence and Characteristics of Autism Spectrum Disorder Among

- Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2018 ». *MMWR. Surveillance Summaries* 70(11):1-16. doi: 10.15585/mmwr.ss7011a1.
- Malliani, A., M. Pagani, F. Lombardi, et S. Cerutti. 1991. « Cardiovascular Neural Regulation Explored in the Frequency Domain. » *Circulation* 84(2):482-92. doi: 10.1161/01.CIR.84.2.482.
- Markram, Kamila, et Henry Markram. 2010. « The Intense World Theory – A Unifying Theory of the Neurobiology of Autism ». *Frontiers in Human Neuroscience* 4. doi: 10.3389/fnhum.2010.00224.
- Mathew, Roy J. 1995. « Sympathetic Control of Cerebral Circulation: Relevance to Psychiatry ». *Biological Psychiatry* 37(5):283-85. doi: 10.1016/0006-3223(94)00232-R.
- Matson, Johnny L., et Daniene Neal. 2009. « Diagnosing High Incidence Autism Spectrum Disorders in Adults ». *Research in Autism Spectrum Disorders* 3(3):581-89. doi: 10.1016/j.rasd.2009.01.001.
- Mayes, Susan D., Susan L. Calhoun, et Errin W. Crowell. 2000. « Learning Disabilities and ADHD: Overlapping Spectrum Disorders ». *Journal of Learning Disabilities* 33(5):417-24. doi: 10.1177/002221940003300502.
- McCormick, Carolyn E. B., Stephen J. Sheinkopf, Todd P. Levine, Linda L. LaGasse, Ed Tronick, et Barry L. Lester. 2018. « Diminished Respiratory Sinus Arrhythmia Response in Infants Later Diagnosed with Autism Spectrum Disorder: RSA Response in Infants with ASD Outcome ». *Autism Research* 11(5):726-31. doi: 10.1002/aur.1929.
- McCorry, Laurie Kelly. 2007. « Physiology of the Autonomic Nervous System ». *American Journal of Pharmaceutical Education* 71(4):78. doi: 10.5688/aj710478.
- Ming, Xue, Ritesh Patel, Victor Kang, Sudhansu Chokroverty, et Peter O. Julu. 2016. « Respiratory and Autonomic Dysfunction in Children with Autism Spectrum Disorders ». *Brain and Development* 38(2):225-32. doi: 10.1016/j.braindev.2015.07.003.
- Molfinio, A., A. Fiorentini, L. Tubani, M. Martuscelli, F. Rossi Fanelli, et A. Laviano. 2009. « Body Mass Index Is Related to Autonomic Nervous System Activity as Measured by Heart Rate Variability ». *European Journal of Clinical Nutrition* 63(10):1263-65. doi: 10.1038/ejcn.2009.35.
- Neuhaus, Emily, Raphael A. Bernier, et Theodore P. Beauchaine. 2015. « Electrodermal Response to Reward and Non-Reward Among Children With Autism: Electrodermal Response to Reward with Autism ». *Autism Research* 8(4):357-70. doi: 10.1002/aur.1451.
- Neuhaus, Emily, Raphael Bernier, et Theodore P. Beauchaine. 2014. « Brief Report: Social Skills, Internalizing and Externalizing Symptoms, and Respiratory Sinus Arrhythmia in Autism ». *Journal of Autism and Developmental Disorders* 44(3):730-37. doi: 10.1007/s10803-013-1923-7.

- Nyström, Marcus, et Kenneth Holmqvist. 2010. « An Adaptive Algorithm for Fixation, Saccade, and Glissade Detection in Eyetracking Data ». *Behavior Research Methods* 42(1):188-204. doi: 10.3758/BRM.42.1.188.
- Pagani, M., F. Lombardi, S. Guzzetti, O. Rimoldi, R. Furlan, P. Pizzinelli, G. Sandrone, G. Malfatto, S. Dell'Orto, et E. Piccaluga. 1986. « Power Spectral Analysis of Heart Rate and Arterial Pressure Variabilities as a Marker of Sympatho-Vagal Interaction in Man and Conscious Dog. » *Circulation Research* 59(2):178-93. doi: 10.1161/01.RES.59.2.178.
- Panju, Sakeena, Jessica Brian, Annie Dupuis, Evdokia Anagnostou, et Azadeh Kushki. 2015. « Atypical Sympathetic Arousal in Children with Autism Spectrum Disorder and Its Association with Anxiety Symptomatology ». *Molecular Autism* 6(1):64. doi: 10.1186/s13229-015-0057-5.
- Parma, Valentina, Nicola Cellini, Lisa Guy, Alana J. McVey, Keiran Rump, Julie Worley, Brenna B. Maddox, Jennifer Bush, Amanda Bennett, Martin Franklin, Judith S. Miller, et John Herrington. 2021. « Profiles of Autonomic Activity in Autism Spectrum Disorder with and without Anxiety ». *Journal of Autism and Developmental Disorders* 51(12):4459-70. doi: 10.1007/s10803-020-04862-0.
- Patriquin, Michelle A., Elizabeth M. Hartwig, Bruce H. Friedman, Stephen W. Porges, et Angela Scarpa. 2019. « Autonomic Response in Autism Spectrum Disorder: Relationship to Social and Cognitive Functioning ». *Biological Psychology* 145:185-97. doi: 10.1016/j.biopsycho.2019.05.004.
- Patriquin, Michelle A., Jill Lorenzi, et Angela Scarpa. 2013. « Relationship Between Respiratory Sinus Arrhythmia, Heart Period, and Caregiver-Reported Language and Cognitive Delays in Children with Autism Spectrum Disorders ». *Applied Psychophysiology and Biofeedback* 38(3):203-7. doi: 10.1007/s10484-013-9225-6.
- Picard, Rosalind W., Szymon Fedor, et Yadid Ayzenberg. 2016. « Multiple Arousal Theory and Daily-Life Electrodermal Activity Asymmetry ». *Emotion Review* 8(1):62-75. doi: 10.1177/1754073914565517.
- Pomeranz, B., R. J. Macaulay, M. A. Caudill, I. Kutz, D. Adam, D. Gordon, K. M. Kilborn, A. C. Barger, D. C. Shannon, R. J. Cohen, et al. et. 1985. « Assessment of Autonomic Function in Humans by Heart Rate Spectral Analysis ». *American Journal of Physiology-Heart and Circulatory Physiology* 248(1):H151-53. doi: 10.1152/ajpheart.1985.248.1.H151.
- Porges, Stephen W. 1995. « Orienting in a Defensive World: Mammalian Modifications of Our Evolutionary Heritage. A Polyvagal Theory ». *Psychophysiology* 32(4):301-18. doi: 10.1111/j.1469-8986.1995.tb01213.x.
- Porges, Stephen W. 2001. « The Polyvagal Theory: Phylogenetic Substrates of a Social Nervous System ». *International Journal of Psychophysiology* 42(2):123-46. doi: 10.1016/S0167-8760(01)00162-3.
- Porges, Stephen W. 2009. « The Polyvagal Theory: New Insights into Adaptive Reactions of the Autonomic Nervous System ». *Cleveland Clinic Journal of Medicine* 76(4 suppl 2):S86-90. doi: 10.3949/ccjm.76.s2.17.

- Porges, Stephen W., Matthew Macellaio, Shannon D. Stanfill, Kimberly McCue, Gregory F. Lewis, Emily R. Harden, Mika Handelman, John Denver, Olga V. Bazhenova, et Keri J. Heilman. 2013. « Respiratory Sinus Arrhythmia and Auditory Processing in Autism: Modifiable Deficits of an Integrated Social Engagement System? » *International Journal of Psychophysiology* 88(3):261-70. doi: 10.1016/j.ijpsycho.2012.11.009.
- Posada-Quintero, Hugo F., John P. Florian, Alvaro D. Orjuela-Cañón, Tomas Aljama-Corrales, Sonia Charleston-Villalobos, et Ki H. Chon. 2016. « Power Spectral Density Analysis of Electrodermal Activity for Sympathetic Function Assessment ». *Annals of Biomedical Engineering* 44(10):3124-35. doi: 10.1007/s10439-016-1606-6.
- Reimer, Jacob, Emmanouil Froudarakis, Cathryn R. Cadwell, Dimitri Yatsenko, George H. Denfield, et Andreas S. Tolias. 2014. « Pupil Fluctuations Track Fast Switching of Cortical States during Quiet Wakefulness ». *Neuron* 84(2):355-62. doi: 10.1016/j.neuron.2014.09.033.
- Reyes del Paso, Gustavo A., Wolf Langewitz, Lambertus J. M. Mulder, Arie van Roon, et Stefan Duschek. 2013. « The Utility of Low Frequency Heart Rate Variability as an Index of Sympathetic Cardiac Tone: A Review with Emphasis on a Reanalysis of Previous Studies: LF HRV and Sympathetic Cardiac Tone ». *Psychophysiology* 50(5):477-87. doi: 10.1111/psyp.12027.
- Schell, Anne M., Michael E. Dawson, et Diane L. Filion. 1988. « Psychophysiological Correlates of Electrodermal Lability ». *Psychophysiology* 25(6):619-32. doi: 10.1111/j.1469-8986.1988.tb01899.x.
- Schultz, Robert, Fred Volkmar, et Katarzyna Chawarska. 2006. « The Social Brain in Autism: Perspectives from Neuropsychology and Neuroimaging ». P. 323-48 in *Understanding Autism*, édité par S. Moldin et J. Rubenstein. CRC Press.
- Shaffer, Fred, et J. P. Ginsberg. 2017. « An Overview of Heart Rate Variability Metrics and Norms ». *Frontiers in Public Health* 5:258. doi: 10.3389/fpubh.2017.00258.
- Shaffer, Fred, Rollin McCraty, et Christopher L. Zerr. 2014. « A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability ». *Frontiers in Psychology* 5. doi: 10.3389/fpsyg.2014.01040.
- Shaffer, Fred, et Zachary M. Meehan. 2020. « A Practical Guide to Resonance Frequency Assessment for Heart Rate Variability Biofeedback ». *Frontiers in Neuroscience* 14:570400. doi: 10.3389/fnins.2020.570400.
- Sinnreich, R., J. D. Kark, Y. Friedlander, D. Sapoznikov, et M. H. Luria. 1998. « Five Minute Recordings of Heart Rate Variability for Population Studies: Repeatability and Age-Sex Characteristics ». *Heart* 80(2):156-62. doi: 10.1136/hrt.80.2.156.
- Staib, Matthias, Giuseppe Castegnetti, et Dominik R. Bach. 2015. « Optimising a Model-Based Approach to Inferring Fear Learning from Skin Conductance Responses ». *Journal of Neuroscience Methods* 255:131-38. doi: 10.1016/j.jneumeth.2015.08.009.
- Suess, Patricia E., Stephen W. Porges, et Dana J. Plude. 1994. « Cardiac Vagal Tone and Sustained Attention in School-Age Children ». *Psychophysiology* 31(1):17-22. doi: 10.1111/j.1469-8986.1994.tb01020.x.

- Tarvainen, Mika P., Juha-Pekka Niskanen, Jukka A. Lipponen, Perttu O. Ranta-aho, et Pasi A. Karjalainen. 2014. « Kubios HRV – Heart Rate Variability Analysis Software ». *Computer Methods and Programs in Biomedicine* 113(1):210-20. doi: 10.1016/j.cmpb.2013.07.024.
- Taylor, Julie Lounds, et Marsha Mailick Seltzer. 2010. « Changes in the Autism Behavioral Phenotype During the Transition to Adulthood ». *Journal of Autism and Developmental Disorders* 40(12):1431-46. doi: 10.1007/s10803-010-1005-z.
- Tessier, Marie-Pierre, Marie-Hélène Pennestri, et Roger Godbout. 2018. « Heart Rate Variability of Typically Developing and Autistic Children and Adults before, during and after Sleep ». *International Journal of Psychophysiology* 134:15-21. doi: 10.1016/j.ijpsycho.2018.10.004.
- Thapa, Rinku, Gail A. Alvares, Tooba A. Zaidi, Emma E. Thomas, Ian B. Hickie, Shin H. Park, et Adam J. Guastella. 2019. « Reduced Heart Rate Variability in Adults with Autism Spectrum Disorder ». *Autism Research* 12(6):922-30. doi: 10.1002/aur.2104.
- Thapa, Rinku, Izabella Pokorski, Zahava Ambarchi, Emma Thomas, Marilena Demayo, Kelsie Boulton, Slade Matthews, Shrujna Patel, Indra Sedeli, Ian B. Hickie, et Adam J. Guastella. 2021. « Heart Rate Variability in Children With Autism Spectrum Disorder and Associations With Medication and Symptom Severity ». *Autism Research* 14(1):75-85. doi: 10.1002/aur.2437.
- Usher, Marius, Jonathan D. Cohen, David Servan-Schreiber, Janusz Rajkowski, et Gary Aston-Jones. 1999. « The Role of Locus Coeruleus in the Regulation of Cognitive Performance ». *Science* 283(5401):549-54. doi: 10.1126/science.283.5401.549.
- Valentino, Rita J., et Elisabeth Van Bockstaele. 2008. « Convergent Regulation of Locus Coeruleus Activity as an Adaptive Response to Stress ». *European Journal of Pharmacology* 583(2-3):194-203. doi: 10.1016/j.ejphar.2007.11.062.
- Wang, Yao, Marie K. Hensley, Allan Tasman, Lonnie Sears, Manuel F. Casanova, et Estate M. Sokhadze. 2016. « Heart Rate Variability and Skin Conductance During Repetitive TMS Course in Children with Autism ». *Applied Psychophysiology and Biofeedback* 41(1):47-60. doi: 10.1007/s10484-015-9311-z.
- Wu, Guo-Rong, et Daniele Marinazzo. 2016. « Sensitivity of the Resting-State Haemodynamic Response Function Estimation to Autonomic Nervous System Fluctuations ». *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 374(2067):20150190. doi: 10.1098/rsta.2015.0190.
- Young, Fiona L. S., et Anthony S. Leicht. 2011. « Short-Term Stability of Resting Heart Rate Variability: Influence of Position and Gender ». *Applied Physiology, Nutrition, and Metabolism* 36(2):210-18. doi: 10.1139/h10-103.
- Zhao, Sijia, Yajie Liu, et Kunlin Wei. 2022. « Pupil-Linked Arousal Response Reveals Aberrant Attention Regulation among Children with Autism Spectrum Disorder ». *The Journal of Neuroscience* 42(27):5427-37. doi: 10.1523/JNEUROSCI.0223-22.2022.
- Zheng, Zhen, Li Zhang, Shiping Li, Fengyan Zhao, Yan Wang, Lan Huang, Jinglan Huang, Rong Zou, Yi Qu, et Dezhi Mu. 2017. « Association among Obesity, Overweight and

Autism Spectrum Disorder: A Systematic Review and Meta-Analysis ». *Scientific Reports* 7(1):11697. doi: 10.1038/s41598-017-12003-4.



## Supplementary Information

	Children		Children subgroups		Adults	
	TD <sub>C</sub>	ASD <sub>C</sub>	TD <sub>C-PUP</sub>	ASD <sub>C-PUP</sub>	TD <sub>A</sub>	ASD <sub>A</sub>
<i>BMI [range]</i>	21 [13-35]	18 [12-26]	18 [14-22]	18 [13-22]	24 [19-31]	22 [17-29]*
<i>Coffee drinker Yes:No</i>					7:14	1:20*
<i>Smoker Yes:No</i>					9:12	2:19*

**Table S1 : Characteristics of TD and ASD children and adults regarding BMI, coffee drinking and smoking.** BMI (Body Mass Index) is expressed in range from minimum to the maximum value. Coffee drinking and smoking is reported in number of individuals. \*:  $p < 0.05$  for the comparison between TD and ASD groups.

### Effect of BMI on autonomic parameters:

As there was no BMI difference between the ASD and TD children groups (Table S1; ASD<sub>C</sub> vs. TD<sub>C</sub>, ASD<sub>C-PUP</sub> vs. TD<sub>C-PUP</sub>), we did not explore the influence of BMI on the ASD parameters in these groups.

However, as the ASD and TD adults' BMI differed (Table S1; ASD<sub>A</sub> vs. TD<sub>A</sub>), we tested the effect of BMI as a continuous factor on the autonomic parameters within a General Linear Model (GLM). When there was an effect of the group on the autonomic parameters in the first ANOVA analysis, we explored the possible existence of a main effect of BMI or an interaction between BMI and group, completed, when necessary, by post-hoc LSD tests. When there was no effect of the group on the autonomic parameters in the first ANOVA analysis, we explored the possible existence of a main effect of BMI that could mask a group effect.

- *Pupil tone*

When controlling for BMI, we found a tendency of a main effect of BMI on hippus amplitude ( $F_{3,98} = 1.35$ ,  $p = 0.053$ ,  $\eta^2p = 0.10$ ), with BMI increasing as hippus amplitude decreases. As ASD participants exhibited a smaller BMI than TD participants (Table S1), we cannot exclude that the group effect observed on hippus amplitude reflected this difference in BMI. No main

effect of BMI or interaction with the group was observed for median pupil diameter or hippus frequency ( $p > 0.11$ ).

- *Cardiac tone*

Only the RR interval was influenced by BMI (main effect:  $F_{1,35} = 4.33$ ,  $p = 0.044$ ,  $\eta^2p = 0.11$ ), but without interaction with the group. When BMI increased, RR interval decreased. As there was a significant difference in BMI between the ASD<sub>A</sub> and TD<sub>A</sub> groups (Table S1), with ASD participants exhibiting a smaller BMI, we cannot exclude that the tendency for a group effect observed on RR interval reflected this difference in BMI.

- *EDA parameters*

For all the EDA parameters, we observed no main effect of the BMI ( $p > 0.19$ ), but a significant BMI x group interaction (tonic component:  $F_{34} = 4.88$ ,  $p = 0.03$ ,  $\eta^2p = 0.12$ ; number of EDA peaks:  $F_{33} = 5.11$ ,  $p = 0.03$ ,  $\eta^2p = 0.13$ ; EDA AUC :  $F_{34} = 4.19$ ,  $p = 0.05$ ,  $\eta^2p = 0.10$ ; amplitude of EDA peaks:  $F_{33} = 7.06$ ,  $p = 0.01$ ,  $\eta^2p = 0.17$ ), with BMI positively correlated with the EDA parameters in ASD<sub>A</sub> group, and negatively in TD<sub>A</sub> group.

### **Effect of coffee drinking and smoking on autonomic parameters:**

The same approach was used for smoking and coffee drinking status as for BMI.

No effect of smoking or coffee drinking status was found for any of the pupil or EDA parameters.

For cardiac parameters, only the RR interval was influenced by being a smoker or not (but not by being a coffee drinker): we observed a significant interaction between smoker status and group ( $F_{1,35} = 4.57$ ,  $p = 0.039$ ,  $\eta^2p = 0.11$ ). Post-hoc tests revealed a significant difference between ASD<sub>A</sub> non-smokers and TD<sub>A</sub> non-smokers ( $p = 0.02$ ), with RR interval higher in TD<sub>A</sub> non-smokers, while the reverse pattern was observed for smokers (not significant, only 2 ASD adult smokers). No main effect of smoking or coffee drinking status was found for any of the other cardiac parameters.