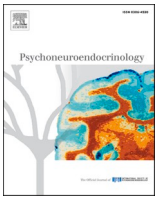
Psychoneuroendocrinology 128 (2021) 105217



Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen





The repeated Montreal Imaging Stress Test (rMIST): Testing habituation, sensitization, and anticipation effects to repeated stress induction

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ARTICLE INFO

Keywords:
Stress
Heart rate
Cortisol
Habituation
Sensitization
Anticipation

ABSTRACT

Background: A psychosocial task that can induce comparable levels of stress repeatedly is fundamental to effectively study changes in stress reactivity over time or as a result of an intervention. However, existing tasks have struggled to provide consistent stress responses across repeated trials.
Aim: The goal was to assess the efficacy of two different designs of the repeated Montreal Imaging Stress Test (rMIST) in reproducing the same pattern of reactivity over two separate sessions.
Methods: In two different studies, stress was induced using the rMIST on two separate sessions, one week apart. Each study used a different task design. In the first study (53 participants [45 women]; mean age=24.16 [SD=3.29]), the rMIST consisted of a single-longer stress exposure, while the second study (30 participants [27 women]; mean age=21.81 [SD=2.09]) consisted of several shorter stress exposures per session. Self-reported (i.e perceived stress [PS] and negative affect [NA]), physiological (i.e heart rate [HR], root mean square of successive differences [RMSSD]) and hormonal (i.e. salivary cortisol) measures of stress were used.
Results: Stress reactivity was comparable across the two repeated stress sessions in both studies. However, baseline HR in the second session increased relative to the first session in the first study, and there was no cortisol response. Additionally, there was a decrease in HR and HRV reactivity within the session on the second study, suggesting a habituation effect not between but within the session itself.
Conclusion: The rMIST overcomes some of the challenges associated with repeated stress induction. However, an anticipation effect and a lack of cortisol response indicate that further adjustments to the task are necessary. Finally, task design is important for repeated stress reactivity.

1. Introduction

Reactivity to minor daily stressors is understood to play a central role in the development of various psychiatric and somatic disorders (Turner et al., 2020). It is therefore important to assess markers of the stress response over time in order to effectively identify the risk for and the development of these disorders longitudinally or to evaluate the success of an intervention. To that end, it is essential to have a valid stress induction task that is able to elicit a significant stress response not just once but repeatedly. Moreover, stress reactivity must be comparable across repeated sessions so that any change in reactivity be attributed to the individual rather than the repeated exposure to the same task. Currently there are various psychosocial stress-induction tasks that fulfill the necessary criteria to elicit stress in the laboratory (i.e. socio-evaluative threat, uncontrollability, and novelty) (Dickerson and Kemeny, 2004), albeit to our knowledge none has succeeded in overcoming the challenges presented by their repeated use, that is, task-related habituation, sensitization, and anticipation to provide consistent average responses across repeated measures.

Studies repeatedly using the same stress-induction task have shown that independent of the time between exposures (e.g. 24 h, one week, or four weeks) (Kirschbaum et al., 1995; Gerra et al., 2001; Jönsson et al.,

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https://doi.org/10.1016/j.psyneuen.2021.105217
Received 24 November 2020; Received in revised form 22 February 2021; Accepted 30 March 2021
Available online 18 April 2021
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2010; Schommer et al., 2003; Boesch et al., 2014; Petrowski et al., 2012), individuals habituate to the task with a significant lower increase in salivary cortisol and, to a lesser degree, a lower increase in heart rate (HR) over repeated instances of stress induction (Boesch et al., 2014; Jönsson et al., 2015; Schommer et al., 2003). A lack of novelty during the second stress induction using the same task may explain this effect (Dickerson and Kemeny, 2004; Stein, 1966).

On the contrary, stress that is intense or prolonged can exacerbate the stress response to the second stress induction. Sensitization is most common when the initial stressor is especially strong and triggers hyper-arousing reactivity (Belda et al., 2015; Groves and Thompson, 1970). Laboratory stress induction tasks thus need to induce enough stress to elicit a stress response but not so much as to cause sensitization during subsequent task exposures.

Finally, anticipation effects become evident when a stress response is observed prior to (i.e. in the anticipation of) the second stress induction (Boyle et al., 2016). This is particularly problematic because an already high stress level at baseline on the second session may influence reactivity and therefore reduce comparability between sessions. Here, manipulating the expectation about the second stress induction may facilitate or reduce anticipatory stress effects.

For these reasons, well-known protocols such as the Trier Social Stress Test (TSST) have yet to successfully induce comparable levels of stress on repeated trials. Furthermore, they are often laborious and difficult to control and standardize (Skoluda et al., 2015), potentially compromising their reliability. In an attempt to overcome these challenges, we have developed the repeated Montreal Imaging Stress Test (rMIST) – a modified version of the original MIST (see Dedovic et al., 2005 for details) with an added element of novelty to the second exposure to limit the sense of familiarity to the task so that it may be used repeatedly.

In order to test the efficacy of the rMIST in inducing comparable stress reactivity over two separate sessions, we performed two studies testing two different designs of the rMIST: (1) a single-run design, meaning that each session consisted of a single longer stress exposure, and (2) a multiple-run design that consisted of multiple shorter stress exposures per session. The latter multiple-run design is a typical fMRI design for which the original MIST was initially intended for. However, it is not easily comparable to other psychosocial stress induction tasks that generally consist of a single stress exposure. In line with previous research, we have formulated the following hypotheses:

1. The rMIST elicits a robust stress response, with significant increases in mean heart rate (HR), cortisol, perceived stress (PS), and negative affect (NA), and a significant decrease in heart rate variability (RMSSD) from the control to the stress condition.
2. The stress response to the rMIST is consistent across both trials, meaning that there is no interaction between condition (i.e. stress vs control) and session (i.e. session one vs session two), that is, no habituation or sensitization effects, for any of the measures.
3. There is no anticipation effect on the second session, meaning that there is no difference in baseline HR, RMSSD, cortisol, PS, and NA between sessions.

2. Methodology

2.1. Participants

2.1.1. Study 1

Fifty-three participants between the ages of 19 and 35 took part in the study. 60% of participants were university students from various fields (e.g. medicine, biomedical science, history, arts, etc.) and the remaining 40% were young working adults. Participants were recruited via flyers spread throughout the city, and shared online. All participants signed an informed consent prior to taking part in the study, and were rewarded 30€ per session. Ethical approval was granted by the Sociaal-Maatschappelijke Etische Commissie (SMEC) of KU Leuven.

2.1.2. Study 2

Thirty participants between the ages of 19 and 27 took part in the study. Participants were first year students from the Faculty of Health, Medicine and Life Science at Maastricht University. Participants were recruited via email to participate in a study on mental capacity. All signed an informed consent prior to taking part in the study. Ethical approval was granted by the Ethic Review Committee Psychology and Neuroscience (ERCPN).

2.2. Procedures

2.2.1. Stress induction

The repeated Montreal Imaging Stress Task (rMIST) was used to induce socio-evaluative stress. The rMIST is a modified version of the original MIST, an arithmetic task where the participant is made to feel pressured to perform well (see Dedovic et al., 2005 for details). Following each stress condition, the participant receives negative feedback on their performance from the experimenter. To be able to use this method repeatedly over the course of different sessions, in this specific case twice one week apart, the protocol was modified to enhance the social defeat component and add an element of novelty on any subsequent trials. We tested two participants at the time, in two adjacent rooms after having them meet each other briefly prior to the task. In case there was only one participant available at that time, we used a confederate pretending to be a second participant. Instead of the "average" performance, the participants were told that they were competing against one another. In reality, there was no direct competition between both individuals. The bar atop the screen indicated how they fared against their opponent specifically, by labeling both arrows with names. Moreover, the task was manipulated so that each participant underperformed compared to their opponent.

Participants were not debriefed about the nature of the task after the first laboratory session, as doing so would likely affect their performance during the following session. In view of that, participants were told that they performed well but that their opponent was exceptionally skilled. As a consequence, they were told that on the next session, they would be paired with an opponent that better matched their skillset. We made sure that no two participants were coupled with each other twice. By changing the social context on the following session, we aimed to provide the necessary novelty factor to use the task repeatedly.

The rMIST induces stress via socio-evaluative threat, which works best if the participant is unaware of its purpose. To that effect, participants were told that they were participating in a study on mental effort where they were asked to take part in two laboratory sessions composed of a highly effortful task (i.e the stress condition) and a low effortful task. Participants in the first laboratory session did not know that they would be competing against another participant until they were asked to compete (i.e. following the control period). The competitive aspect of the stress condition was described as being necessary to force participants into engaging in greater mental effort, their performance being, therefore, a direct indicator of their effort and abilities.

2.2.2. Study 1: single-run design

Baseline measurements were taken immediately preceding the control period (*baseline*), that is 25 min ensuing their arrival; a standard time for physiology to stabilize (Petrowski et al., 2012). The testing phase was composed of a control period, followed by a 300-second break, and a stress period. Lastly, participants were asked to remain seated in the room while watching a neutral muted video (i.e. BBC documentary *Spy in the wild*) for an hour. For the purpose of this study we will focus on data from the testing phase (i.e. minute 20–25: *baseline*) to half an hour post stress (i.e. minute 80: *post+1*) (see Fig. 2). All laboratory sessions took place from 13 h to 15 h in order to minimize circadian variations in cortisol measures (Kudielka et al., 2009).

The testing phase was composed of a single-run, which means only a single stress exposure per session. One run includes 600 s of control and 600 s of stress separated by a 300-second break.

In addition, participants were given scripted negative feedback during the stress period. Feedback was framed to motivate them to perform better, similar to that used in the traditional MIST. Feedback was given four times in total for each laboratory session (see Fig. 1).

2.2.3. Study 2: multiple-run design

The testing phase consisted of a multiple-run design, each run representing one stress exposure. Here, each session included four runs of the rMIST, reflecting a standard functional magnetic resonance imaging (fMRI) design (Dedovic et al., 2005). Each 8-minute run was composed of each of the following conditions: rest (60 s), control (60 s), and stress (120 s) in that order, repeated twice. Negative feedback was given between runs. Five measures of self-reported stress were collected for each laboratory session: upon arrival (*1*: *baseline*), three times between each run of the rMIST (*2*, *3*, and *4*: *stress*), and a final sample post-stress (*5*: *post*) (see Fig. 1).

2.3. Measures

2.3.1. Perceived stress and affect

Participants completed a self-reported questionnaire seven times during each laboratory session. This questionnaire is composed of items adapted from Experience Sampling Methodology studies (Myin-Germeys et al., 2009) and that have been used in laboratory studies before (Lataster et al., 2011) to assess perceived stress (PS), and negative affect (NA). Items are scored on a 7-point Likert scale from 1- *not at all* to 7- *extremely*.

2.3.1.1. Study 1. A factor analysis identified two factors: (1) PS, composed of the items: *at the moment, I feel tense*, and *at the moment, I feel under pressure*, and (2) NA, composed of *at the moment, I feel down* and *at the moment, I feel annoyed*.

2.3.1.2. Study 2. The items *at the moment, I feel relaxed* (reversed), *at the moment, I'm under pressure* are averaged to form PS, and likewise *at the moment, I feel down* and *at the moment, I feel annoyed* form NA.

2.3.2. Heart rate and heart rate variability

Heart rate was measured continuously across both laboratory sessions. Mean heart rate (HR) and Root Mean Square of the Successive Differences (RMSSD) were computed to investigate heart rate and heart rate variability respectively. RMSSD is a time-domain measure sensitive to high frequency heart rate period, which is indicative of parasympathetic activity, and often used for short-term measurements (Shaffer and Ginsberg, 2017) and it is calculated as follows: $\mathrm{RMSSD} = \sqrt{(RRinterval_{n-1} - RRinterval_n)^2}$.

2.3.2.1. Study 1. ECG was measured using Kendall H66LG disposable electrodes (Medtronic, USA) at lead type II locations and a LabLinc Isolated Bioamplifier with Bandpas Filter (V75–04; Coulbourn). An analog lowpass filter was applied at 150 Hz. The module's analog output was sampled at 1000 Hz and digitized by a National Instruments Multifunction IO device (PCI-621 and BNC-2111; National Instruments, Austin, TX, USA). Affect 5 software was used for stimulus presentation as well as data acquisition (Spruyt et al., 2009).

ECG was preprocessed using the Kubios HRV Analysis Software (Niskanen et al., 2004). The ECG signal was cut into multiple 5-minute segments and digitized at 1000hz. All segments were manually checked, and a very low artifact correction was applied when necessary. Time-domain parameters were calculated using Kubios HRV Premium software 3.3.1 released in August 2019. Interpolation rate was set at 4 Hz, and frequency bands VLF (0–0.04 Hz), LF (0.04–0.15 Hz), and HF (0.15–0.4 Hz), with average beats per minute (HR) and RMSSD values extracted for each segment. Four segments had artifacts ≥5% and were hence excluded from the analyses.

2.3.2.2. Study 2. Heart rate was measured using 3-lead Electrocardiography (ECG) at lead type II locations. ECG signals were amplified, sampled and stored on a portable amplifier Vitaport System (Temec Instruments B.V., Kerkrade, Netherlands) (Anderson and Lyons, 2001). Mean heart rate in beats per minute (bpm) was calculated for every 20-second segment. Root Mean Square of the Successive Differences (RMSSD) was computed per run, for each condition.

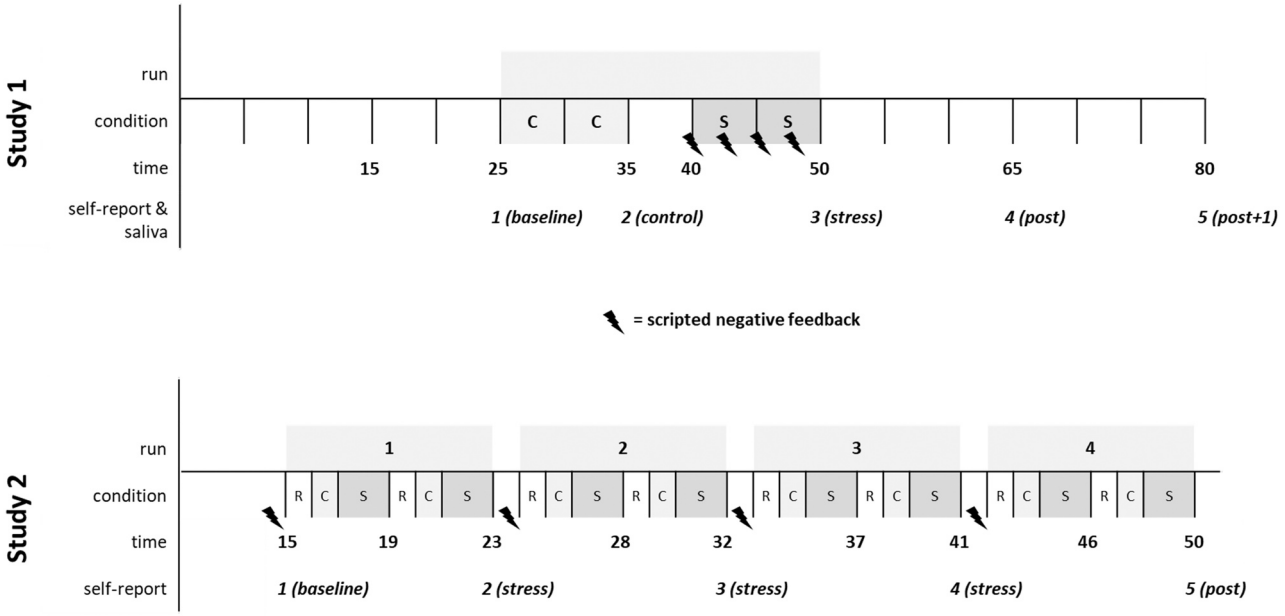


Fig. 1. rMIST single-run design in study 1 and multiple-run design in study 2, with R=rest, C=control, S= stress, with the time from arrival in minutes, the five sampling measures of self-reported stress and saliva samples, and the moments where feedback was given.

2.3.3. Salivary cortisol

2.3.3.1. Study 1. Five samples of salivary cortisol were collected during each laboratory session. A sample was collected following every self-reported stress rating questionnaire. Saliva samples were collected using cotton Salivettes (Sarstedt, Germany). Participants chewed on each sample, on the same side of their mouth, with the same intensity, for 60 s. In addition, participants were instructed not to eat or drink anything aside from water in the two hours prior to testing.

Saliva samples were analyzed at Clemens Kirschbaum's laboratory in Dresden, Germany: LabService Gmbh. Time-resolved radio-immunoassays with fluorescence detection were determined in single (Dressendörfer et al., 1992). Saliva samples were frozen and stored at −20 degrees Celsius until analysis. After thawing, Salivettes were centrifuged at 3000 rpm for 5 min, which resulted in a clear supernatant of low viscosity. Salivary concentrations were measured using commercially available chemiluminescence immunoassay with high sensitivity (IBL International, Hamburg, Germany). The intra and interassay coefficients for cortisol were both below 6%.

Salivary cortisol measurements were log transformed to correct for skewness, resulting in a normally distributed variable. Moreover, we controlled for age, gender, use of the contraceptive pill, and days since the last period. We also computed the area under the curve with respect to the ground (AUC_g) and the area under the curve in respect to increase (AUC_i) per participant per session. These are often used when addressing cortisol over time as they are understood to provide a more objective measure of reactivity than the raw values (see Pruessner, et al., 2003 for details).

2.3.3.2. Study 2. No salivary cortisol was collected.

2.4. Statistical analyses

Analyses were carried-out in STATA 13.1. First, to test hypotheses 1, that the rMIST induced a stress response, multi-level analyses with random intercepts and random slopes were conducted to test the effect of time/condition on all stress measures (i.e. PS, NA, HR, RMSSD, and cortisol). Individual data points (level 1) were nested within subjects (level 2), across both sessions of testing. Time/condition included five measurements: *baseline*, *control*, *stress*, *post*, and *post+1* for study 1, and three measurements for study 2: *baseline*, *stress*, and *post*. Analyses were run separately for every stress measure as a dependent variable. To control for age and gender, they were added to all models as covariates.

To test hypothesis 2, whether there are habituation or sensitization effects, we added an interaction term of session and time to the multi-level models. Session is composed of two levels: session 1 and session 2.

To test hypothesis 3, whether there is an anticipation effect, we ran a paired *t*-test for every stress measure comparing baseline in session 1 and session 2.

3. Results

3.1. Sample and descriptive statistics

3.1.1. Study 1

Fifty three participants took part in this study (45 women). 40% of participants were working and the remaining 60% were university students engaged in various degrees (i.e. biomedical science, history, sexology, etc.). Out of the 45 women taking part in the study, 31 were taking the contraceptive pill at the time of the study (see Table 1 for further descriptive statistics). In addition, two participants were unable to take part in the second session.

3.1.2. Study 2

Thirty students took part in this study (27 women). However, heart rate data of 20 participants from at least one of the sessions could not be collected due to a lack of available equipment. Therefore, analyses on the cardiovascular response were run only on the data from the remaining 10 participants.

Table 1
Descriptive statistics.

	Study 1				Study 2			
	N	Mean (SD)	Min	Max	N	Mean (SD)	Min	Max
Age	53	24 (3.29)	19	35	30	22 (2.09)	19	27
PS	53	2.56 (1.61)	1	7	30	3.51 (1.32)	1	6.5
NA	53	2.08 (1.27)	1	7	30	1.45 (0.77)	1	4.5
HR	53	74 (11)	47	125	10	73 (13)	51	104
RMSSD	53	49 (25)	7	174	10	52 (32)	9	160
Cortisol	52	3.29 (1.96)	.68	14.05	–	–	–	–
AUC_g	52	13.07 (7.01)	3.92	37.87	–	–	–	–
AUC_i	52	-1.81 (3.47)	-12.07	20.31	–	–	–	–

PS: perceived stress, NA: negative affect, HR: mean heart rate, RMSSD: heart rate variability, AUC_g: area under the curve in respect to the ground, AUC_i: area under the curve in respect to increase

3.2. Stress reactivity

Mixed models indicate that there is a significant effect of time on both PS and NA across both studies (see Table 2). More specifically, pairwise comparisons show a significant increase from *baseline* to *stress* (PS: B= 2.18, $p \leq .01$) (NA: B= 1.71, $p \leq .01$) as well as from *control* to *stress* (PS: B= 1.67, $p \leq .01$) (NA: B= 1.49, $p \leq .01$) in study 1, and a similar increase from *baseline* to *stress* (PS: B= 1.67, $p \leq .01$) (NA: B=0.20, $p \leq .01$) in study 2.

In addition, there is a significant effect of time on HR and RMSSD on both studies (see Table 2), with pairwise comparisons showing a significant increase in HR from *control* to *stress* in both studies (study 1: B= 3.82, $p \leq .01$; study 2: B= 2.26, $p \leq .01$), and a parallel decrease in RMSSD (study 1: B= −3.53, $p \leq .01$; study 2: B= −2.08, $p \leq .01$).

Lastly, there is a significant negative effect of time on salivary cortisol in study 1, with a significant decrease from *control* to *stress* (B= −0.07, $p \leq .01$).

Together, findings show that the rMIST induced a significant self-reported and cardiovascular stress response. However, it did not elicit a neuroendocrine response.

3.3. Repeated stress

To test hypothesis 2, whether stress reactivity was comparable across both sessions, the interaction term of session and time was added to the models (see Table 2).

There was no significant interaction between session and time for neither PS nor NA in study 1, meaning that the self-reported response to the rMIST was comparable across sessions with no indication of habituation or sensitization effects.

In contrast to study 1, there was a significant interaction effect between session and time on PS and NA in study 2. However, pairwise comparisons showed that the significance was not in the reactivity itself but in the recovery to the stressor, with participants in session two not returning to baseline following the rMIST (see Fig. 2).

There were also no significant interactions between session and time for neither HR nor RMSSD indicating that cardiovascular reactivity was comparable across both sessions for both studies (see Fig. 2).

Finally, there was no significant interaction between session and

Table 2
Results from mixed models testing stress reactivity and repeated stress.

			Study 1				Study 2			
	Dependent	Predictor	N	df	chi2	*p*	N	df	chi2	*p*
1. Stress reactivity	PS	time	53	4	588.00	≤0.01	30	2	182.94	≤0.01
	NA	time	53	4	289.59	≤0.01	30	2	6.81	.03
	HR	time	53	4	617.04	≤0.01	10	1	12.40	≤0.01
	RMSSD	time	53	4	87.47	≤0.01	10	1	1.90	0.17
	cortisol	time	53	4	60.79	≤0.01	–	–	–	–
2. Repeated stress	PS	session*time	53	4	9.25	.06	30	2	8.26	.02
	NA	session*time	53	4	2.14	.71	30	2	6.95	.03
	HR	session*time	53	4	5.70	.22	10	1	.32	.57
	RMSSD	session*time	53	4	1.20	.88	10	1	1.67	.20
	cortisol	session*time	53	4	3.35	.50	–	–	–	–

PS: perceived stress, NA: negative affect, HR: mean heart rate, and RMSSD: heart rate variability

time when added to the model with cortisol in study 1. In addition, to further compare cortisol reactivity across sessions we performed a paired *t*-test with AUC_g and AUC_i between S_1 (AUC_g: M=13.69; SD=7.91) (AUC_i: M=−1.55; SD=4.02) and S_2 (AUC_g: M=12.52; SD=6.15) (AUC_i: M=−2.11; SD=2.90) according to AUC_g $t(50) = -1.15 = 0.25$ and AUC_i $t(50) = -0.77$, $p = .45$ respectively, and found no significant difference respectively, suggesting that cortisol reactivity is also comparable across both sessions.

3.4. Anticipation

Paired *t*-tests with baseline measures were computed to test hypothesis 3, whether participants showed any signs of anticipating the stressor (see Table 3).

Paired *t*-tests with baseline measures of PS and NA were all non-significant, indicating that there were no self-reported anticipation on neither studies.

In contrast, results from the paired *t*-test conducted on the baseline measures of HR and RMSSD in study 1 found a significant difference in HR (see Fig. 2). This effect was not found in study 2.

Lastly, there was no significant difference in baseline measures of cortisol.

3.5. Post hoc analyses

Owing to the fact that the multiple-run design of the rMIST in study 2 contains four runs, and thus participants are exposed to the stressor multiple times as opposed to the single exposure from the single-run design in study 1, we repeated the analyses with run as an interaction term with session and time. Where we previously collapsed the three stress time points, we now regarded all of them. Again, there was a main effect of time on PS ($X^2(4, 30) = 185.50$, $p \leq .01$) but not on NA ($X^2(4, 30) = 7.50$, $p = .11$) indicating that PS but not NA was significantly different between runs. Adding an interaction between time and session, revealed a significant effect for both PS ($X^2(4, 30) = 13.88$, $p \leq .01$) and NA ($X^2(4, 30) = 14.33$, $p \leq .01$) indicating that PS and NA reactivity was not the same between days. However, pairwise comparisons revealed no significant difference in run 2 between session 1 and session 2 for neither PS (B = −0.32, $p = .24$) nor NA (B =0.20, $p = .23$), suggesting that the initial reactivity is the same between sessions (see Fig. 3). Instead, the interaction seemed driven by differences between sessions at time-point 5 (i.e. *post*) (see Fig. 3).

Adding run as a main effect with session and condition showed a significant effect of run on both HR ($X^2(3, 10) = 47.32$, $p \leq .01$) and RMSSD ($X^2(3, 10) = 7.70$, $p = .05$). Pairwise comparisons indicate that there is a habituation effect within sessions with smaller cardiovascular reactivity across runs during both sessions. On the other hand there was no interaction between session, condition, and run with neither HR ($X^2(3, 10) = 1.82$, $p = .61$) nor RMSSD ($X^2(3, 10) = 3.63$, $p = .30$) (see Fig. 3), meaning that the habituation effect found within sessions is comparable between sessions.

4. Discussion

The present study aimed to investigate the effectiveness of the newly developed rMIST in inducing self-reported and physiological stress over repeated trials, using a single-run and a multiple-run design. Our findings indicate that the task is able to induce a significant self-reported and cardiovascular, but not neuroendocrine stress response. These responses are largely comparable across two separate sessions.

4.1. Repeated stress

The added element of novelty in the rMIST appears to fulfill the necessary requirements to render the second session equally novel and as stressful as the first session without making any substantial change to the task itself. This can be evidenced by the self-reported and cardiovascular responses to the task that are consistent across laboratory sessions for both studies. This is in contrast to other studies that found significant habituation effects for both self-reported (Boesch et al., 2014; Jönsson et al., 2015; Schommer et al., 2003) and cardiovascular (Boesch et al., 2014; Jönsson et al., 2015; Schommer et al., 2003) measures.

In the same way there was also no sensitization effect, meaning that while the task is sufficiently stressful to engage the stress system, it is not so intense as to trigger an exacerbated response on the second session.

It should be noted that while the first run of the second study was comparable across the two sessions, post hoc analyses point to a habituation effect within the task itself, with significantly smaller increases in HR with every run. These findings are consistent with other studies that have induced the stressor repeatedly within the same session (Al'Absi et al., 1997; Schiweck et al., 2019), and underscore the significance of selecting the correct task-design. In this particular case, a shorter design with a single-run appears to be more suitable for more consistent and comparable cardiovascular reactivity across repeated sessions.

Finally, while the change in social context between sessions seems to also have largely prevented self-reported anticipation effects, it did not suffice to prevent anticipation of the cardiovascular system on the first study. Moreover, potential differences in anticipation may have been obscured by the small sample size for the cardiovascular analyses of the second study. These findings are in line with other studies that assessed anticipation to repeated stress (Boyle et al., 2016). The expectation to compete is likely to be driving the increase in the cardiovascular baseline levels we see on the second session. Consequently, in addition to the social element of the rMIST (i.e. the change in opponent), it could also prove beneficial to slightly alter the cover story so that participants only discover that they are competing again on the second session at the moment of competing. One way to achieve this is by pretending that data from the first session was poorly saved, thus requiring the participant to repeat the task once more, as was done by Schiweck et al., 2019.

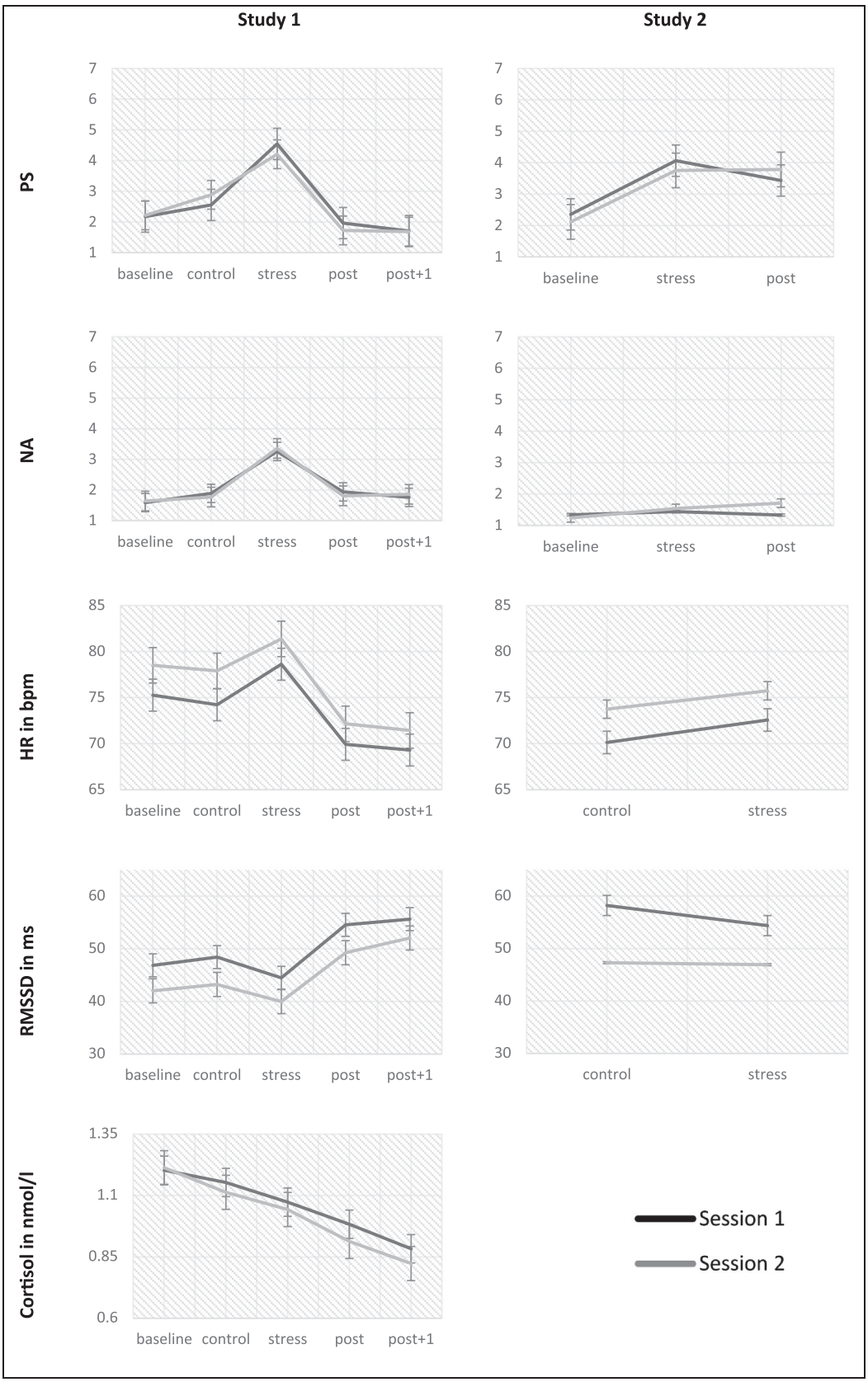


Fig. 2. Stress measures across time for session 1 and session 2 with PS: perceived stress, NA: negative affect, HR: mean heart rate, RMSSD: heart rate variability, with standard error bars.

Table 3
Paired *t*-tests with baseline measures of PS, NA, HR, RMSSD, and cortisol at S_1 and S_2.

	Study 1					Study 2				
	Session 1 Mean (SD)	Session 2 Mean (SD)	df	t	*p*	Session 1 Mean (SD)	Session 2 Mean (SD)	df	t	*p*
PS	2.14 (1.05)	2.21 (1.20)	49	.64	.52	2.35 (0.83)	2.12 (1.10)	29	-1.16	.26
NA	1.55 (0.88)	1.63 (0.86)	49	.55	.59	1.33 (0.55)	1.2 (0.55)	29	-0.71	.48
HR	74.50 (9.21)	78.20 (10.95)	40	-3.41	≤0.01	75.72 (15.33)	73.75 (14.21)	7	1.02	.34
RMSSD	49.74 (25.31)	42.98 (22.98)	40	-1.95	.06	46.21 (34.01)	40.74 (18.96)	7	-0.70	.51
Cortisol	3.83 (2.15)	3.89 (2.28)	48	.16	.87	–	–	–	–	–

PS: perceived stress, NA: negative affect, HR: mean heart rate, RMSSD: heart rate variability

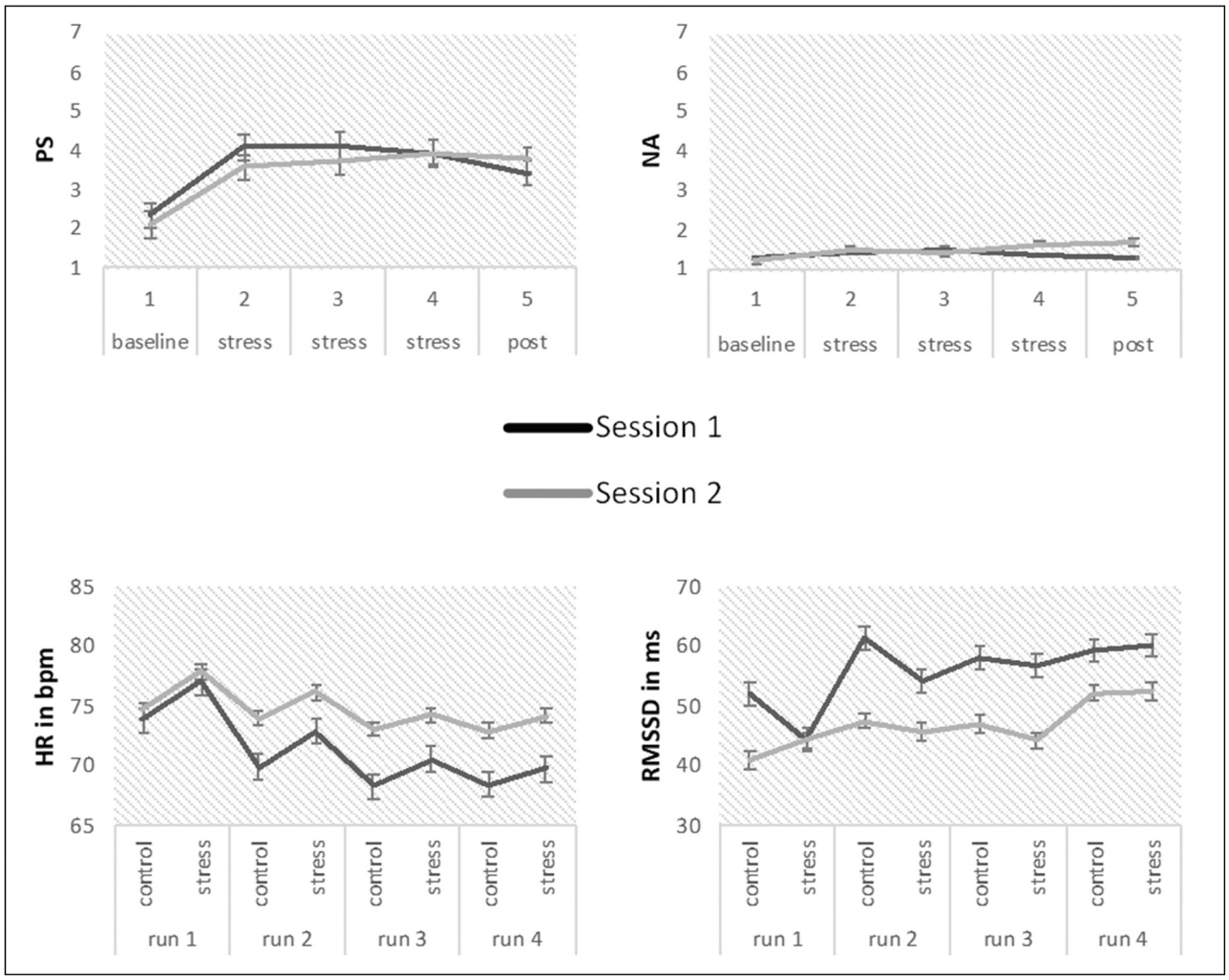


Fig. 3. Self-reported measures (perceived stress, and NA) across all five time-points and cardiovascular measures (heart rate, and RMSSD) between conditions (control and stress) per run (1,2,3, and 4) for session 1 and session 2 in study 2, with standard error bars.

4.2. Cortisol

The rMIST did not elicit a cortisol response, to the contrary, salivary cortisol levels decreased across both laboratory sessions. While several studies using the MIST have found a cortisol response (Dedovic et al., 2009; Pruessner et al., 2008), the opposite has also been reported (Chung et al., 2016; Kogler et al., 2017). Additionally, studies that do find a cortisol increase rarely find it for the group as a whole but only for a percentage of responders (Noack et al., 2019; Pruessner et al., 2008). Here, we do not report on responders and non-responders as it is beyond the scope of this study.

It is well established that cortisol is highly sensitive, with gender, childhood trauma, menstrual cycle, food intake, time of data collection, to name a few, all showing a robust effect on cortisol measurements (Kudielka et al., 2009). However, the current study design addressed the aforementioned confounders in both the design (e.g. timing of data collection, baseline time for physiology to settle, no food or drinks in the two hours preceding testing) and in the analyses (e.g. controlled for age, gender, menstrual cycle). Consequently, findings add to existing literature showing that psychosocial stressors do not consistently induce a significant neuroendocrine response.

By contrast, psychophysiological stressors are better able than psychosocial tasks to elicit a cortisol response, with approximately 60–70% compared to 30–50% of responders respectively (Noack et al., 2019), which raises the question of whether psychosocial stress tasks are unable to induce a robust neuroendocrine stress response, or whether

psychophysiological stressors are simply not comparable to the psychosocial hassles individuals encounter in their daily lives (Almeida et al., 2002) and therefore may not be ecologically valid. The latter being more probable than the former as there is sound evidence that while psychosocial stress tasks do not always induce a cortisol response, they are in fact able to do so (Dickerson and Kemeny, 2004; Peters et al., 1998).

It should be noted that there continues to be a large percentage of participants that do not qualify as cortisol responders to any type of laboratory stress task. Cortisol reactivity is complex with high or low responses affected by context, personality traits, coping to name a few. Moreover, both high and low responses can signal both good and bad clinical outcomes (Koolhaas et al., 2011; Shirtcliff et al., 2014) making the interpretation of these responses highly challenging. Hence, the question may be not whether psychophysiological tasks are more or less suited to study daily stress reactivity, but what to expect from and how to interpret the cortisol response.

4.3. Limitations

There are several important limitations in our study that need to be addressed. First, samples from both studies were relatively small and consisting mostly of healthy individuals. Therefore, results, especially from the cardiovascular response to the stressor on the second study, should be carefully considered. Cardiovascular data from a mere ten participants were of satisfactory quality to be used in the analyses. While laboratory studies where stress is induced in the laboratory often have small sample sizes, in some cases also ten (Jönsson et al., 2010), or 21 participants (von Känel et al., 2006), the current sample remains relatively small and therefore susceptible to type II error. Nevertheless, given that findings from the second study align with the first study which has a more robust sample size, we expect it to be sufficiently representative.

Finally, even though the rMIST uses a computer-based stressor and is thus easy to standardize, there remain aspects of the task that are difficult to control such as the interaction between researcher and participant. In the same way as this human factor makes it difficult to standardize the TSST (Skoluda et al., 2015), there is also the possibility that minor changes in tone or body language may have an effect on how the participant experiences the rMIST.

5. Conclusion

The rMIST is a psychosocial stress task that is able to elicit consistent and comparable self-reported and cardiovascular stress reactivity in the laboratory on repeated instances. Notably, the change in social context between sessions seems to provide the necessary novelty to prevent habituation and sensitization, but not anticipation. More specifically, the cardiovascular system shows an anticipation effect on the second session, meaning that the task must be further refined to address that effect. It is also of consequence to highlight that the rMIST may not be suitable in instances where cortisol is the measure of interest as it proved unable to elicit a significant cortisol response in the sample as a whole. Finally, task design is important. In order to maximize comparability, preference for a design with a single stress exposure should be given over a multiple-run design.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the FWO Odysseus grant G0F8416N.

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