Acknowledgement

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Research context

Today, one of the most frequently studied conditions in the field of neurodevelopmental disorders is Autism Spectrum Disorder (ASD) (Matson & Kozlowski, 2011). It is a common disorder, which starts in early childhood and is expressed in terms of limitations in three domains, namely difficulties in social interaction, problematic communication skills and unusual repetitive behavior (Fakhoury, 2015). In addition to these problems in social and cognitive behavior, there is also a dysregulation of other biological systems including the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis (Corbett, Mendoza, Abdullah, Wegelin, & Levine, 2006).

The ANS and the HPA-axis are key stress response systems, which generate an adaptive response to physiological and psychological stress (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014). Exposure to acute stress leads to an activation of the ANS and a potentiation of the HPA-axis. The ANS is divided into sympathetic and parasympathetic branches. Increased HR is caused by a sympathetic activity, while a decrease in HR is caused by a parasympathetic activity. The interaction between the two circuits is reflected in the heart rate variability (HRV), which are the variations in inter-beat (RR) intervals (Taelman, Vandeput, Spaepen, & Huffel, 2009). Therefore, stress responses are often examined by measuring HR and HRV.

Some studies have investigated the effect of a social stressor on HR in ASD (Jansen et al., 2003, 2006). The results show that ASD subjects have a significantly lower HR response compared to typically developing (TD) subjects (Jansen et al., 2006, 2003). Yet, it is unclear whether the ASD specific differences in HPA-axis or ANS function are associated with a higher stress response and anxiety in subjects with ASD (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014). Besides, it is difficult to compare the different studies because various designs were used. One explanation for this lack of evidence is the variety of stress tasks (social versus nonsocial) used in different studies.

Furthermore, the measurements to investigate the stress responses differed. The most frequently used measurements are cortisol (derived from blood samples, saliva or urine), mean HR and HRV measurements (derived from ECG recordings or heart rate monitor recordings) and questionnaires. Information from adrenocorticotropic hormone (ACTH), epinephrine, norepinephrine, vasopressin and oxytocin, collected from blood samples can be used to measure the stress response as well (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014, Taylor

& Corbett. 2014). Currently, little is known about the influence of temperament on HR(V), but it is assumed that a negative temperament is correlated with a higher stress response (Karam et al., 2010).

The present master's thesis is part of the 'Yes I drive study' in association with IMOB. It consists of two parts and focuses on stress in adolescents with ASD. In part one, driving behavior in adolescents with ASD is investigated. In part two, described in this paper, the stress response during different tasks in adolescents with ASD is compared with the stress response of TD subjects. The stress response is examined during three different tasks (the MINI-interview, an informal conversation and a mental arithmetic task (MAT) by measuring HR and HRV using a Polar V800. In contrast to other research, any differences in stress response between these three tasks will be investigated. Potential covariates, including gender, age and Body Mass Index (BMI), that might influence the stress response will be investigated as well. Furthermore, temperament and anxiety will be taken into account when looking at the stress response in adolescents with ASD because it's influence was investigated too little in other studies. ASD is a neurodevelopmental disorder which starts in early childhood and therefore, this master's thesis is situated in the field of the pediatric rehabilitation.

This research is a dual-master's thesis, performed by two master students physiotherapy and rehabilitation sciences of the department medicine and life sciences at the university of Hasselt. It is executed under the supervision of Dr. M. Braeken. The topic and data of this experiment were provided by Dr. M. Braeken. Subsequently, the data was converted to usable date for statistical analysis by the two students. The statistical analysis was performed by master student Lien Santermans in consultation with the other master student Neel Vanrusselt and under supervision of Dr. M. Braeken. The introduction was written by Neel Vanrusselt in consultation with Lien Santermans and under supervision of Dr. M. Braeken. The rest of the article was written by the two master students together. Dr. M. Braeken made suggestions and corrections during the whole process.

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Abstract

Background

Previous literature shows a decreased HR response to stress in subjects with autism spectrum disorder (ASD) compared to typically developing (TD) subjects. Yet, it is unclear whether these ASD specific differences are associated with an altered autonomic nervous system (ANS) in general and what the role of psychological factors and the type of stress tasks are.

Objectives

The objective is to investigate autonomic stress response in TD subjects and subjects with ASD during different tasks. This stress response is measured by heart rate (HR) and heart rate variability (HRV).

Participants

48 subjects (29 male; 19 female) between 17 and 30 years old were recruited. They were classified into a TD group (n=26) and an ASD group (n=22).

Measurements

The Polar V800 was applied to measure the HR of all participants during an informal conversation, a structured interview and a mental arithmetic task (MAT). They were asked to fill in an anxiety (STAI) and temperament (ATQ) questionnaire.

Results

This study showed a significantly higher HRV in TD subjects. There is an effect of gender as the mean HR is significantly lower in male subjects compared to females. The HRV is significantly higher in subjects with a higher score on effortful control (EC) (RMSSD HRV: $p<.019$, HF HRV: $p<.027$) and in the TD group the effect of EC is less compared to the ASD group. Thus a lower autonomic stress response is associated with a greater EC. Looking at the HF, the effect of anxiety in the TD group is less compared to the ASD group. In addition, this study showed a significant difference between the tasks and the recovery for mean HR, RMSSD and HF.

Conclusion

It can be concluded that there is a lower autonomic stress response in TD subjects compared with ASD subjects considering their higher HRV. Next, this study suggests important influences of gender, anxiety and temperament. Finally, using different tasks to analyze the stress response is important.

Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder (Ratto & Mesibov, 2015) characterized by impairments in two domains, including persistent deficits in social communication and social interaction across multiple contexts as well as restricted, repetitive patterns of behavior, interests or activities (Fakhoury, 2015). Because ASD is a developmental disorder, the symptoms and the individual's functioning are not static across the lifespan, but change in the developmental course of the individual (Ratto & Mesibov, 2015). In 2011, the prevalence in adults from 18-27 years old with ASD in Sweden was estimated at 1.76% (Idring et al., 2015). Patients with ASD have an increased vulnerability to stress due to the deficits in social skills, communication and adapting to unfamiliar situations (Jansen et al., 2006). In addition to the problems in social and cognitive behavior, there is also a dysregulation of other biological systems including the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis (Corbett, Mendoza, Abdullah, Wegelin, & Levine, 2006).
"The autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis are both part of a dynamic stress response system, that is essential in generating adaptive responses to both physiological and psychological threats. It is involved in the well-defined "fight-or-flight response" (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014). During stress situations, a body response is created by the activation of the sympathetic branch of the ANS to heighten the arousal and prepare the person to deal with the stressor (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014). At the same time, a parasympathetic control on the sinoatrial node of the heart limits the duration of the arousal state (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014). Under normal circumstances, exposure to acute stress is associated with the potentiation of the HPA-axis and an increase of stress markers, such as cortisol and heart rate (HR). Stress is also associated with a decline of the heart rate variability (HRV) (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014). HRV is a non-invasive method to measure ANS regulation (Sztajzel, 2004). It is an index of the dynamic relationship between sympathetic and parasympathetic innervation of the heart. It has been suggested as a marker of affective disorders such as anxiety as well (Friedman, 2007).

Some studies have investigated the effect of a social stressor on HR in ASD. (Jansen et al., 2006, 2003). The results show that individuals with ASD have a significantly decreased HR response compared to typically developing (TD) subjects (Jansen et al., 2006, 2003). The impact of stress on HRV in ASD and TD adolescents has been scarcely studied**.** Only one study reported that there was no difference in HRV between ASD and TD adolescents (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014).

It is currently unclear whether ASD specific differences in HPA-axis or ANS function are significantly associated with the increased rates of stress and anxiety observed in this population (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014). It is difficult to compare the different studies because various designs were used. One problem is the wide variety of stress tasks (social versus nonsocial) that were used and no comparison was made between these different (stress) tasks. Also the measurements to investigate the stress response differed. The findings of the literature are based on measurements of cortisol, mean HR or HRV and questionnaires. Outcomes can be based on measurements of adrenocorticotropic hormone (ACTH), epinephrine, norepinephrine, vasopressin and oxytocin, collected from blood samples as well (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014, Taylor & Corbett. 2014). Furthermore, the influence of temperament on the stress response has not been adequately studied in ASD and TD adolescents. It is assumed that a negative temperament is correlated with a higher stress response (Karam et al, 2010).

The present study was designed to provide insights in the difference in ANS responses in three different tasks between TD subjects and subjects with ASD. It can be hypothesized that there are significant differences in HR and HRV between TD subjects and ASD subjects during all tasks. Another hypothesis is that the stress response varies between different stress tasks. The first task is an informal conversation. The stress response in this situation should be lower in TD subjects because they are more comfortable during a conversation with an unknown. The second task is the MINI-interview, a structured questionnaire. Therefore the stress response should fall in the ASD group and remain unchanged in the TD group. Afterwards a mental arithmetic task (MAT) will be executed. Due to stress provoked by the MAT, the stress level should rise in both groups, but there might be a difference because of the increased vulnerability to stress of ASD adolescents compared to TD adolescents (Chauhan & Chauhan, 2006). Finally, the association between temperament and the ANS response is investigated.

Methods

Design

This study is part of the ‘Yes I drive’ study in association with IMOB. It is divided into two separate studies. In one study, the participants perform a driving test using a driving simulator and in the other study, the participants undergo several motor tests, a structured interview, an informal conversation, a mental arithmetic task (MAT) and fill in a few questionnaires while wearing a heart rate monitor. Our research focuses on the HR and HRV during the interview, the informal conversation, the MAT and the questionnaires. The researchers were not blinded for the grouping of the participants.

Medical ethics

This study was approved by the ethics committees of Hasselt University before the data collection.

Selection and description of participants

There were 48 subjects (29 male; 19 female) recruited for this study through local advertisements at university ground, including via mailing lists for students with special needs. In addition, subjects with ASD were recruited via local clinical institutions and hospital centers. They were classified into two groups. One group (n=26, 14 male; 12 female) with TD subjects and one group (n=22, 15 male; 7 female) with ASD. The mean length of the subjects is 176.1 cm and the mean weight is 68.6 kg. The average age of the participants is 20.77 years (SD = 2.537) and the average BMI is 22.1 (SD = 2.972).

Inclusion and exclusion criteria are listed in table 1.

Table 1
Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age: 17-30 years	Acute psychiatric diagnosis (except for ASD)
Novice driver (provisional license or license less than 2 years)	Motor impairment
Some driving experience (20 hours with a driving school or 60 hours with a constant companion)	
Normal vision (possibly corrected with glasses / contact lenses)	
At least a degree of secondary school (i.e. high school)	
Participants in the ASD group diagnosed according to a multidisciplinary clinical consensus classification for ASD (APA, 2013)	
TD participants screen negative for ASD on the Social Responsiveness Scale for Adults (SRS-A) (Constantino and Gruber, 2012; Noens et al. 2012)	

Procedure

After a complete description of the procedure, a written informed consent was obtained from all participants. The first step was to apply the heart rate monitor (Polar V800). Then a motor test was completed where the participants had to observe a motor action and imitate the movements. Thereafter, an informal conversation of five minutes took place with questions about the motor task, what they do in their everyday life, their studies and the weather. The participants were not aware that during this conversation their autonomic response was measured as part of the test. After the conversation, the participants completed the MINI-interview ('Mini International Neuropsychiatric Interview'). The first ten minutes of the MINI were used to measure the autonomic stress response of the participants during a structured interview.

Subsequently, the MAT was performed. This was a calculation task in which the participants start at 2034 and count backwards with 17. At each incorrect answer, they had to start again. This task was performed for five minutes. The five minutes after the MAT were considered as a recovery phase. The overview of the different stress tasks is displayed in figure 1. Finally, the participants filled in the State-Trait Anxiety Inventory (STAI). Then, the heart rate monitor was disconnected. The participants also filled in a few other questionnaires such as 'the Social Responsiveness Scale for Adults (SRS-A)', 'the adult temperament questionnaire (ATQ)' and the 'ASD quotient (AQ)'.

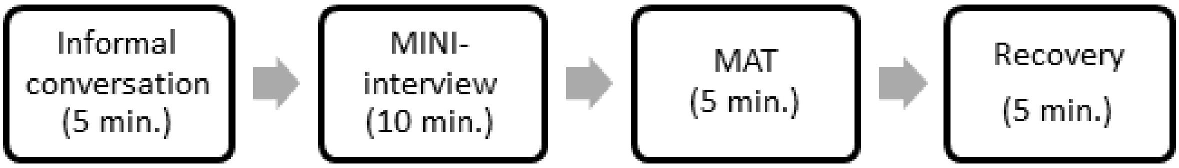

Figure 1
Overview of the different stress tasks

Outcomes

Primary outcomes

The primary outcomes during the whole procedure are the mean HR, the root mean square successive difference (RMSSD) and the high-frequency (HF), these two are measurements of the HRV, derived from the variation in inter-beat (RR) interval, reflecting ANS activity (Electrophysiology, 1996). A higher HF indicates a higher stress level while a higher HRV is associated with a lower stress level.

Secondary outcomes

The secondary outcomes are the results of the questionnaires.

Material and questionnaires

The questionnaires used in the second part of the study to measure ASD characteristics and anxiety are:

The Social Responsiveness Scale-adult (SRS-A)

The SRS-A is a quantitative screening tool for ASD by assessing participant's level of social responsiveness. The questionnaire determines the different dimensions of interpersonal behavior, communication and rigid, repetitive behaviors and interests, which characterize ASD. It focuses on the behavior of adults with or without a mental disability within the age range of 18 to 65 years. The questionnaire consists of 64 questions, scored on a four-point Likert scale through self-reporting. For the reliability, the manual implies a Cronbach's Alpha of .95. This refers to an excellent internal consistency. Based on our own calculations, there is a Cronbach's Alpha of .88. This refers to a good internal consistency.

In this study, all participants filled in a self-report form of the Dutch version of the SRS-A (Noens et al. 2012). Higher T-scores on social responsiveness correspond to more severe impairments in social responsiveness (Constantino and Gruber, 2012; Noens et al, 2012). Participants with ASD reported significantly lower levels of social responsiveness on the total SRS-A score than TD participants (ASD mean: 66.73; TD mean: 47.88). The same was true for all SRS-A subscales. SRS-A scores confirmed the diagnostic classification (ASD versus TD) in 68% of the participants. Seven participants with ASD scored negative for ASD, with scores varying between 3 and 40% under diagnosis cut-off. As required, all TD participants scored negative for ASD. There was a significant difference between the TD and the ASD group ($p<.000$), which means that the negative scores for ASD have no effect on the outcome measurements.

Autism Spectrum Quotient (AQ)

The AQ is a self-administered questionnaire for behavior and personality in ASD used in adults. The questionnaire is comprised of 50 items that are scored on a four-point Likert scale ('strongly disagree', 'disagree', 'agree', 'strongly agree'). "A total AQ score is calculated by summing all the scores for each of the items, with a maximum score of 200"(Stewart & Austin, 2009). It can be used as an assessment or as a screening tool. The AQ consists of different subscales namely social skills, communication, imagination, attention to detail and attention-switching. The literature implies a Cronbach's Alpha of .81. This refers to a good internal consistency.

Adult Temperament Questionnaire (ATQ)

The ATQ is a self-report questionnaire for adults (ages 16-85 years old) to measure temperament and personality based on self-regulatory processes in addition to constitutionally based individual reactivity. It includes general constructs of effortful control (EC), negative affect (NA), extraversion/surgency and orienting sensitivity. Participants have to rate how well the items describes themselves on a 7-point Likert scale: '1-extremely untrue, 2-quite untrue, 3-slightly true, 4-neither true or false, 5-slightly true, 6-quite true, 7-extremely true'. The short form of the ATQ includes 77 items (Evans & Rothbart, 2007). For the reliability, the literature implies a Cronbach's Alpha of .72 - .84 which refers to an acceptable to good internal consistency. Based on the calculations in this study, there is a Cronbach's Alpha of .81, which refers to a good internal consistency.

The Spielberger State-Trait Anxiety Inventory (STAI)

The STAI is a 40-item measure (Spielberger, Gorsuch, & Lushene, 1970). The Dutch, psychometrically validated version of the STAI (van der Ploeg, Defares, & Spielberger, 1981) is used in this study. It is a self-report scale for adults to measure two different concepts of anxiety, namely transient and enduring levels of anxiety. The overall anxiety level is scored on a four-point Likert Scale (Kvaal, Ulstein, Nordhus, & Engedal, 2005). The reliability of the STAI is divided into two parts, namely the TRAIT and the STATE. In this study, the focus is on the STATE. For the STATE, the literature reported a Cronbach's Alpha of more than .89 (Charles D Spielberger, 2010). This refers to a good internal consistency. In this study, a Cronbach's Alpha of .61 is found for the STATE, which refers to a questionable internal consistency.

Mini-International Neuropsychiatric Interview (MINI)

Current and lifetime psychopathology was assessed using the MINI (Sheehan & Lecrubier, 2010). This is a short structured diagnostic psychiatric interview to identify Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). During this interview the HR(V) of the participants was measured.

Polar V800

The Polar V800 is an instrument that measures the HR and HRV in a non-invasive way through the measurement of variations in inter-beat (RR) intervals. This allows the exploration of the cardiovascular function of the subjects (Thayer et al, 2012). The polar V800 contains a smartwatch and a chest strap (polar H7), which comprises a heart rate sensor (Giles, Draper, & Neil, 2016). The chest strap must be attached tightly but comfortably just below the chest muscles (Giles, Draper, & Neil, 2016). Signals, captured by the sensor, are transmitted to the receiver by an electromagnetic field. Recorded data are then transferred to Kubios HRV analysis software for further analysis (Da Luz Goulart et al, 2017). The Polar V800 measures the stress response because it is sensitive to physiological and psychological changes (Thayer et al, 2012). HRV shows an insight in the potential of subjects to function effectively in complex environmental, physiological and psychological conditions (Thayer et al, 2012). It is a valid instrument to record the RR interval. (Giles, Draper, & Neil, 2016). In this study, HR, RMSSD HRV and HF HRV were included as stress measures. The HR is a measurement that contains combined sympathetic and the parasympathetic input, while the HRV (RMSSD and HF) are measurements of the parasympathetic branch. HRV analysis are divided into time-domain analysis and frequency-domain analysis (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014). The time-domain analysis refers to the RMSSD measurement and the frequency-domain analysis refers to the HF measurement.

Data analysis

The HR data (beat-to-beat RR interval data) recorded with the Polar V800 were converted into HR and two HRV measures (RMSSD HRV and HF HRV) using Kubios HRV version 2.2 software. Kubios HRV is an advanced software package for HRV analysis, which computes time-domain and frequency-domain HRV parameters (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2014).

These measures were calculated for all tasks and the recovery phase, which was defined as the first five minutes after the MAT. The statistical analysis was performed using IBM SPSS Statistics 22. The significance response was set on $P<.05$. In SPSS, all data was analyzed using explore. The mean, median, SD, percentiles and the extreme values were calculated. Next, the outliers were calculated and deleted. An outlier is defined as a value deviating more than three SD's from the mean. Sixteen values were excluded.

Then the data was examined on normality using the Kolmogorov-Smirnov, the Shapiro-Wilk test and the Q-Q plot. After a log transformation, all data was normally distributed. Subsequently, the homoscedasticity was verified using the Levene's test for equality of variances. Next an independent t-test was used to analyze the potential differences in age, weight, length, BMI, social responsiveness, temperament and STATE anxiety between the ASD group and the TD group. A chi-square test was used to analyze the difference in gender between the ASD and the TD group. Finally, linear mixed models were used to analyze the associations between ANS responses, temperament scales, and STATE anxiety. It was used to examine differences in boys and girls, ASD and TD and the different tasks as well. An interaction effect (group * task) was added to test whether ASD and TD differ in ANS responses depending on the tasks. Additionally, interaction effects were included to test whether associations between ANS response, temperament scales and state anxiety differ between ASD and TD (i.e. respectively group * temperament and group * STATE anxiety). In each model, BMI and age were added as covariates. As estimation method was chosen for maximum likelihood (ML) instead of restricted maximum likelihood (REML) because it gave a better estimation of the model. As covariance structure 'unstructured' resulted into the best fitness for this linear mixed model.

Results

Participants

The characteristics of the participants of both groups are listed in table 2. There was a significant difference ($p<.000$) for the SRS-A total score. The SRS score was significantly higher in the ASD group compared to the TD group. There was also a difference in ATQ score. The ATQ, subtest extraversion was significantly lower ($p<.002$) in the ASD group. The ATQ, subtest EC was also significantly lower ($p<.006$) in the ASD group. The ATQ, subtest NA was significantly higher ($p<.000$) in the ASD group. The score on the STAI was significantly higher in the ASD group ($p<.029$).

Table 2
Overview of the participants characteristics.

	TD (n=26)	**ASD (n=22)**	***p*-value**
Gender: male/female, n	14/12	15/7	.283
Age: mean, (SD) years	21 (2.37)	20 (2.671)	.175
Age range	18-25	17-29	/
Weight: mean, (SD) kg	67.88 (10.43)	69.55 (10.40)	.585
Length: mean, (SD) cm	175.62 (6.27)	176.59 (7.03)	.614
BMI: mean, (SD)	21.96 (7.96)	22.34 (3.20)	.670
SRS total score: mean, (SD)	49.54 (8.07)	66.45 (14.67)	**.000**
ATQ extraversion: mean, (SD)	82.28 (11.77)	70.19 (14.30)	**.002**
ATQ sensitivity: mean, (SD)	58.57 (10.97)	61.98 (9.50)	.260
ATQ EC: mean, (SD)	87.16 (16.90)	73.84 (14,53)	**.006**
ATQ NA: mean, (SD)	78.46 (16.36)	100.68 (20.32)	**.000**
STAI: STATE: mean, (SD)	29.72 (5.50)	34.17 (8.13)	**.029**

Analysis of mean HR, RMSSD and HF HRV within the two groups and the different tasks

Looking at table 3, this study shows a significant difference in stress response between the two groups (ASD/TD) based on the measurements of the RMSSD HRV ($p<.016$) and the HF HRV ($p<.004$). The two HRV measurements are significantly higher in TD subjects. This indicates a lower stress level in TD subjects.

There is also a significant effect of gender based on the measurement of the mean HR ($p<.008$). From this it can be deduced that the mean HR is significantly lower in male subjects compared to female subjects. A trend significance effect of gender based on the measurement of the RMSSD HRV (p<.094) is reported. Next, there is a significant difference between the three tasks (MAT/MINI/conversation) and the recovery. The mean HR is significantly lower ($p<.015$) during the MINI-interview compared to the MAT. Additionally, the mean HR is significantly higher ($p<.000$) and RMSSD HRV ($p<.006$) and HF HRV ($p<.009$) are significantly lower during the informal conversation than during the MAT.

When these tasks are further examined (table 4), a significant difference in stress response based on the measurement of the mean HR is found between the MINI and the recovery ($p<.038$), the MINI and the informal conversation ($p<.000$), the MINI and the MAT ($p<.000$), the conversation and the recovery ($p<.000$) and the conversation and the MAT ($p<.000$). The mean HR is higher during the recovery, the MAT and the informal conversation compared to the MINI. The mean HR is also higher during the informal conversation compared to the MAT and the recovery. There is also a significant difference in RMSSD HRV between the conversation and the recovery ($p<.000$), the conversation and the MAT ($p<.000$), and the conversation and the MINI ($p<.005$). The RMSSD HRV is higher during the recovery ($p<.000$), the MINI ($p<.005$) and the MAT ($p<.000$) compared to the conversation. Looking at the measurement of the HF HRV, a significant difference is found between the conversation and the recovery ($p<.001$), between the conversation and the MINI ($p<.002$) and between the conversation and the MAT ($p<.000$). The HF HRV is higher during the recovery ($p<.001$), the MAT ($p<.000$) and the MINI ($p<.002$) compared to the conversation.

Subsequently, there is a positive effect of the ATQ, subtest effortful control for the RMSSD ($p<.019$) and the HF HRV ($p<.027$). This implies a significantly higher HRV in subjects with a higher score on the ATQ, subtest EC. Thus, subjects with more EC, have a lower stress level.
Next, there is a significant negative interaction between the ATQ, subtest EC and group (ASD/TD) for RMSSD HRV ($p<.027$) and HF HRV ($p<.043$). This implies that the effect of EC in the TD group is less compared to the ASD group.

Finally, table 3 shows a significant negative interaction between group (ASD/TD) and the STAI: STATE (p<.006) for HF HRV. This implies that the effect of the STAI: STATE, and thus the effect of anxiety on the stress response in TD subjects is less compared to ASD subjects.
There was no significant interaction between group and task, indicating that any differences in ANS responses between ASD and TD were not dependent on the task.
Furthermore, besides EC, all other temperament scales (sensitivity, NA and extraversion) were not significantly related to ANS responses. There were also no significant interactions between group and the other temperament scales. Finally, there was a trend-significant positive interaction between the groups and NA on the measurement of both RMSSD HRV (p<.050) and HF HRV (p<.053) indicating that the effect of the ATQ, subtest NA is less in the ASD group compared to the TD group.

Table 3
Mixed model analysis comparing mean HR, RMSSD and HF HRV within the groups and the different tasks

	Mean HR			RMSSD HRV			HF HRV		
Parameter	**Estimate**	**SD error**	**Sig.**	**Estimate**	**SD Error**	**Sig.**	**Estimate**	**SD error**	**Sig.**
Intercept	76.646	21.791	**.001**	1.231	.423	**.005**	2.117	.748	**.007**
TD group (vs. ASD)	-19.459	20.612	.350	1.007	.404	**.016**	2.140	.712	**.004**
Male (vs. female)	-6.748	2.428	**.008**	.082	.048	.094	.128	.084	.135
Recovery condition (vs. MAT)	-1.530	1.345	.261	.027	.026	.302	-.052	.080	.520
MINI condition (vs. MAT)	-.840	1.128	**.015**	.0006	.032	.986	-.074	.065	.256
Conversation condition (vs. MAT)	8.376	1.448	**.000**	-.089	.031	**.006**	-.255	.093	**.009**
ATQ: EC	-.142	.140	.314	.007	.003	**.019**	.011	.005	**.027**
STAI: STATE	.036	.222	.871	.004	.004	.402	.013	.008	.105
BMI	.824	.420	.057	-.011	.008	.208	-.019	.015	.191
Age	.031	.528	.953	-.001	.010	.904	-.004	.018	.841
Recovery * TD group	.875	1.812	.631	-.023	.035	.524	-.071	.108	.518
MINI * TD group	-.622	1.518	.684	.002	.044	.956	.042	.088	.630
Conversation * TD group	.844	1.967	.670	.014	.042	.750	.008	.125	.947
TD group * ATQ EC	.114	.181	.530	-.008	.003	**.027**	-.013	.006	**.043**
TD group * STAI: STATE	.189	.357	.600	-.011	.007	.134	-.036	.012	**.006**

Table 4
Pairwise comparisons of the different tasks

Task condition	Task condition	Mean HR			RMSSD HRV			HF HRV		
		Mean difference	Std. error	Sig.	Mean difference	Std. error	Sig.	Mean difference	Std. error	Sig.
Recovery	MINI	2.059	.962	**.038**	.014	.022	.542	-.034	.046	.465
	Conversation	-9.890	1.071	**.000**	.098	.021	**.000**	.164	.046	**.001**
	MAT	-1.092	.906	.234	.016	.018	.380	-.087	.054	.115
MINI	Recovery	-2.059	.962	**.038**	-.014	.022	.542	.034	.046	.465
	Conversation	-11.949	1.028	**.000**	.084	.029	**.005**	.198	.059	**.002**
	MAT	-3.151	.759	**.000**	.002	.022	.935	-.053	.044	.232
Conversation	Recovery	9.890	1.071	**.000**	-.098	.021	**.000**	-.164	.046	**.001**
	MINI	11.949	1.028	**.000**	-.084	.029	**.005**	-.198	.059	**.002**
	MAT	8.798	.984	**.000**	-.083	.021	**.000**	-.251	.063	**.000**
MAT	Recovery	1.092	.906	.234	-.016	.018	.380	.087	.054	.115
	MINI	3.151	.759	**.000**	-.002	.022	.935	.053	.044	.232
	Conversation	-8.798	.984	**.000**	.083	.021	**.000**	.251	.063	**.000**

Discussion

This study compared the autonomic stress responses between TD adolescents and adolescents with ASD during different tasks. To compare the stress responses, different ANS outcomes (mean HR, RMSSD HRV, HF HRV) derived from the Polar V800 were used. Secondly, the association between temperament, anxiety and the stress responses were examined.

The main findings of this study is that TD adolescents have higher values of RMSSD and HF HRV but, there was no significant interaction between the two groups (ASD/TD) and the tasks, indicating that any differences in ANS responses between ASD and TD were not dependent on the task. There were also no significant differences in mean HR between the two groups and no significant interaction between the two groups and the tasks. Although, based on our literature study, a significant difference in mean HR between the ASD group and the TD group was expected. Looking at the findings of Jansen et al. (2003, 2006), a significant increase in mean HR for the ASD group was expected, especially in the tasks with a social factor. This was not the case in this analysis. Possible explanations for this are the differences in the characteristics of the population. In this study, participants were between 17 and 30 years old and were in possession of a provisional driver's license or a driver's license less than two years and a degree of secondary school. Based on these properties, it can be deduced that the ASD subjects in this study had few problems in daily life and were highly independent. On the other hand, little can be said about the intelligence quotient (IQ). In the study of Jansen et al. (2003), participants between eight and ten years old with a mean IQ of 86 (range: 62-107) were included. In this range, there is a wide variety in the level of independent functioning. Evidence shows that there is a possible decline in symptoms severity of ASD over time, especially in adolescents with rather mild ASD symptoms (Seltzer, Shattuck, Abbeduto, & Greenberg, 2004). Possibly, the severity of the ASD symptoms has an important influence on the results. Further research to investigate the influence of symptom severity on stress is needed. The lack of significant group effect for mean HR can also be attributed to the limitations of this study.

In our study, a significant difference in mean HR was found between male and female subjects. The mean HR was lower in male subjects. There was no significant difference in RMSSD HRV or HF HRV between male and female participants. In contrary, Uhart, Chong, Oswald, Lin, & Wand. (2006) found that male subjects had higher HPA-axis responses to a psychological stressor than female subjects. But, the influence of gender on the HR(V) was barely studied in prior research.

Looking at the pairwise comparison of the data, a significant difference between the different tasks was found. It can be deduced from the analysis that there was a significant difference between the MINI and the recovery, the MINI and the conversation, the MINI and the MAT, the conversation and the recovery and between the conversation and the MAT for the mean HR. The results from the RMSSD HRV measurements demonstrate that there was a significant difference between the conversation and the recovery, the conversation and the MAT and the conversation and the MINI. For the HF HRV, a significant difference is found between the conversation and the recovery, the conversation and the MINI and the conversation and the MAT. Prior the informal conversation, all subjects had to walk a short distance to go to another room. This could have an influence on the measurements of stress. A difference in stress response between the different tasks was expected. However, a difference between the MAT and the recovery was not found. This could be attributed to the fact that the recovery measurement took place immediately after the MAT. At the same time, the participants filled in the anxiety questionnaire. Thereby, there was insufficient recovery of the HR. Next, there was indeed a difference in temperament between the ASD and the TD group. The TD group had a higher score on the ATQ, subtest extraversion and subtest EC. This implies higher sociability and a higher attentional control in TD adolescents. The ASD group had a higher score on the ATQ, subtest NA. This implies that ASD adolescents are more withdrawn and anxious. There was no significant difference for the ATQ, subtest sensitivity. There was a positive effect of EC on HRV. This indicates a lower stress response in subjects with more EC and thus more attentional control. There is also a negative interaction between the groups (ASD/TD) and temperament. This means that the effect of EC is less in the TD group compared to the ASD group. According to Konstantareas & Stewart (2006), a child with higher ASD symptom had a lower EC.

Finally, anxiety is associated with physiological changes and therefore, there is an overlap between anxiety and stress (Groden, Cautela, Prince, & Berryman, 1994; Shin & Liberzon, 2010). Based on the measurements of the HF HRV, the results of this study suggest that there is a significant negative interaction between anxiety and the groups (ASD/TD). This implies that the effect of anxiety on the stress response is less in TD adolescents compared to ASD adolescents. There was also a higher anxiety level in ASD subjects compared to TD subjects.

Some limitations need to be addressed. A first limitation is the small sample sizes (TD group n=26; ASD group n=22). The groups were not matched based on specific patient characteristics, but the analysis shows that there were no significant differences between the two groups. There were also less female subjects in the ASD group. This difference can be explained by the ratio boys/girls with ASD. This ratio is 4/1, this means that there are four more males diagnosed with ASD than females (Head, McGillivray & Stokes).

Furthermore, the reliability of the STAI, subtest STATE can be questioned in this study. Previous studies reported a good to excellent reliability for the STATE (De Vries & Van Heck, 2013; Charles D Spielberger, 2010). Another limitation is the blinding of the researchers. The researchers were not blinded for the grouping of the participants, which means that they were aware which subjects had an ASD diagnosis. The absence of a baseline measurement, is also a weakness. Normally, the HR(V) during the different tasks should be compared with the HR(V) in rest. Thereby, the measurement of the recovery is not the real recovery because it cannot be compared with a baseline measurement. Normally, a recovery measurement is the time the subjects need to lower their HR to the baseline response. Another limitation is the lack of information of the sympathetic branch. The sympathetic branch is mostly activated during stress. In further research, direct measurements of the sympathetic branch have to be taken into account. Moreover, differences in HPA-axis response and ANS response should be investigated together in further research.

Next, this master's thesis has some strengths. The first strength is that the stress response of the subjects was measured during different tasks. The inconsistent findings in the literature can be explained by the wide variety of stress tasks (social versus nonsocial) that are used to compare the stress response between ASD and TD subjects. In the analyses of the data revealed that there was indeed a difference in mean HR, RMSSD HRV and HF HRV between the different tasks. According to Taylor & Corbett. (2014), there is a difference in HR depending on gender, pubertal stage, experience and severity of impairment for individuals with ASD. In this study, gender, BMI and age were included as covariates. This is also a strength. On the other hand, experience and severity of impairment were not included in the analysis. The influence of stress on temperament has not been adequately studied in adolescents with ASD and typically developing adolescents. Another strength of this research is that the relationship between temperament, anxiety and stress response is examined.

Recommendations for further research are to include a baseline measurement. In this way, a comparison can be made between the stress response during the baseline measurement and the stress response during different tasks. In this manner, a recovery measurement can also be carried out. Another recommendation is that the severity of ASD, the experience with stressful situations and the IQ of the subjects should be included in the analysis. Subsequently, the ASD group and the TD group should be matched based on age and gender. Next, a larger sample size should be used so that the power of the study increases. Lastly, the researchers should be blinded for the grouping of the participants.

The social value of this research is great because it is very important to take into account the deviating stress level in ASD adolescents. In this way, the treatment of ASD can be adapted and therapist can pay more attention to the stress level. Also, temperament and anxiety play an important role in the stress level of ASD adolescents. When the stress level is under control, the ASD adolescents should function better in the society and their reactions to stressful situations should be more adapted.

Conclusion

This study was one of the first studies that compared the reaction on stress during different tasks between ASD adolescents and TD adolescents. It can be concluded that the kind of task has an important influence on the autonomic stress response. There was a lower stress response in TD subjects compared to ASD subjects. Subsequently, there is a lower stress level in male subjects compared to female subjects. Next, the analysis showed a higher sociability and a higher attentional control in TD adolescents compared to ASD adolescents. Furthermore, the stress response is lower in subjects with a higher attentional control and the effect of attentional control in the TD group is less compared to the ASD group.

Moreover, ASD adolescents seem more withdrawn and anxious than TD adolescents and have higher anxiety levels compared to TD adolescents. The effect of anxiety on stress response in the TD group is less compared to the ASD group.

Thus, it can be concluded that gender, temperament and anxiety are important variables to take into account.

In prior research, stress response in ASD adolescents in general has been poorly studied, but can be important for their daily life functioning and their treatment adaptations. Still some questions remain unanswered and require further research.

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