

RESEARCH ARTICLE

Psychological characteristics and physiological reactivity to acute stress in mothers of children with autism spectrum disorder

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Abstract

Stress related to parenting a child with autism spectrum disorder can differently affect caregiver's physiological reactivity to acute stress. Here, parental stress levels, psychological characteristics, and coping strategies were assessed alongside measures of heart rate, heart rate variability, and cortisol during a psychosocial stress test in mothers of children with ASD (M-ASD, $n = 15$) and mothers of typically developing children ($n = 15$). M-ASD reported significantly higher levels of parental stress, anxiety, negative affectivity, social inhibition, and a larger preference for avoidance strategies. M-ASD showed larger heart rate and cortisol responses to the psychosocial stress test. A positive relationship was found between parental stress levels and the magnitude of the cortisol stress response in both groups. The present findings indicate exaggerated physiological reactivity to acute psychosocial stress in M-ASD and prompt further research to explore the role of individual differences in mediating the effects of parental stress on physiological stress responses.

KEYWORDS

anxiety, autism, caregiving, cortisol, heart rate, stress

1 | INTRODUCTION

Stress is a natural experience related to parenting and the associated responsibilities of caring for a child (Bornstein, 2002). Parents of children with autism spectrum disorder (ASD), a lifelong neurodevelopmental disorder characterized by persistent deficits in social communication, social interaction, and restricted and repetitive patterns of behaviour or interests (American Psychiatric Association, 2013), show a higher psychosocial burden than parents of typically developing children (Baker-Ericzén, Brookman-Frazee, & Stahmer, 2005; Bromley, Hare, Davison, & Emerson, 2004; Davis & Carter, 2008). Indeed, high incidence rates of depression and anxiety have been documented in this population of caregivers (Kuusikko-Gauffin et al., 2013; van Steijn, Oerlemans, van Aken, Buitelaar, & Rommelse, 2014). In addition to psychological effects, parents of children with

ASD may experience a number of changes in various physiological systems that may lead to health impairments, as observed in other populations of caregivers (Corrêa et al., 2015). The polyvagal theory has indeed proposed a theoretical model to explain the coexistence of dysregulated physiological systems in psychiatric and behavioural disorders characterized by difficulties in regulating appropriate social, emotional, and communication behaviours (Porges, 2007). Specifically, a few studies have investigated physiological reactivity to acute stress in parents of adolescents with ASD and reported a lower stress-induced sympathetic activation in this population compared with noncaregivers, as indexed by electrodermal activity (Ruiz-Robledillo & Moya-Albiol, 2015) and heart rate variability (HRV; Ruiz-Robledillo, Bellosta-Batalla, & Moya-Albiol, 2015). Interestingly, a positive relationship was found between the magnitude of sympathetic stress reactivity and somatic symptoms severity in caregivers only,

suggesting that a blunted stress-induced sympathetic activation may be indicative of an adaptive habituation to stress (Ruiz-Robledillo et al., 2015; Ruiz-Robledillo & Moya-Albiol, 2015). Similarly, a buffered cortisol response to a mental psychosocial stressor was found in parents of offspring with ASD in comparison with noncaregiver parents (De Andrés-García, Moya-Albiol, & González-Bono, 2012).

An important issue in research addressing the psychological and physiological consequences of parenting children with ASD (or other disabilities) is the study of individual differences: Personality factors, coping strategies, cognitions, and psychological trait characteristics may have a profound impact on individuals' psychological and physiological consequences of parenting-related stress (Bitsika, Sharpley, & Bell, 2013; Davis & Carter, 2008). For example, in a longitudinal study, depressive symptoms in mothers of ASD children were found to be influenced by individual levels of parental anxiety and efficacy, coping styles, and social support (Carter, Martínez-Pedraza, & Gray, 2009). Moreover, a significant positive correlation was found between parental stress levels and anxiety and depression symptom severity in parents of children with ASD, even though these self-reported measures were not correlated to 24-hr physiological (heart rate and blood pressure) and neuroendocrine (cortisol) measures (Padden & James, 2017). Interestingly, a recent study assessed the association between resilient coping and cortisol response to acute stress in caregivers of adolescents with ASD and demonstrated that stress-induced cortisol response was lower in high-resilient caregivers than low-resilient caregivers (Ruiz-Robledillo, Romero-Martínez, & Moya-Albiol, 2017).

The aim of the present study, which was conducted in mothers of children with ASD (M-ASD) and mothers of typically developing children (M-TD), was twofold. First is to extend previous findings on the consequences of parenting a child with ASD on physiological reactivity to acute stress. To reach this goal, heart rate, HRV, and salivary cortisol levels were assessed during a psychosocial stress challenge alongside measures of state anxiety and non-verbal behaviour. Based on the existing literature (De Andrés-García et al., 2012; Ruiz-Robledillo et al., 2015; Ruiz-Robledillo et al., 2017; Ruiz-Robledillo & Moya-Albiol, 2015), we hypothesized that M-ASD would show a blunted physiological stress reactivity compared with M-TD. Second, we explored the extent to which parenting stress levels would predict physiological responses to an acute psychosocial challenge as a function of group and/or individual psychological characteristics and behavioural coping strategies.

2 | MATERIALS AND METHODS

2.1 | Participants

Thirty M-ASD and 23 M-TD received written information and a verbal explanation about the purpose of the study and were invited to take part in it. M-ASD were recruited from the Center for Care, Diagnosis and Study of Communication and Socialization Disorders of the Local Health Authority of Parma and from the Children Autism Foundation

of Fidenza, Italy. Inclusion criteria for this group were the following: (a) being a mother of and living with a 3- to 11-year-old child diagnosed by a trained neuropsychiatrist with a moderate level of ASD according to DSM-5 (American Psychiatric Association, 2013) and ADOS-2 criteria (Lord et al., 2012); (b) 30 to 50 years of age. Exclusion criteria for this group were the following: (a) having other children or a partner with disabilities or chronic diseases; (b) having suffered from particularly stressful events such as deaths, serious accidents, dismissal, or divorce during the previous year; (c) a history of cardiovascular disease or previous diagnosis of psychological disorders; (d) obesity, menopause, and pregnancy or childbirth within the last 12 months; and (e) use of drugs that may affect cardiovascular or neuroendocrine functions. Fifteen subjects failed to meet these prerequisites and were excluded from the study. M-TD were recruited from kindergartens and primary schools of the region through an awareness campaign carried out by the parents' representatives, with the cooperation of the principals of the schools. Inclusion and exclusion criteria for the M-TD group were the same as for the other group, except for the fact that M-TD had to be mothers of and live with a child of 3 to 11 years of age without any diagnosed chronic condition or disability. The final sample consisted of 15 M-ASD (time since the diagnosis of the child = 2.7 ± 0.3 years) and 15 M-TD. Their demographic, anthropometric, and lifestyle characteristics are reported in Table 1. Participants were not compensated for their time. The protocol was approved by the Bioethical Committee of the University of Parma, Italy (Prot. N. 27135). All participants signed an informed consent.

2.2 | Procedure

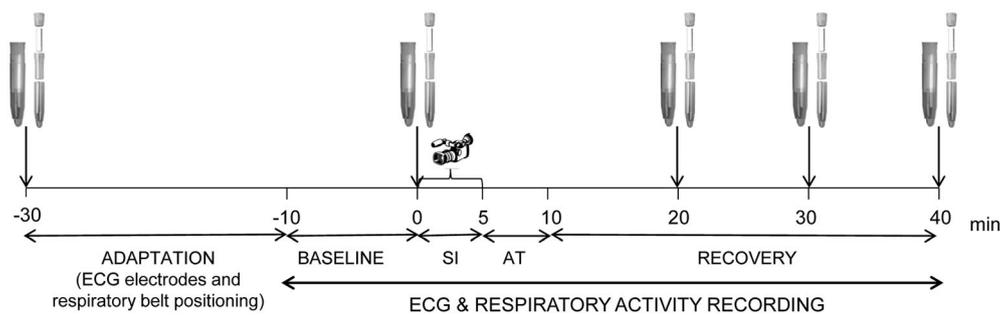
Participants were first invited to the lab to complete sociodemographic, anthropometric, and lifestyle questionnaires, as well as a series of psychometric scales assessing parenting stress levels, personality and psychological characteristics, and coping strategies (see below). Three days later, participants returned to the lab and were submitted to a psychosocial stress test (PST), following a previously adopted procedure (Pico-Alfonso et al., 2007) that was based on an adapted version of the Trier Social Stress Test (Kirschbaum, Pirke, & Hellhammer, 1993). Participants were asked to refrain from caffeine, alcohol, and nicotine consumption, as well as strenuous exercise for at least 2 hr prior to the experimental session, as these variables may have transient effects on cardiovascular/neuroendocrine measurements (e.g., Zimmermann-Viehoff et al., 2016). Figure 1 depicts the sequence of events included in the PST protocol of this study. Upon their arrival to the lab, a saliva sample was collected from each participant using oral swabs and swab storage tubes (Salimetrics, UK). Participants were then fitted with a BT16Plus device (Francesco Marazza Hardware & Software, Monza, IT), which allows real-time acquisition of ECG (sampling frequency: 250 Hz), respiratory (sampling frequency: 25 Hz), and gross physical activity signals in freely moving subjects. Subsequently, they were allowed to settle down in the new environment for 20 min while sitting on a comfortable chair in front

TABLE 1 Sociodemographic, anthropometric, and lifestyle characteristics

	M-TD (n = 15)	M-ASD (n = 15)	t or χ^2	p	Effect size (Cohen's d)
Age (years)	38.3 ± 0.8	39.5 ± 0.7	1.10	N.S.	.41
Marital status	12 M 3 D	14 M 1 D	1.15	N.S.	.40
Number of children at home	1 (60%) 2 (40%)	1 (67%) 2 (33%)	0.14	N.S.	.14
BMI (kg/m ²)	21.0 ± 1.6	22.3 ± 0.5	0.75	N.S.	.28
Education (years)	7 H 8 U	10 H 5 U	1.22	N.S.	.41
Smoking status	6 Y 9 N	10 Y 5 N	2.14	N.S.	.55
Use of oral contraceptives	7 Y 8 N	7 Y 8 N	0	N.S.	.00
Child age (years)	5.7 ± 0.2	5.3 ± 0.2	-1.53	N.S.	.65

Note. Data are expressed as means ± SEM.

Abbreviations: M, married; D, divorced; H, high school; U, university; Y, yes; N, no.

**FIGURE 1** Outline of the psychosocial stress test protocol. SI, stress interview; AT, arithmetic task

of two familiar experimenters. Continuous ECG recordings were performed during the following phases: baseline (10 min), stress interview task (5 min), arithmetic task (5 min), and recovery (30 min; Figure 1). In baseline conditions, participants sat quietly in the presence of the same experimenters. During the social interview phase, they were asked to answer a series of questions about how they behave and feel in different social contexts (e.g., “What are the social situations that make you most uneasy?”). Subsequently, they were asked to complete a 5-min mental arithmetic task by counting aloud backwards from 2083 by 13’s. The stress tasks were administered by an unfamiliar male interviewer, with a small unfamiliar audience (two people) sitting behind the participants to heighten the socially evaluative character of the stress context. During both tasks, the interviewer applied pressure by instructing participants to “please continue” or “go faster” when they paused. The interviewer also corrected participants’ math responses, invited them to restart from 2083 after an incorrect response, and encouraged them to “try to do better.” Upon completion of the stress phases, the unfamiliar interviewer and audience left the room, and participants remained seated and quiet in the presence of the two familiar experimenters for the following 30-min recovery phase. Saliva samples were collected immediately before, and 20, 30, and 40 min after the beginning of stress interview (Figure 1). Moreover, the behaviour of the participants during the stress interview was videotaped with a camera positioned in front of them (Figure 1).

Finally, before and after the PST participants completed the state version of the State-Trait Anxiety Inventory (STAI; Spielberger & Gorsuch, 1983). The PST took place between 14:30 and 16:30 hr, in a quiet room at a comfortable temperature (21 ± 2°C).

2.3 | Psychometric assessment

Stress related to the role of parenting was assessed using the Parenting Stress Index-Short Form (PSI-SF; Abidin, 1995), which is a 36-item, self-report instrument that contains statements such as “I feel trapped by my responsibilities as a parent” and “My efforts for child aren’t appreciated.” Individuals rate their level of agreement with each statement on a 5-point Likert scale from 1 (*strongly agree*) to 5 (*strongly disagree*). The PSI-SF yields an overall parenting stress score from three different subscales: parental distress (PD), parent-child dysfunctional interaction, and difficult child. However, an item analysis has identified PD as the only valid subscale for parents of children with ASD (Zaidman-Zait et al., 2010). Therefore, only the PD subscale was considered in the present study. PD examines the extent to which the parent experiences stress in his/her role as a parent. The PSI-SF shows high internal consistency (Cronbach’s α .92), and its validity has been established in parents of children with chronic medical conditions, including diabetes and asthma (Abidin, 1995; Wysocki, Huxtable, Linscheid, & Wayne, 1989).

The severity of trait anxiety was measured using the trait version of the STAI (Spielberger & Gorsuch, 1983), which is a 4-point Likert scale consisting of 20 items assessing how the patient feels, independent from the status and circumstances (e.g., "I feel secure," "I feel troubled"). The lowest score that can be obtained is 20 and the highest is 80: Higher scores indicate higher anxiety levels. The validity of this scale has been repeatedly confirmed, with reliability coefficients ranging from 0.71 to 0.86 and internal consistency and homogeneity coefficients between 0.83 and 0.87 (Aydemir & Köroğlu, 2000). State anxiety was measured using the state version of the STAI, which asks how respondents feel "right now" using 4-point Likert scale items that measure subjective feelings of apprehension, tension, nervousness, worry, and activation/arousal of the autonomic nervous system. The reliability coefficient is 0.62.

The Type D Personality Scale (DS14) was used to assess the presence of Type D personality (Denollet, 2005). The DS14 consists of two 7-item subscales: negative affectivity (e.g., "I am often irritated") and social inhibition (e.g., "I find it hard to start a conversation"). Each item is rated on a 5-point Likert-type scale ranging from 0 to 4. Subjects scoring above the cut-off score of 10 on both subscales are categorized as Type D (Denollet, 2005). The Italian version of the negative affectivity and social inhibition subscales show high internal consistency (Cronbach's α .82 and .80) and reliability ($r = 0.62$ to 0.81 ; Gremigni & Sommaruga, 2005).

Coping strategies were assessed using the Italian version of the Coping Orientation to Problems Experienced (COPE-NVI; Sica et al., 2008), which is a 60-item, self-report instrument that evaluates four independent dimensions of coping: social support, avoidance strategies, positive attitude, task-oriented, and transcendental orientation. All items are rated on a 4-point scale: 1 (*I usually don't do this at all*); 2 (*I usually do this a little bit*); 3 (*I usually do this a medium amount*); 4 (*I usually do this a lot*). The COPE-NVI shows high internal consistency (Cronbach's α from .62 to .92) and reliability (coefficients ranging from 0.48 to 0.77; Sica et al., 2008)

2.4 | ECG analysis

ECG signals were recorded by means of three electrodes secured to the right and left parasternal regions and the left groin area, respectively. ECG signals were amplified, converted to digital, transferred to a laptop computer via Bluetooth technology, preprocessed in a Matlab environment, and stored for off-line analysis (BT16Plus Acquisition Software 1.9.0, Francesco Marazza), which was performed by means of Chart 5 software (ADInstruments, Sydney, Australia). Initially, each raw ECG signal was manually inspected to ensure that all R-waves were correctly detected. Across participants, less than 1% of RR intervals were identified as artifacts and excluded before further analysis. For each recording period, ECGs were split in 5-min epochs. For each epoch, we then calculated heart rate (HR) by plotting the number of R waves per unit time (reported in beats per minute; bpm) and quantified time- and frequency-domain indexes of HRV. In the time-domain, we calculated the root mean square of successive

beat-to-beat interval differences (RMSSD, ms), which reflects vagal regulation of HR (Laborde, Mosley, & Thayer, 2017). For the frequency-domain analysis, the power spectrum was obtained with a fast Fourier transform-based method (Welch's periodogram: 256 points, 50% overlap, and Hamming window) to quantify the power of the high frequency band (HF; 0.15–0.4 Hz, ms^2), which is due to the activity of the parasympathetic nervous system and includes respiration-linked oscillations of HR (Laborde et al., 2017). HR and HRV responses to the PST were also quantified by computing the area under the response time curve with respect to baseline values (AUCb; Pruessner, Kirschbaum, Meinschmid, & Hellhammer, 2003).

2.5 | Saliva collection and cortisol determination

Immediately after collection, saliva samples were frozen at -20°C until analysis. Salivary cortisol levels were determined by radioimmunoassay. Samples were thawed, brought to room temperature and centrifuged ($1,500 \text{ g} \times 10 \text{ min}$), resulting in a clear supernatant of low-viscosity. Cortisol levels were assayed in duplicates following kit instructions with a 96-well plate enzyme-linked immunosorbent assay (High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit: Salimetrics LLC, State College, PA), using Infinite F50 plate reader and Magellan software (TecanGroup Ltd, Männedorf, Switzerland). Cortisol responses to the PST were also quantified by computing the AUCb (baseline reference value was calculated as the mean value between min -30 and min 0 time values; Pruessner et al., 2003).

2.6 | Non-verbal behaviour analysis

Non-verbal behaviour during the stress interview of the PST was analysed by means of the Ethological Coding System for Interviews by a trained observer. The Ethological Coding System for Interviews is a modified version of Grant's ethogram (Grant, 1968; Troisi, 1999) and is designed to measure 37 patterns of non-verbal behaviours, which are grouped in eight macro categories that reflect the subject's intention to communicate a certain mood or intention and show different aspects of individual's psychosocial attitude (Dixon & Fisch, 1998). Before the beginning of the study, the observer was trained until she reached an adequate level of inter-observer reliability. Assessment of inter-observer reliability was based on a sample of 30 interviews not including those with the subjects of this study. Recording method was one-zero sampling. The recording session was divided into successive 15-s sample intervals. The instant of time at the end of each sample interval, referred to as the "sample point," was identified by a beeper. On the instant of each sample point, the observer recorded whether or not the behaviour pattern had occurred during the preceding sample interval. The score of each behaviour for each subject was expressed as the proportion of all sample intervals during which that behaviour occurred (see Troisi, 1999 for details). One-zero scores are highly correlated with both frequency and duration measures of the same behaviour, which means that they give a composite measure of "amount" of behaviour. Using a short sample interval (e.g., 15 s), the scores obtained using

TABLE 2 Psychometric assessment

	M-TD (n = 15)	M-ASD (n = 15)	t or χ^2	p	Effect size (Cohen's d)
PSI-SF					
Parental distress (score)	23.0 ± 1.5	38.4 ± 1.2	8.07	<.05	2.93
STAI					
Trait anxiety (score)	33.8 ± 1.9	49.5 ± 2.1	5.57	<.05	2.02
DS-14					
Negative affectivity (score)	8.4 ± 1.3	12.6 ± 0.7	2.94	<.05	1.04
Social inhibition (score)	5.4 ± 0.9	12.1 ± 1.1	4.71	<.05	1.72
Type-D personality (incidence)	2/15	11/15	11.0	<.05	1.52
COPE-NVI					
Social support (score)	29.4 ± 1.9	34.1 ± 1.6	1.86	N.S.	.69
Avoidance (score)	21.1 ± 1.1	32.4 ± 2.7	3.85	<.05	1.42
Positive attitude (score)	30.8 ± 1.0	28.1 ± 1.4	1.57	N.S.	.57
Task oriented (score)	32.4 ± 1.0	36.7 ± 1.5	2.35	<.05	.87
Transcendent orientation (score)	21.1 ± 1.2	17.2 ± 1.0	2.47	<.05	.91

Note. Data are expressed as means ± SEM.

Abbreviations: COPE-NVI, New Italian Version of the Coping Orientation to Problems Experienced; PSI-SF, Parenting Stress Index-Short Form; STAI, State-Trait Anxiety Inventory; DS14, Type D Personality Scale.

one-zero sampling and continuous recording are highly correlated (Troisi, 1999). Briefly, the following categories were considered: (a) *eye contact*, which expresses involvement and attention, (b) *affiliation*, a positive attitude that promotes social interaction; (c) *submission*, whose purpose is to maintain not hostile social interaction; (d) *flight*, which includes all the non-verbal behaviours aimed at avoiding social stimuli that are perceived as harmful or stressful, (e) *assertion*, which includes all the facial expressions and hand movements indicating a low level of aggressiveness, (f) *gesture*, which is a reliable index of global psychomotor activity and includes all the hand movements that illustrate and reinforce the contents of a speech, (g) *displacement*, which includes all the movements that are focused on one's own body or addressed to manipulate external objects, and (h) *relaxation*, which indicates a low level of emotional arousal.

2.7 | Statistical analysis

All statistical analyses were performed using SPSS 24 software package (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p < .05$. Assumptions for normality were tested for all continuous variables using the Shapiro-Wilk test. We accounted for non-normal distribution of RMSSD and HF values ($p < .05$) by calculating their natural logarithm (lnRMSSD and lnHF). Sociodemographic, anthropometric, and lifestyle characteristics were compared between the two groups using independent *t* tests and Chi-square tests, as appropriate. PSI-SF, STAI-T, COPE-NVI, and DS-14 scores were compared between the two groups using independent *t* tests. Correlations among personality variables were assessed using partial correlation analysis, controlling for the effects of group, with a Bonferroni correction for multiple

comparisons. Two-way ANOVAs for repeated measures, with "group" as the between-subject factor (two levels: M-TD and M-ASD) were applied for (a) HR and HRV data (within-subject factor "time": 10 time points), (b) cortisol levels (within-subject factor "time": five time points during the PST), and (c) STAI-S scores (within-subject factor "time": two time points). Follow-up analyses were conducted using Student's *t* tests, with a Bonferroni correction for multiple comparisons. Differences in AUCb values and non-verbal behaviour between M-TD and M-ASD subjects during the PST were statistically evaluated via Student's *t* tests. Additionally, correlations among AUCb values and non-verbal behaviour were assessed using partial correlation analysis, controlling for the effects of group, with a Bonferroni correction for multiple comparisons. To test the hypothesis that parental stress levels would predict physiological (HR, HRV, and cortisol) responses to PST as a function of group and/or individual psychometric characteristics and coping strategies, a series of GLMs were performed using AUCb HR, HRV, and cortisol values, incorporating as predictors (a) parental distress score, and the interaction terms of parental distress with (b) group, (c) STAI-T score, (d) negative affectivity score, (e) social inhibition score, and (f) percentage of avoidance strategies.

3 | RESULTS

3.1 | Sociodemographic, anthropometric, and lifestyle data

M-TD and M-ASD subjects were well matched regarding all sociodemographic, anthropometric, and lifestyle characteristics, which are listed in Table 1.

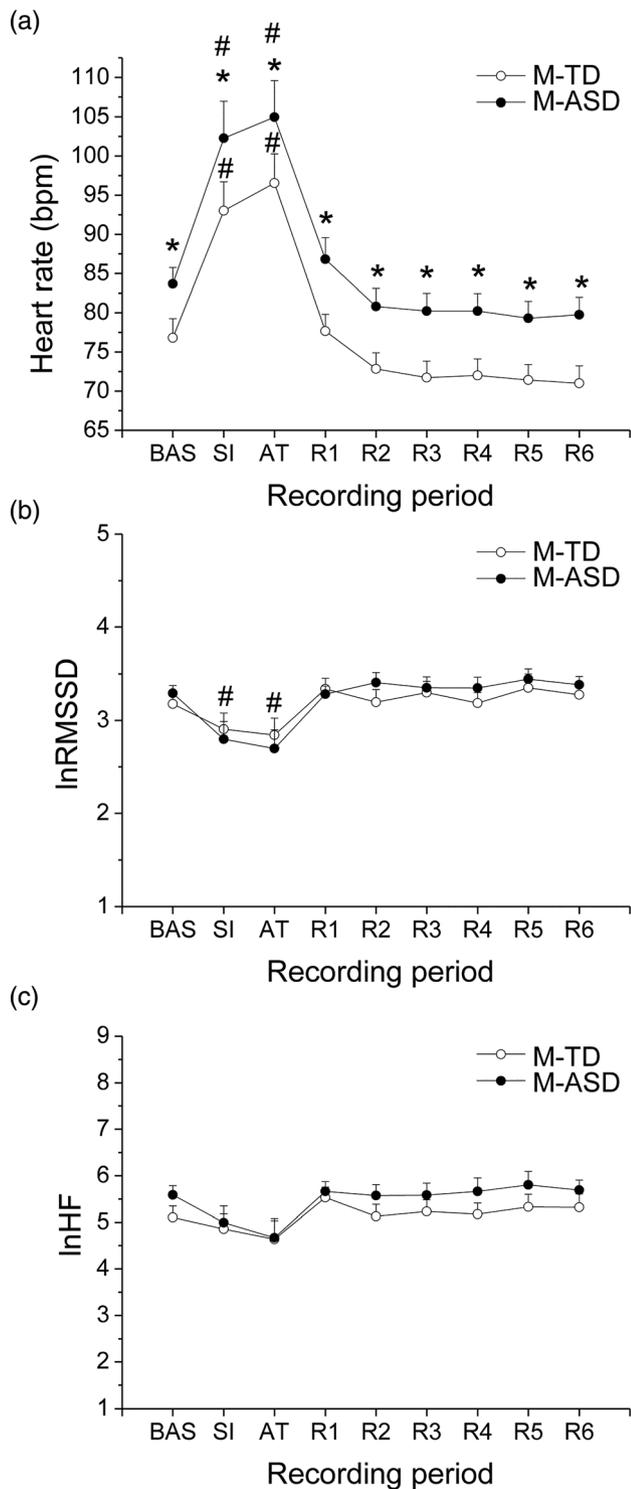


FIGURE 2 Time course of changes in heart rate (panel a), lnRMSSD (panel b), and lnHF (panel c) values during the psychosocial stress test, in mothers of typically developing children (M-TD, $n = 15$) and mothers of children with autism spectrum disorder (M-ASD, $n = 15$). Data are reported as means \pm SEM. BAS, baseline; SI, stress interview; AT, arithmetic task; R, recovery; lnRMSSD, natural logarithm of the root mean square of successive beat-to-beat interval differences; lnHF, natural logarithm of the high frequency band. * indicates a significant difference between the two groups, and # indicates a significant difference compared with the respective baseline value ($p < .05$)

3.2 | Psychometric assessment

Psychological and personality characteristics of the two groups are summarized in Table 2. M-ASD reported higher levels of parental distress than controls. In addition, M-ASD reported higher levels of trait anxiety and significantly higher scores in both the negative affectivity and social inhibition scales of the DS14 than M-TD. Interestingly, we found a significantly higher incidence of Type D personality among M-ASD compared with M-TD. With respect to coping strategies, M-ASD reported significantly higher scores than M-TD in the avoidance, task-oriented and transcendent orientation subscales. Moreover, partial correlation analysis revealed a significant positive association between trait anxiety levels and negative affectivity scores ($r = .61$, $p < .05$). No other significant relationships among psychometric measurements were found.

3.3 | Physiological responses to the PST

HR and HRV values during the PST are depicted in Figure 2. Two-way ANOVA yielded a significant effect of group ($F = 5.61$, $p < .05$) for HR values, with M-ASD having significantly higher mean HR values than M-TD in all recording periods (Figure 2a). The overall HR response to the PST, calculated as the AUCb, was significantly larger in M-ASD than M-TD (M-ASD = 3691 ± 440 beats vs. M-TD = 3337 ± 361 beats; $t = 2.40$, $p < .05$, $d = .87$). However, there were no group differences in vagally mediated HRV parameters both during resting conditions and in response to stress (Figure 2b,c).

Salivary cortisol levels during the PST are depicted in Figure 3. Two-way ANOVA yielded a significant effect of time ($F = 9.69$, $p < .05$) and a time \times group interaction ($F = 5.76$, $p < .05$). Prestress cortisol levels were similar between the two groups. At min 20, we found a significant stress-induced increment in cortisol levels in M-ASD, but not in M-TD, compared with baseline levels ($t = 2.65$, $p < .05$, $d = .97$). However, absolute cortisol values did not significantly differ between the two groups. In addition, M-ASD showed significantly higher cortisol values compared with (a) their respective time 0 value at min 30 ($t = 2.94$, $p < .05$, $d = .99$) and (b) M-TD at min 30 ($t = 2.22$, $p < .05$, $d = .81$) and min 40 ($t = 2.25$, $p < .05$, $d = .92$). The overall cortisol response to the PST, as calculated as the AUCb, was larger in M-ASD than M-TD ($t = 2.03$, $p = .053$, $d = .63$).

Moreover, a significant positive relationship was found between parental distress scores and AUCb cortisol values ($r^2 = 0.752$), independently from group (M-TD: $r^2 = 0.607$; M-ASD: $r^2 = 0.381$), personality characteristics and coping strategies (Table 3 and Figure 4). No significant interactions were found between parental distress levels and the other outcome variables tested in this study.

3.4 | Non-verbal behaviour

Figure 5 reports non-verbal behaviour during the PST. M-ASD reported higher frequencies of affiliation ($t = 3.5$; $p < .05$, $d = 1.32$), submission ($t = 2.1$; $p < .05$, $d = .79$), flight ($t = 2.5$; $p < .05$, $d = .96$),

FIGURE 3 Time course of changes in salivary cortisol levels during the psychosocial stress test, in mothers of typically developing children (M-TD, $n = 15$) and mothers of children with autism spectrum disorder (M-ASD, $n = 15$). Data are reported as means \pm SEM. * indicates a significant difference between the two groups and # indicates a significant difference compared with the respective baseline value ($p < .05$)

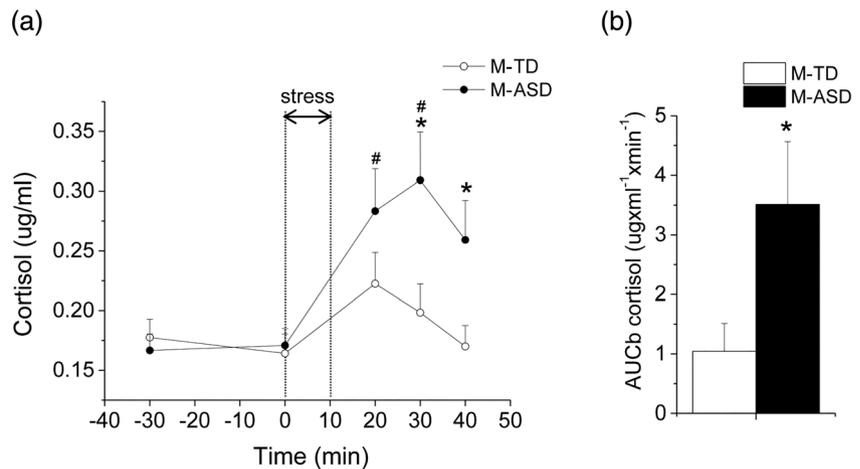


TABLE 3 General linear model for the prediction of cortisol response (indexed by AUCb values) to the psychosocial stress test by parental distress scores (PD subscale), as a function of group, trait anxiety (STAI-T scale), personality characteristics (DS14 scale), and coping strategies (COPE-NVI scale)

Parameter	β	SE	t	p	95% CI
Intercept	-2.87	3.13	0.92	N.S.	-9.354-3.608
PD (score)	0.41	0.19	2.11	<.05	0.009-0.823
PD*Group (M-TD, M-ASD)	-0.09	0.09	-1.01	N.S.	-0.280-0.097
PD*STAI-T (score)	-0.01	0.01	-1.13	N.S.	-0.009-0.003
PD*negative affectivity (score)	0.01	0.01	0.18	N.S.	-0.015-0.018
PD*social inhibition (score)	-0.01	0.01	-0.82	N.S.	-0.016-0.007
PD*avoidance (score)	-0.01	0.01	-0.47	N.S.	-0.007-0.004

Abbreviations: AUCb, area under the response curve with respect to baseline; PD, parental distress; STAI-T, State-Trait Anxiety Inventory, Trait version; DS14, Type D Personality Scale-14; COPE-NVI, New Italian Version of the Coping Orientation to Problems Experienced; M-TD, mothers of typically developing children; M-ASD, mothers of children with autism spectrum disorder.

and displacement ($t = 2.4$; $p < .05$, $d = .92$) behaviours than M-TD. Partial correlation analysis between physiological stress responses and non-verbal behavioural patterns did not reveal any significant relationship.

3.5 | State anxiety

Two-way ANOVA applied to state-anxiety scores yielded significant effects of group ($F = 25.2$, $p < .05$) and time ($F = 17.2$, $p < .05$). Specifically, M-ASD reported higher levels of state-anxiety than M-TD both prior to (M-ASD = 41.3 ± 1.9 vs. M-TD = 32.8 ± 0.8 , $t = 4.05$, $p < .05$, $d = 1.51$) and at the end of (M-ASD = 39.5 ± 1.8 vs. M-TD = 28.5 ± 1.0 , $t = 5.34$, $p < .05$, $d = 1.95$) the PST. Furthermore, state anxiety levels were significantly lower at the end of the PST compared with pre-PST levels in M-TD ($t = 3.34$, $p < .05$), but not in M-ASD.

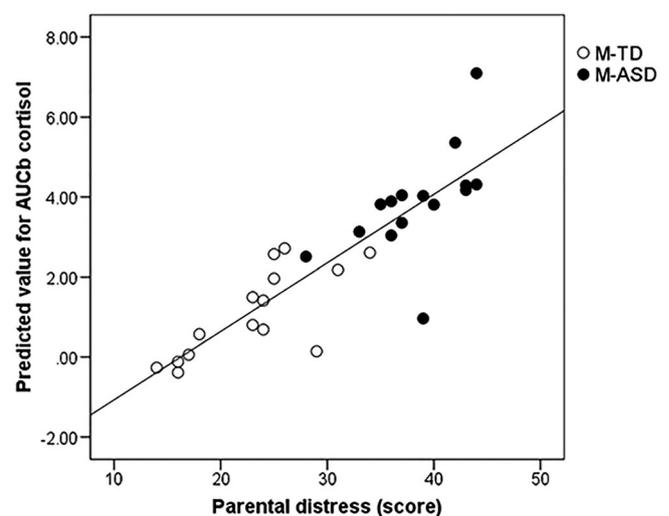


FIGURE 4 Scatter plot of the prediction of cortisol response (indexed by the area under the response curve with respect to baseline [AUCb]) to the psychosocial stress test by parental stress score, in mothers of typically developing children (M-TD, $n = 15$) and mothers of children with autism spectrum disorder (M-ASD, $n = 15$). Figure shows the strong positive relationship between parental distress levels and cortisol stress reactivity

4 | DISCUSSION

4.1 | Physiological reactivity to psychosocial stress

M-ASD exhibited significantly higher mean HR values than M-TD during the resting period that preceded the PST. Other studies reported significantly higher mean 24-hr HR values in caregivers of patients with dementia (Von Känel, Dimsdale, Patterson, & Grant, 2003), but not in parents of children with ASD (Padden & James, 2017). The apparent discrepancy between our results and those reported for 24-hr recordings in parents of children with ASD (Padden & James, 2017) might be due to the fact that M-ASD perceived the laboratory assessment as a stressful situation per se, and therefore HR values prior to the PST did not reflect true resting state values. This is

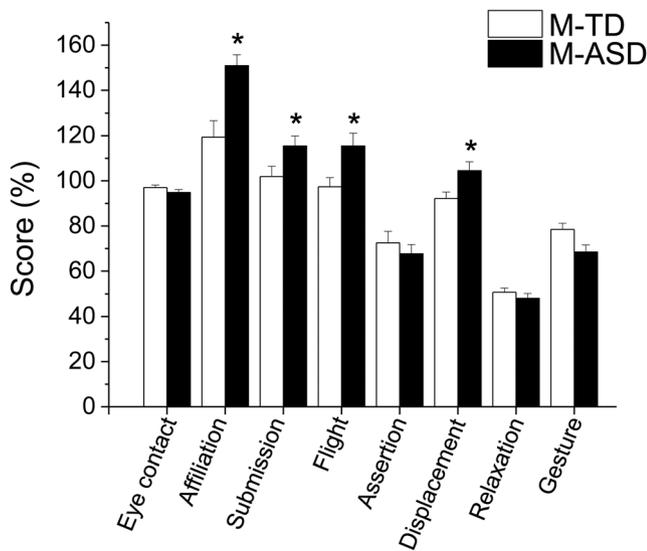


FIGURE 5 Non-verbal behaviour during the social interview phase of the psychosocial stress test, in mothers of typically developing children (M-TD, $n = 15$) and mothers of children with autism spectrum disorder (M-ASD, $n = 15$). Data are reported as means \pm SEM. * indicates a significant difference between the two groups

somewhat supported by the higher levels of state anxiety found in M-ASD compared with M-TD prior to the beginning of the PST. However, M-ASD showed higher HR values throughout all the phases of the PST. Notably, this chronotropic effect was not explained by differences in the autonomic modulation of cardiac function (indexed by HRV analysis) between M-ASD and M-TD. Therefore, further investigation is warranted to investigate the biological determinants of higher HR in mothers of children with ASD. Contrary to our initial hypothesis, M-ASD showed greater HR and cortisol responses to the PST compared with noncaregivers, which is in apparent contradiction with previous reports of blunted sympathetic and cortisol responses to acute stress in parents of adolescents with ASD (De Andrés-García et al., 2012; Ruiz-Robledillo et al., 2015; Ruiz-Robledillo et al., 2017; Ruiz-Robledillo & Moya-Albiol, 2015). The authors of these studies argued that a dampened physiological stress reactivity in caregivers could be explained by processes related to habituation to stress. In other words, the daily stressors associated with their caring role could change their perception of the laboratory stressor, which may be perceived as less threatening, and dampen their physiological stress response. The major difference between the present investigation and the above cited studies is represented by the age of the care recipient (i.e., 5 years old vs. adolescents). It is therefore tempting to speculate that the number of years spent caring for a child with ASD, and the associated build-up of stress, may represent a critical factor in determining the extent to which caregiving stress influence physiological reactivity to an acute heterotypic stressor, as also previously suggested (De Andrés-García, Sariñana-González, Romero-Martínez, Moya-Albiol, & Gonzalez-Bono, 2013). Notably, we found that the magnitude of the cortisol response to the PST was predicted by self-reported levels of parental distress in both groups, further suggesting that stress-related to parenting a young child may bring about

excessive cortisol responses to an acute heterotypic stressor. Moreover, M-ASD failed to reduce their levels of state anxiety at the end of the PST, supporting previous results (Conner, Maddox, & White, 2013), and showed a slower return of cortisol levels towards baseline levels after the PST, suggesting excessive emotional arousal and difficulties in deactivating the anxious and neuroendocrine stress responses in this population of M-ASD.

4.2 | Psychological and personality characteristics of M-ASD

Raising a child with a severe disability represents a major burden in various domains of life. Not surprisingly, in this study M-ASD reported larger levels of parental stress than M-TD, replicating previous findings (Davis & Carter, 2008; Padden & James, 2017; Ruiz-Robledillo & Moya-Albiol, 2013). M-ASD showed higher levels of trait anxiety than M-TD. High trait anxiety is a clinical feature of several anxiety spectrum disorders, including generalized anxiety disorder (Chambers, Power, & Durham, 2004). Some of the clinical symptoms of generalized anxiety disorder (American Psychiatry Association, 2013), such as being easily fatigued, difficulty concentrating, muscle tension, sleep disturbance, are found in many parents of children with ASD (Hastings, 2003). These symptoms, even when they do not lead to the development of an anxiety disorder, can affect mental health and daily activities, thus negatively impacting the quality of life of parents of children with ASD.

The present findings on personality factors indicate that M-ASD are characterized by higher levels of negative affectivity and social inhibition and a much larger incidence of Type-D personality than M-TD. This personality trait is typified by a general propensity to experience psychological distress (Pedersen & Schiffer, 2011), thus representing a risk factor for the development of anxiety and depressive symptoms and poor health outcomes (Pedersen & Schiffer, 2011). The positive correlation found between trait anxiety and negative affectivity scores further indicates that greater levels of anxiety in M-ASD are related to the expression of negative emotions. Notably, to the best of our knowledge, this is the first study to report a higher incidence of Type D personality in mothers of children with ASD. Given the documented negative relationship between Type D personality and health outcomes (Denollet, Pedersen, Vrints, & Conraads, 2006), further investigation on the presence of this personality type among mothers of children with ASD is warranted. However, we failed to demonstrate the presence of significant relationships between personality and psychological characteristics and physiological stress reactivity.

4.3 | Coping strategies

Anxiety has also been linked to avoidance coping. For example, people with panic disorder engage in avoidance coping (including not leaving their home in some cases) in order to try to avoid panic feelings (Feldner, Zvolensky, & Leen-Feldner, 2004). Accordingly, M-ASD reported a higher use of avoidance strategies when facing stressful

situations. A recent study demonstrated a higher use of a number of adaptive coping strategies, including emotional support, positive reframing, planning, humour, acceptance, and religion in parents of children with ASD than parents of TD, which could act as a buffer to the negative impact of parenting-related stress (Padden & James, 2017). Moreover, the present results indicate a greater frequency of prosocial behaviours in M-ASD than M-TD during the social interview phase of the PST. Prosocial behaviours reflect an attempt to maintain a non-hostile interaction with and to elicit empathy in the interviewer. This result appears to contradict the relatively high levels of social inhibition reported by M-ASD. However, another important feature of Type-D personality is the fear of being criticized and this could explain a greater effort by M-ASD to maintain a good interaction with the interviewer via prosocial behaviours. Notably, a higher frequency of flight and displacement behaviours was also observed in M-ASD, which may reflect their higher levels of anxiety and their tendency to use avoidance strategies during stressful situations (Troisi, 2002). It must be acknowledged; however, that no significant relationships were found between non-verbal behaviour and physiological responses to the PST. This is in contrast with a previous study documenting a positive relationship between submissive behaviour scores and the magnitude of sympathetic and cortisol responses to a psychosocial stress test in healthy university students (Sgoifo et al., 2003).

4.4 | Study limitations, conclusions, and perspectives

There are limitations to this study. First, due to difficulties in recruiting mothers of children with ASD who met the inclusion criteria and consented to participate, the sample size is rather small and limits the extent to which the results can be interpreted. Moreover, we included only mothers in order to abolish gender-related variation, and therefore, these results cannot be generalized to fathers. However, the M-ASD group was homogeneous, because the age of the child and the time since diagnosis were very similar among these mothers.

The present results indicate that mothers of children with ASD show larger heart rate and cortisol responses to an acute psychosocial stress. Moreover, we document a strong positive relationship between parental distress levels and cortisol stress reactivity independently from group. Further research with larger samples and a longitudinal design is needed to investigate whether physiological stress reactivity in M-ASD undergoes a progressive decline over time, as suggested by previous reports in parents of older children with ASD (De Andrés-García et al., 2012; Ruiz-Robledillo et al., 2015; Ruiz-Robledillo et al., 2017; Ruiz-Robledillo & Moya-Albiol, 2015). We failed to conclusively demonstrate whether personality and psychological characteristics and coping strategies mediate the effects of parental stress on individual physiological responses to stress. However, the high incidence of Type-D personality among M-ASD is an intriguing finding and requires further investigation. Future studies employing larger samples will likely answer these open questions.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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