HRV and Stress Regulation: Sex Differences in Heart Rate Variability and Perceived Stress During Participation of IAT

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Abstract

The experiment used a racial implicit association test (IAT) as a stress inducing factor. The IAT consisted of four rounds, with the first two being practice rounds, in which subjects pressed the corresponding (either right or left) button in response to positive or negative adjectives, and native or Moroccan names, respectively. For the third and fourth round the sequence was randomised, and subjects had to either pair the native names with positive adjectives and the Moroccan names with negative adjectives or vice versa. Subjects consisted of 49 German and Dutch University students, of which 33 had usable data. Thus, subjects included 23 cisgender women, 9 cisgender men and 1 transgender man. A low-threshold ECG device measured participant's HRV; perceived stress was self-reported using the Self-Assessment-Manikin (SAM). We hypothesised (1) A decrease of HRV during IAT, (2) Higher SAM scores correlating with lower HRV, (3) The effect found for 1) to be more pronounced for female participants and (4) A higher resting HRV in females. Our results did not show a decreased HRV during the IAT, instead an increment of HRV was found. Of the SAM, only a positive correlation between Arousal and HRV was established. The study did find a higher HRV during stress in males (p =.04), but no sex differences were found in perceived stress and resting HRV. We concluded that our baseline might have led to a skewed course of HRV levels, and the participation of the IAT was perceived as rather tiresome than stressful.

Keywords: Heart Rate Variability, Implicit Association Test, Perceived Stress, Autonomic Nervous System, Sex Differences, Self-Assessment Manikin, Emotion Regulation

HRV and Stress Regulation: Sex Differences in Heart Rate Variability and Perceived Stress During Participation of IAT

The association between stress, heart rate variability (HRV) and emotion regulation, has been the focal point of various psychophysiological studies throughout the past decades. Stressors are either psychological or physiological stimuli, which are interpreted as a potential threat to homeostasis. Homeostasis refers to the balance of body systems, such as blood pressure and glucose levels, that are necessary for the organism to function properly (Cannon, 1932). In particular, for a stimulus to be interpreted as threatening, it is suggested that it has to be perceived as uncontrollable and unpredictable, which makes the process of experiencing stress not merely biological and physiological in nature, but supports the idea that coping with stress is generally possible and its interpretation is subjective (Koolhaas, 2011). Psychological stress can be caused by situations that pose social evaluation, exclusion and, thus, social threat and can be experienced more extremely by individuals susceptible towards these social evaluations (Dickerson, 2004; Pruessner, 1999). Physiological stress is characterised by an aversive sensoric experience that could lead to potential harm to an individual's body, such as hunger or pain (Kogler, 2015).

Both the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) are crucial in regulating the body’s response to stress (Mueller, 2022; Dunlavey, 2018). The ANS consists of the sympathetic nervous system (SNS), parasympathetic nervous system (PNS) and enteric nervous system (ENS). A balance between these subsystems is necessary in order to maintain homeostasis (Purves et al., 2008). Whereas the ENS is primarily concerned with digestive processes, the SNS and PNS reflexively react to stressors within seconds (Mueller, 2022). The HPA-axis in comparison, has a longer response time and a rather long-term effect. The stress response starts with the amygdala perceiving a stressor as potentially harmful based on anterior experiences, and thus

it begins signalling to the hypothalamus (Baretta, 2009). The hypothalamus consequently communicates with the adrenal glands and initiates the activation of the SNS, which results in the adrenal glands releasing epinephrine (also known as adrenaline) into the bloodstream, and axon terminals starting to release norepinephrine (Goldstein, 1987; Mueller, 2022). This process leads to changes in bodily functions, such as faster heart beat, dilated pupils and inhibition of digestion, which are related to fight-or-flight behaviours. If an individual is faced with a dangerous stressor, the body tries to prepare for the potential threat, by inhibiting currently unnecessary functions (e.g., digestion) and facilitating functions crucial in overcoming the threat (e.g., increased blood flow to muscles). This is known as the fight-or-flight response (McCarty, 2016).

The hypothalamic-pituitary-adrenal (HPA) axis is the second and main mechanism involved in stress regulation (Dunlavey, 2018). The signalling of the amygdala to the hypothalamus, as a consequence of perceiving a stressor, results in the hypothalamus' release of corticotropin-releasing hormone (CRH). Consequently, CRH triggers the pituitary gland to release adrenocorticotropic hormone (ACTH), which in turn stimulates the adrenal glands to release cortisol. This process causes the body to maintain alert until the stressor subsides. After passing the stressor, cortisol levels decrease and provide negative feedback to the hypothalamus to discontinue releasing CRH, which leads to the cessation of the stress response. To facilitate the recovery of homeostasis, the PNS releases acetylcholine, which further decreases the body's stress response (Mueller, 2022; Dunlavey, 2018)

Chronic and intense stress can lead to dysfunction of the HPA-axis and structural plasticity within the basolateral amygdala, affecting the encoding of emotional memories (Roozendaal, 2009).

Biological Sex Differences in Stress Response

With cisgender women being approximately twice as likely to develop stress related disorders (Bangasser & Valentino, 2014), many studies suggest that stress responses vary across biological sexes. One example is, that reproductive females are more susceptible to develop affective disorders than cisgender men, however, this difference changes after menopause onset, which indicates a possible influence of ovarian hormones on stress response (Gert, 2009). Ovarian hormones (e.g., oestrogen) are shown to be able to bind to cytoplasmic oestrogen receptors (ER)-beta and (ER)-alpha within the limbic system, which consequently can influence the stress response, based on the oestrogen concentration. Furthermore, male rats displayed an increment in neuronal activity in limbic regions when confronted with chronic stress, whereas female rats showed lowered limbic activity (Gert, 2009). Regarding the HPA-axis, female rats exhibit an increased HPA function when confronted with an acute stressor, compared to male rats (Heck & Handa, 2019). This variation seems to be caused by the influence of gonadal hormones, such as testosterone and estradiol, which are generated by the hypothalamic-pituitary-gonadal (HPG) axis. Additionally, female rats exhibit a delay in restoration of baseline corticosterone (CORT) and ACTH levels post acute stressor confrontation, which suggests a sex based difference for the negative feedback regulation necessary for the HPA axis (Heck & Handa, 2019).

Heart Rate Variability and Stress

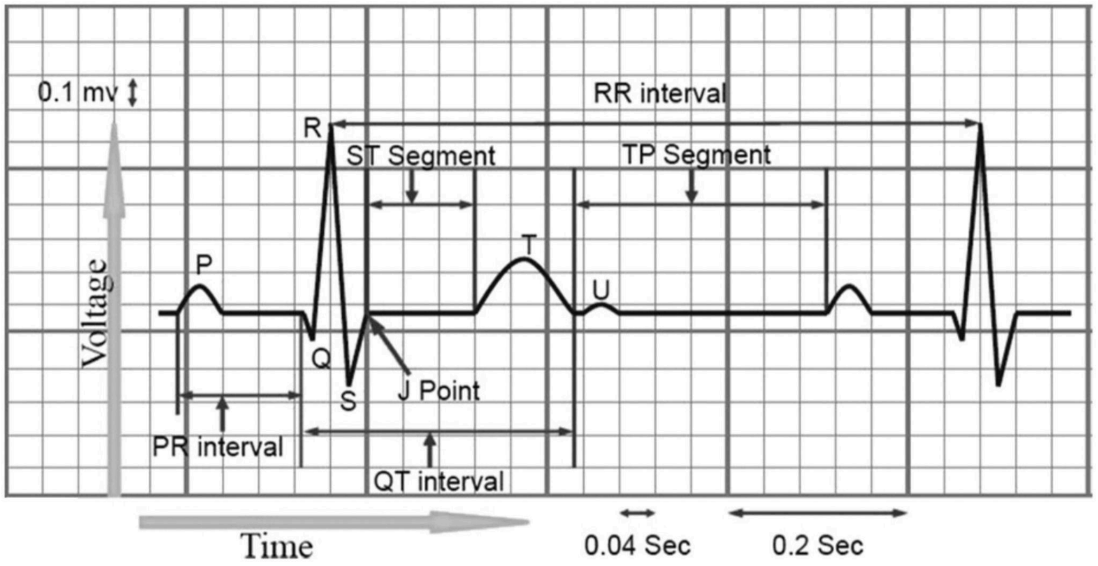
Heart rate variability represents the “fluctuation of the length of heart beat intervals” (Kim et al., 2018) and works in response to both environmental and physiological stimuli. It represents parasympathetic and sympathetic activity, in particular, the low-frequency component (<0.15 Hz) represents overall ANS activity, including a sympathetic predominance, and the high-frequency component (>0.20 HZ) demonstrates vagally mediated activity and adaptability of the autonomic nervous system (Rajendra Acahrya et al., 2006). Low HRV is therefore associated with a high level of perceived stress, heightened

sympathetic activity and low parasympathetic activity, which leads to a decreased ability to handle internal and external stressors. High HRV, however, shows a relaxed state, low sympathetic activity, and thus, increased regulatory control and ability to cope with stressors effectively (Kim et al., 2018; da Estrela et al., 2021).

This study measures HRV by using an electrocardiogram (ECG) signal, which "corresponds to the electrical activity of the heart" (Berkaya et al., 2018). Measuring heart rate via ECG is an effective and completely non-invasive way to examine individuals for cardiovascular diseases, such as cardiac arrhythmias, or, in the case of this study, to inspect HRV. ECG signals have five main components, namely, P, Q, R, S and T waves, as well as the minor deviation, the U wave (see Figure 1).

Figure 1

Standard Fiducial Points in ECG



Note. From "A survey on ECG analysis," by S. K. Berkaya, A. K. Uysal, E. S. Gunal, S. Ergin, S. Gunal, M. B. Gulmezoglu, 2018, Biomedical Signal Processing and Control, 43, pp. 216-235. Copyright 2018 by Elsevier. https://doi.org/10.1016/j.bspc.2018.03.003. Copyright 2018 by Elsevier.

Heart rate variability represents the fluctuations of time between consecutive R peaks. Thus, we are interested in the interbeat interval, which reflects the duration between successive R peaks, also known as the RR interval, as indicated in Figure 1. This variation in heart beat intervals allows the cardiovascular system to flexibly adapt to acute physical and psychological threats to homeostasis (Shaffer & Ginsberg, 2017). Therefore, a high HRV is associated with increased regulatory control of stressors, and low HRV with high stress and, in the long term, serious health problems.

Heart Rate Variability and Biological Sex Differences

Prior research on HRV also includes discussions on potential sex-specific differences. In a study by Vanderhasselt et al. (2018) participants received both negative and positive social feedback. Female participants self-reported greater stress after the negative feedback than male participants did, and even though both female and male participants showed an increment of HRV subsequent to the social feedback, the increment was greater for the male participants. This suggests that male participants showed higher self-regulatory control, were therefore possibly better at adapting to the presented stressor than female participants and consequently experienced less stress, which is in line with the self-reports. Interestingly, the resting HRV was found to be higher in female participants, which is consistent with other findings regarding sex differences in baseline HRV (Min et al., 2023).

Implicit Association Tests and Self-Regulation

Since the introduction of Implicit Association Tests (IATs) 1998 by Greenwald and colleagues, IATs are a widely accepted measurement tool used to supposedly show individual differences in implicit constructs among people. Some researchers claim that most cognition happens apart from consciousness and control, and therefore IATs would offer a better measurement of human cognition than self-reports do (Nosek et al., 2011). Despite the

frequent use and acceptance of IATs, there is a substantial amount of criticism among researchers concerning the validity of IATs. For instance, Schimmack (2021) criticises IAT's construct validity, stating that outcomes provided no psychometric support for IAT's apparent measurement of implicit constructs, and there also seems to be no evidence for significant incremental predictive validity. More specifically, there is no evidence to support the idea that IATs perform better at measuring implicit constructs than self-reports or other explicit measures do (Oswald, 2013; Schimmack, 2021).

For our research we are not interested in the actual outcomes of the IAT but use it merely as a psychological stress inducing factor of social conformity. This study aims to investigate how Heart Rate Variability (HRV), representing physiological stress, is affected when individuals are confronted with a stressful situation, how this effect correlates with the perceived stress individuals experience and whether outcomes are sex distinctive. To measure perceived stress in subjects, a non-verbal pictorial self-assessment manikin (SAM) indicating perceived pleasure, arousal and dominance was used (Lang, 1980). The IAT consisted of connecting negative or positive adjectives to either native (Dutch/German), or Moroccan common names. Hereby, it is important to note that the study was conducted with only Dutch and German participants, and therefore placing Moroccan names in the out-group social category to investigate racial bias.

Since our study used a racial bias IAT to induce social pressure in participants, besides the correlation between HRV and stress, the self-regulatory effort associated with HRV is interesting as well, because participants might be eager to perform in a social norm conforming way, i.e. regulating their behaviour to not seem discriminatory. Thayer and Lane (2000) explain this connection between HRV and self-regulatory effort in their 'Neurovisceral Integration Model". The 'central autonomic network' connects different brain areas involved in motor, interoceptive, perceptual and memory functioning and integrates

these with the ANS to promote adaptation to different stimuli. Vagally mediated heart rate variability (vmHRV) functions as a biomarker of CAN activity, and therefore works as an indicator of activity related to adaptation in emotion regulation and dysregulation (Thayer & Lane, 2002).

Corroborating this theory, Di Palma et al. (2019) explored how vmHRV correlates with empathic pain ratings for both White and Black actors. They found a significant increment of vmHRV in participants when rating Black actor's perceived pain, as well as participant's resting vmHRV predicting the lag-time they later needed to rate actor's pain during the study. The researchers explained these outcomes with respect to previous research investigating the connection between cognitive and self-regulatory effort and racial biases. According to the "Contrasting forces model" (Berlingeri et al., 2016) cognitive effort is necessary in order to self-regulate implicit biases, so one can behave in a social norm conforming way. The time needed to self-regulate is reflected by lag-time. In the study conducted by Di Palma et al. (2019) participants needed approximately 100 milliseconds longer to rate the pain of Black actors than White actors, indicating, indeed, a possible sign of additional time needed for emotion regulation, which is in line with the findings of a higher vmHRV as well.

Our specific hypotheses are therefore: (1) An overall decrease of HRV during participation in the IAT, (2) Higher SAM scores correlating with lower HRV, (3) The effect found for hypothesis 1 to be more pronounced for female as compared to male participants and (4) higher resting HRV in female participants compared to male participants.

Methods

Participants

The participants for this study consisted of 49 young adults, of which 33 had usable data. Due to a technical error, the program did not always mark the beginning of the questionnaires and the IAT in the recording of the ECG, which led to the omission of data from 16 participants. The 33 participants consisted of 23 cisgender women, 9 cisgender men and one transgender man. All of the subjects were either native German or native Dutch. The study was done using a convenience sample; participants were recruited through the SONA research pool of the University of Groningen, in which first year psychology students apply for participation in studies in order to achieve credits and complete their propaedeutic year. Students decide for themselves in which studies they want to participate in; the inducement for participation in this study was 1.4 SONA-credits. Besides first year psychology students, participation via private invitation was also possible, leading to five participants joining the study who were invited by the student researchers. All of them were students of the University of Groningen as well and they received no inducements. All participants agreed for their data to be used by signing an informed consent form.

Description of Parameters

The RMSSD measures the short-term variability in differences between successive normal heartbeats and is used to administer HRV. RMSSD reflects parasympathetic (vagal) activity, with increased RMSSD values reflecting higher parasympathetic activity, which is linked to a relaxed mental state. Decreased RMSSD value on the other hand can indicate stress and potential health issues. RMSSD is calculated as followed:

$$\mathrm{RMSSD} = \sqrt{\frac{1}{N-1}\sum_{n=1}^{N-1}(\mathrm{RR}_{n+1} - \mathrm{RR}_n)^2}.$$

RR represents the interval between consecutive R-peaks in the electrocardiogram, *N* refers to the total number of RR intervals.

To compare the HRV of different tests and participants, we used the Corrected Root Mean Square of the Successive Differences (cRMSSD). This allows us to account for the differences in baselines of HRV between participants and cRMSSD is robust against possible biases, such as outliers, which makes it the preferred option over the Root Mean Square of the Successive Differences (RMSSD). The cRMSSD is calculated by dividing the RMSSD by the mean Inter-Beat Interval (IBI). For the statistical analysis, JASP Version 0.18.3 was used.

Materials and Apparatus

The laboratory was equipped with two soundproof rooms, one in which the participant underwent the study, the other one for the researchers. The room for the experiment consisted of one computer, a keyboard, a computer mouse and a button box with two buttons. To measure the subjects heart beat, a Polar H10 Heart Rate Sensor waistband was utilised, a wireless low-threshold ECG measurement device. The waistband was put on by the participants themselves and was worn under their clothing around the ribcage. After sufficient arrangement, the band sent the data via Bluetooth to the researcher's computer using a programme, developed at the Rijksuniversiteit Groningen, to display and record the ECG. To administer the subsequent gender questionnaire (see Appendix A), the Big Five Inventory (see Appendix B) and the Implicit Association Test, OpenSesame version 4.0.13 was used, a program designed to construct experimental research. We decided to construct the gender questionnaire as an open format, allowing participants to type their answers in order to conduct research about gender and sex differences in an inclusive manner, which would not be possible to the same extent by simply providing a few options to choose from. We were also interested in differences between gender identity and biological sex regarding HRV, so solely asking for sex would have been misleading for the research. However, since our sample was rather small, it did only include one trans person, making comparisons between

cis- and transgender people not feasible. The Big Five Inventory was administered in a computerised version, and was included to examine possible correlations between Neuroticism and HRV, which was the focal point of other student researchers from the same study. However, these will not be the focus of this research paper.

Procedure

After explaining the process of the study, participants read the instructions and signed the informed consent form before the experiment started. The study was administered under the false pretence to test racial biases within subjects using a Racial Implicit Associations Test. The subject then arranged the Polar H10 band themselves, with the band's sensor in the middle of their ribcage. After confirming the correct attachment of the device by checking the connection to the researcher's computer, the participant could start with the experiment, isolated in the soundproof room. The experiment and questionnaires were conducted in the native tongue of the subject, either Dutch or German. The study started with the gender questionnaire and the subject answered by using the keyboard provided. The participant then continued with the Big Five Inventory, and subsequently the IAT, implemented in OpenSesame, was administered.

The IAT (see Appendix C) consisted of four rounds, with eight practice trials at the beginning of each round. Whenever a mistake was made, a loud buzzer sound appeared in the participant's room, indicating the error. For the first round, subjects were supposed to press the right button every time a positive adjective appeared and the left button for every negative adjective. The second round, the subject had to press the right button each time a common native name (either German or Dutch, depending on the subject's nationality) appeared and the left button everytime a Moroccan name was displayed. These first two rounds functioned as practice to make the participant familiar with the task and each consisted of 36 trials. The

sequence of the third and fourth round were randomised and each consisted of 144 trials. The MN/NP round consisted of pressing the right button each time a native name or positive adjective appeared and the left button for every Moroccan name and negative adjective. For the MP/NN round, the participant had to press the right button when a Moroccan name or a positive adjective was displayed and the left button each time a native name or negative adjective appeared. Here a decrease in performance was expected, since the condition 'Moroccan name' was never placed on the right button during practice rounds, so switching its position could presumably lead to an increase in mistakes. Thus, we expected the most discomfort here, since the participant might notice their decreased performance and the possibility of showing discriminatory behaviour.

After every 16 trials, feedback on the subject's performance appeared, including their average reaction time and the percentage of mistakes made. After the feedback, the SAM (see Appendix D) was displayed, asking the participant to rate their valence, arousal and dominance utilising a likert scale. After approximately 30 minutes, participants completed the tasks, were thanked for participation and asked to remove the heart rate measuring band. Lastly, subjects were debriefed and the actual purpose of the study was disclosed. In particular, that the IAT was only used to induce discomfort and stress in the participant and not to measure racial biases. Potential questions participants had were answered.

Results

Table 1 presents the descriptives of the overall performance on the administered Implicit Associations Test regarding the response time between an item being displayed and the button getting pressed. The p-values of the Shapiro-Wilk test suggest a violation of normality for every variable. The response time was generally longer when participants made a mistake. Regarding the amount and percentage of correctly and incorrectly pressed

responses, Table 2 represents the descriptives across the four conditions of the IAT. Again, the Shapiro-Wilk p-values show that each condition is not normally distributed. Participants pressed correctly for 94.04% of MN/NP round items and 92.27% of MP/NN round items.

Table 1

Descriptive Statistics of Overall Performance on IAT Regarding Response Time

	MN/NP		MP/NN	
	Correct	Incorrect	Correct	Incorrect
N	33	33	33	33
Mean	810.91	1703.97	944.23	1585.42
SD	338.3	908.02	288.1	740.9
p*	< .001	< .001	< .001	.01

Note. p = Shapiro-Wilk p-value. “Correct” and “Incorrect” refer to whether the participant responded correctly or incorrectly. Omitted data not included.

* For interpretation an alpha-level of 0.05 is used.

Table 2

Descriptive Statistics of Overall Performance on IAT Regarding the Amount of Correct and Incorrect Answers

	MN/NP Correct	MN/NP Incorrect	MP/NN Correct	MP/NN Incorrect
N	33	33	33	33
Mean	135.42	8.57	132.88	11.12
SD	3.18	3.18	5.61	5.61
p*	.005	.005	.007	.007

Note. p = Shapiro-Wilk p-value.

*For interpretation an alpha-level of 0.05 is used.

The descriptives for the stress measurements can be found in Table 3, these include the cRMSSD for each test condition, MN/NP and MP/NN, as well as the self-reported stress indicated by the three dimensions of the SAM scale: arousal, dominance and valence. The cRMSSD for both MN/NO and MP/NN seems to be not normally distributed.

Table 3

Descriptive Statistics for Stress Measurements Including Measured Heart Rate and Stress-Assessment via Self-Report

	Mean	SD	p
MN/NP_cRMSSD	58.08	30.37	.001
MN/NP Arousal	2.74	1.39	.925
MN/NP Dominance	3.78	1.6	.286
MN/NP Valence	4.8	1.31	.203
MP/NN_cRMSSD	63.02	30.89	.005
MP/NN Arousal	2.73	1.39	.859
MP/NN Dominance	3.74	1.6	.790
MP/NN Valence	4.694	1.3	.467

To test our first hypothesis, “Overall decrease of HRV during IAT”, we conducted a Paired Samples T-Test with the HRV of the Big Five Inventory as a baseline measurement to compare with the stress inducing variable MP/NN (see Table 4). Since both variables were not normally distributed, we used a Wilcoxon signed-rank test. The p-value of 0.024 suggests that participants had a significantly higher HRV during the stress-inducing part of the IAT than they had when filling out the Big Five Personality Inventory.

Table 4

Paired Samples T-Test to Test Difference Between HRV of Big Five Inventory and Stress Inducing Condition MP/NN

Measure 1	Measure 2	W	p
Big Five cRMSSD	MP/NN_cRMSSD	155	.024

Note. Wilcoxon signed-rank test.

To examine our second hypothesis, “Higher SAM scores correlating with lower HRV”, we first tested the equality of variances across the three dimensions of the SAM scale using Levene’s test (see Table 5). After ensuring homogeneity of variances, we conducted a correlation analysis using Spearman’s rho, examining whether the HRV of the MN/NP and MP/NN round is correlated with each dimension on the SAM scale, as well as whether the individual dimensions are correlated with each other (see Table 6 and 7). For the MN/NP round, valence and dominance are positively correlated (rho = 0.563), valence and arousal are negatively correlated (rho = -0.515) and there seems to be no correlation between any SAM dimension and the HRV measured during this round. Regarding the MN/NP round, Valence and Dominance are again positively correlated (rho = 0.578), as well as Arousal and Valence being again negatively correlated (rho = -0.453). Additionally, HRV measured during the MP/NN round is positively correlated with the Arousal dimension (rho = 0.385). Thus, the only correlation between SAM scores and HRV can be found in the stress inducing block, MP/NN, meaning, when people reported feeling more aroused, their HRV was increased.

Table 5

Test of Equality of Variances (Levene's) for the Three Dimensions on the SAM Scale

	F	df_1	df_2	p
MP/NN Dominance	0.016	1	31	.899
MP/NN Arousal	0.011	1	31	.918
MP/NN Valence	1.435	1	31	.240
MN/NP Dominance	0.309	1	31	.582
MN/NP Arousal	0.132	1	31	.719
MN/NP Valence	0.639	1	31	.430

Table 6

Correlations Between HRV During MN/NP Round and SAM Scale Dimensions

			Spearman's rho	p
MN/NP_cRMSSD	-	MN/NP Valence	-0.188	.295
MN/NP_cRMSSD	-	MN/NP Dominance	0.034	.853
MN/NP_cRMSSD	-	MN/NP Arousal	0.242	.174
MN/NP Valence	-	MN/NP Dominance	0.563	< .001
MN/NP Valence	-	MN/NP Arousal	-0.515	.002
MN/NP Dominance	-	MN/NP Arousal	-0.106	.558

Table 7

Correlations Between HRV During MP/NN Round and SAM Scale Dimensions

			Spearman's rho	p
MP/NN_cRMSSD	-	MP/NN Arousal	0.385	.027
MP/NN_cRMSSD	-	MP/NN Dominance	0.229	.200
MP/NN_cRMSSD	-	MP/NN Valence	-0.037	.837
MP/NN Arousal	-	MP/NN Dominance	-0.192	.285
MP/NN Arousal	-	MP/NN Valence	-0.453	.008
MP/NN Dominance	-	MP/NN Valence	0.578	< .001

For our third hypothesis “The effect found for hypothesis 1 to be more pronounced for female as compared to male participants”, Table 8 shows the group specific descriptives for the stress conditions. We then conducted an Independent Samples T-Test (see Table 9) to see if the differences seen in male and female participants are significantly distinctive. We used a Mann-Whitnex U test, since the normality assumption for MP/NN_cRMSSD is violated. The test indicated that there is a significant difference between female and male participants for HRV within the MP/NN round, meaning, male participants had a significantly higher HRV than female subjects.

Table 8

Group Descriptives for Stress Conditions

	Group	N	Mean	SD
MP/NN_cRMSSD	Male	9	86.03	38.72
	Female	24	54.39	22.82
MP/NN Arousal	Male	9	2.72	1.38
	Female	24	2.73	1.42
MP/NN Dominance	Male	9	3.85	1.69
	Female	24	3.7	1.61
MP/NN Valence	Male	9	5.1	1.59
	Female	24	4.54	1.18

Table 9

Independent Samples T-Test Examining Sex Differences in HRV and Self-Reported Stress

	W	**p**
MP/NN_cRMSSD	159.000	.040
MP/NN Arousal	109.500	.968
MP/NN Dominance	116.500	.746
MP/NN Valence	129.500	.394

Note. Mann-Whitney U test.

Regarding our final, fourth, hypothesis, “Higher resting HRV in female compared to male participants” we used the Big Five Inventory as a the resting HRV variable and compared its values with the ones from the stress inducing part of the IAT, the MP/NN round, by conducting an Independent Samples T-Test (Mann-Whitney U test). The p-values (see Table

10) indicate the difference in baseline between males and females does not seem to be significant.

Table 10

Independent Samples T-Test Examining Sex Differences in Resting HRV

	W	p
bigfive_cRMSSD	153	.072

Note. Mann-Whitney U test.

Discussion

This research aims to contribute to the understanding on the impact of stress on heart rate variability and how we can interpret these effects in regard to emotion regulation, perceived stress and cognitive load. By utilising a racial-bias IAT to induce stress in participants we aimed to investigate if HRV changes, and whether this change is correlated with self-reported stress indicated by the SAM. We were also interested in possible sex-related differences. In particular, we expected a decrease of HRV during the stressful task, higher perceived stress to be correlated with lower HRV and female participants to have a lower HRV during the stressful task, a higher resting HRV in general and a higher self-reported stress.

The findings did not support our first hypothesis, expecting HRV to decrease during participation in the IAT. Instead, the outcomes seem to indicate the opposite, participants showed a significantly higher HRV during the stress inducing part of the IAT when compared to their baseline HRV (filling out the Big Five Inventory). This is a particularly surprising result, since high HRV usually represents a relaxed mental state and the absence of stress, which contradicts associations with a stressful task. One possible explanation for this finding could be that participation in the IAT was simply not stressful enough and the quite long

duration of approximately 30 minutes led to participants becoming overly familiar with the task, making them rather bored than stressed. What further strengthened this effect is the fact that we labelled the very beginning of the study, when participants had to fill out the Big Five Inventory, as the baseline HRV level, even though most participants might be the most stressed at the beginning of a study, regardless of what the study entails. This might have led to a skewed baseline HRV level and thus, facilitating an increase in HRV after the first nervousness of participants goes down.

For our second hypothesis we anticipated SAM scores to correlate with lower HRV, in particular, we expected heightened arousal scores and diminished valence and dominance to correlate with decreased HRV. Whereas we did find a significant correlation for the arousal dimension and HRV, it was contradictory to our hypothesis, indicating that when participants reported feeling more aroused, their HRV also increased. This further supports the explanation that participants were getting bored of the study instead of feeling distressed, and therefore evaluating their annoyance as feeling aroused.

Regarding possible sex differences within our study, the findings suggest that male participants had a significantly higher HRV during the stress-evoking MP/NN round than females did. These findings are in support of our third hypothesis, expecting the effect found for hypothesis 1 to be more pronounced for females as compared to male participants. However, self-reported stress in female participants compared to males, was not supported by our research. A possible explanation for this could be that the SAM was not sufficient in measuring stress in participants, which was evident in the other findings as well.

Moreover, we could not find any evidence for a significantly higher resting HRV in female participants. As mentioned already, utilising the beginning of the study as a baseline,

and therefore resting, HRV might not represent the true HRV resting level of participants, but rather already shows a mental state of nervousness and distress.

Limitations

There are some limitations to this study that are worth considering, as well as resulting recommendations helpful for future research. Since we worked with a convenience sample, mostly consisting of young, white university students, all of them being German or Dutch, the external validity is rather low. Since many participants were first year psychology students, they might have heard of the IAT and its diminished ability to refer anything substantial about one's racial bias before, and therefore were less concerned about behaving in a norm-conforming way, leading to decreased stress levels. Furthermore, the study consisted of a smaller sample (N = 33) with only 9 male participants, making it difficult to draw externally valid conclusions regarding sex differences. It would have also been interesting to investigate differences between gender and sex, however, since we only had one trans person in our sample, making any inferences about differences between them and the 32 other cisgender persons would be entirely case-specific without any external validity possible.

Besides external validity, we also experienced limitations due to our construct validity, in particular, using the SAM scale to examine self-reported stress turned out to not be a good fit. During and after the experiment, many participants voiced being unsure on how to use the SAM to accurately rate their feelings during the trials. Many participants merely used the more neutral ratings on the SAM likert-scale and barely changed the rating throughout, making correlations with possible other variables difficult. This might have led to a discrepancy between what we attempted to measure and how the participant used the measuring tool. Another limitation concerning construct validity was how the HRV

measurements were administered. The issues arising from this were already briefly mentioned above, but in particular, using the Big Five Inventory as a baseline HRV and not measuring HRV after the IAT as well, limited our research immensely. This way any change in HRV can only be interpreted very diffidently.

Recommendations

Specific to this study, we recommend not using the SAM scale for participants to self-report their stress levels. While the SAM was a helpful tool to ask about participants' emotions without specifically mentioning stress and therefore possibly revealing the purpose of the research and making answers biassed, it was confusing for subjects and thus likely not accurate in rating stress levels. We therefore recommend using a short questionnaire paired with a likert scale in between trials including questions about participants distress, how cognitively demanding they think the task is, how they estimate their performance, and whether they like the exercise. These questions could include any aspects important for the research, without only asking about stress and perhaps causing bias.

Regarding the measurement of HRV, we strongly advise researchers to measure HRV before, during and after the stress inducing task. The baseline measurements, before and after the task, should also not include any possible stress-evoking stimuli. Beginning the study with a personality questionnaire could result in increased participants' nervousness. Thus, we recommend the before and after measurement to either not include any task or a very mundane one. Additionally, since our research did not support the idea of the IAT to be stressful enough, we recommend considering using other stress inducing stimuli, such as public speaking, time sensitive tasks or social interaction feedback, since these were evidentially evoking stress during earlier studies (Vanderhasselt et al., 2018; Westenberg, C. L. et al., 2009). It is also recommended for future research to include a wider and larger

sample in order to increase external validity. In particular, different nationalities and ethnicities, a broader age range and individuals with diverse socio-economic backgrounds. We also certainly recommend the use of an open gender questionnaire as it was done for this study, in order to gain more knowledge on gender and sex differences in a truly inclusive way. This could lead to compelling new findings about whether differences shown between men and women are due to biological sex or attributable to growing up within certain gender norms shaping one's behaviour.

Conclusion

Even though the study did not find evidence for the effect stress has on heart rate variability, it contributed to our understanding on how HRV and self-reported stress should be administered. Whereas the research did find that male participants had lower levels of physiological stress, reflected by a higher HRV, this was not correlated to lower perceived stress levels, compared to female participants. Thus, future research focusing on how well perceived and physiological stress correlated with each other would be interesting.

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Appendix A

Gender Questionnaire

English	Nederlands	Deutsch
What sex were you assigned at birth?	Welk geslacht is je toegewezen bij de geboorte?	Welches Geschlecht wurde dir bei der Geburt zugewiesen?
What gender were you raised until the age of 12?	Als welke genderidentiteit ben je opgevoed t/m twaalfjarige leeftijd?	Mit welcher Geschlechtsidentität bist du bis zum Alter von 12 Jahren aufgewachsen?
What is your gender identity right now?	Wat is op dit moment je genderidentiteit?	Mit welchem Geschlecht identifizierst du dich heute?

Appendix B

Big Five Inventory Items in English, Dutch, German

I see Myself as Someone Who...

____1. Is talkative

____2. Tends to find fault with others

____3. Does a thorough job

____4. Is depressed, blue

____5. Is original, comes up with new ideas

____6. Is reserved

____7. Is helpful and unselfish with others

____8. Can be somewhat careless

____9. Is relaxed, handles stress well

____10. Is curious about many different things

____11. Is full of energy

____12. Starts quarrels with others

____13. Is a reliable worker

____14. Can be tense

____15. Is ingenious, a deep thinker

____16. Generates a lot of enthusiasm

____17. Has a forgiving nature

____18. Tends to be disorganised

____19. Worries a lot

____20. Has an active imagination

____21. Tends to be quiet

____22. Is generally trusting

____23. Tends to be lazy

____24. Is emotionally stable, not easily upset

____25. Is inventive

____26. Has an assertive personality

____27. Can be cold and aloof

____28. Perseveres until the task is finished

____29. Can be moody

____30. Values artistic, aesthetic experiences

____31. Is sometimes shy, inhibited

____32. Is considerate and kind to almost everyone

____33. Does things efficiently

____34. Remains calm in tense situations

____35. Prefers work that is routine

____36. Is outgoing, sociable

____37. Is sometimes rude to others

____38. Makes plans and follows through with them

____39. Gets nervous easily

____40. Likes to reflect, play with ideas

____41. Has few artistic interests

____42. Likes to cooperate with others

____43. Is easily distracted

____44. Is sophisticated in art, music, or literature

Ik zie mezelf als iemand die...

____ 1. Spraakzaam is

____ 2. Vaak fouten bij anderen zoekt

____ 3. Zijn werk grondig doet

____ 4. Neerslachtig, somber is

____ 5. Origineel is, nieuwe ideeën bedenkt

____ 6. Gereserveerd is

____ 7. Behulpzaam en onzelfzuchtig is voor anderen

____ 8. Enigszins slordig kan zijn

____ 9. Ontspannen is, goed met stress omgaat

____ 10. Nieuwsgierig is naar veel verschillende dingen

____ 11. Boordevol energie zit

____ 12. Ruzies begint met anderen

____ 13. Betrouwbaar is als werker

____ 14. Gespannen kan zijn

____ 15. Vernuftig is, een denker

____ 16. Veel enthousiasme bij anderen genereert

____ 17. Vergevingsgezind is

____ 18. De neiging heeft om ongeorganiseerd te zijn

____ 19. Zich veel zorgen maakt

____ 20. Een actieve verbeelding heeft

____ 21. De neiging heeft om stil te zijn

____ 22. Over het algemeen goed van vertrouwen is

____ 23. De neiging heeft om lui te zijn

____ 24. Emotioneel stabiel is, niet snel van streek raakt

____ 25. Vindingrijk is

____ 26. Een assertieve persoonlijkheid heeft

____ 27. Koud en afstandelijk kan zijn

____ 28. Volhardt tot de taak is voltooid

____ 29. Soms humeurig kan zijn

____ 30. Kunstzinnige, esthetische ervaringen waardeert

____ 31. Soms verlegen en geremd is

____ 32. Attent en vriendelijk is tegen bijna iedereen

____ 33. Dingen efficiënt doet

____ 34. Kalm blijft in gespannen situaties

____ 35. De voorkeur geeft aan routinematig werk

____ 36. Extravert en sociaal is

____ 37. Soms onbeleefd is tegen anderen

____ 38. Plannen maakt en deze uitvoert

____ 39. Snel nerveus wordt

____ 40. Graag nadenkt, speelt met ideeën

____ 41. Weinig artistieke interesses heeft

____ 42. Graag samenwerkt met anderen

____ 43. Makkelijk afgeleid is

____ 44. Verfijnd is op het gebied van kunst, muziek of literatuur

Ich sehe mich selbst als jemanden, der...

____ 1. Gesprächig ist

____ 2. Dazu tendiert, Fehler bei Anderen zu suchen

____ 3. Einen gründlichen Job macht

____ 4. Depressiv oder bedrückt ist

____ 5. Originell ist, neue Ideen entwickelt

____ 6. Zurückhaltend ist

____ 7. Nicht egoistisch Anderen gegenüber ist

____ 8. Manchmal nachlässig sein kann

____ 9. Entspannt ist, mit Stress gut umgehen kann

____ 10. Neugierig auf viele verschiedene Dinge ist

____ 11. Viel Energie hat

____ 12. Streitereien mit anderen beginnt

____ 13. Ein zuverlässiger Arbeiter ist

____ 14. Angespannt sein kann

____ 15. Genial ist, ein tiefgründiger Denker ist

____ 16. Viel Enthusiasmus erzeugt

____ 17. Dazu neigt, Anderen zu vergeben

____18. Dazu tendiert, unorganisiert zu sein

____ 19. Viel grübelt

____ 20. Eine aktive Vorstellungskraft hat

____ 21. Dazu tendiert, ruhig zu sein

____ 22. Im Allgemeinen vertrauensvoll ist

____ 23. Dazu tendiert, faul zu sein

____ 24. Emotional stabil ist, nicht leicht aus der Fassung zu bringen ist

____ 25. Erfinderisch ist

____ 26. Eine durchsetzungsstarke Persönlichkeit hat

____ 27. Kaltherzig und distanziert sein kann

____ 28. Durchhält, bis die Aufgabe erledigt ist

____ 29. Manchmal launisch sein kann

____ 30. Künstlerische, ästhetische Erfahrungen schätzt

____ 31. Manchmal schüchtern und gehemmt ist

____ 32. Rücksichtsvoll und freundlich gegenüber fast jedem ist

____ 33. Dinge effizient erledigt

____ 34. In angespannten Situationen ruhig bleibt

____ 35. Routinearbeit bevorzugt

____ 36. Offen und gesellig ist

____ 37. Manchmal unhöflich zu anderen ist

____ 38. Pläne macht und diese auch umsetzt

____ 39. Leicht nervös wird

____ 40. Gerne reflektiert, mit Ideen spielt

____ 41. Wenig künstlerische Interessen hat

____ 42. Gerne mit anderen zusammenarbeitet

____ 43. Leicht abgelenkt wird

____ 44. In Kunst, Musik oder Literatur kultiviert ist

Appendix C

Names and Adjectives Used in IAT

Dutch words		German words	
Positive	Negative	Positive	Negative
Vrolijk	Verdrietig	Frölich	Traurig
Vriendelijk	Boosaardig	Freundlich	Bösartig
Samen	Alleen	Zusammen	Allein
Gezond	Ziekte	Gesund	Krankheit
Open	Gesloten	Offen	Geschlossen
Constructief	Destructief	Konstruktiv	Zerstörerisch
Degelijk	Vies	Solide	Schmutzig
Inventief	Zwak	Erfinderisch	Schwach
Lekker	Banaal	Lecker	Unbedeutend

Dutch names	German names	Moroccan names
Daan	Peter	Ahmed
Lucas	Wolfgang	Salma
Anna	Michael	Amina
Bram	Werner	Hassan
Floris	Klaus	Ayoub
Diana	Maria	Adil
Tess	Ursula	Ali
Lars	Monika	Omar
Jan	Petra	Hamza

Appendix D

Self-Assessment Manikin