Effects of the Paced Auditory Serial Addition Task (PASAT) with different rates on autonomic nervous system responses and self-reported levels of stress

Running head: Stress and autonomic responses to the PASAT with different rates

Original research

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Abstract

Objectives: To characterize self-reported levels of stress and autonomic responses in healthy humans evoked by different rates of the Paced Auditory Serial Addition Task (PASAT). Materials and Methods: Fifteen participants performed four PASATs with different rates (3.6, 2.4, 1.6, or 1.2 s intervals) and a control task, in random order. The correct responses and self-reported levels of stress, to the PASATs were analyzed using one-way repeated measures ANOVA. The autonomic responses were analyzed using paired t-test or one-way repeated measures ANOVA followed by Tukey HSD post-hoc test. Results: Increased PASAT rates were associated with decreases in correct responses \( P < 0.001 \) and increases in self-reported levels of stress \( P < 0.001 \). For autonomic responses, significant changes were seen in 10 variables during 2.4 s-PASAT compared with the respective baseline, however, significant differences in the relative changes from baseline were found between the 2.4 s-PASAT and the control task only for mean RR-intervals \( P < 0.001 \), systolic and diastolic blood pressure \( P = 0.002 \) and \( P = 0.006 \), and cardiac output \( P < 0.001 \). Regarding the comparison between the four PASATs, significant differences in the relative changes from baseline were seen between the 3.6 s-PASAT and the faster PASATs for e.g. mean RR-intervals, high-frequency power, and respiration rate, however, there were no differences between the faster PASATs. Conclusions: The autonomic responses during the PASATs with different rates were quite similar for the faster PASATs (intervals < 2.4 s), however, the slowest 3.6 s-PASAT evoked significantly less self-reported stress and autonomic arousal compared with the faster PASATs. Standardization of the PASAT rate may be important for
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studies on autonomic nervous system function and self-reported measures of stress. Future studies
may test more complex interactions between stress, autonomic responses and pain responses.

Keywords: autonomic nervous system, chronic pain, heart rate variability, stress, PASAT, 
temporomandibular disorders
Introduction

Stress interacts with the autonomic nervous system (ANS) and pain, including for example, orofacial pain and painful temporomandibular disorders (TMD), in a complex manner [1, 2]. For instance, an increased urinary level of norepinephrine and epinephrine, catecholaminergic neurotransmitters, released in response to stress, has been demonstrated in patients suffering from TMD [3] suggesting altered sympathetic activity in pain patients [4]. Also, some studies have indicated that experimental acute mental stress affects the ANS responses differently in TMD patients and healthy controls [5], and that additional mental stress could modify autonomic changes induced by experimental myofascial pain [6]. Therefore, it is suggested that there are mutual impacts worthy of further investigations between stress, ANS responses and pain.

As a first approach it is important to focus on the relationship between stress and ANS responses. Initially, Cannon who coined the famous terms “homeostasis” and “fight or flight” described that animals reacted to threats with a general discharge of the sympathetic nervous system. However, mental stress, which was defined first and most generically as the nonspecific response of the body to any demand by Selye [7], is variously interpreted based on each specialized field like psychology, physiology, biology, and neuroendocrinology and widely recognized as a central problem in human life. In order to deal with mental stress in a laboratory, it is necessary to establish a reliable and valid experimental model to evoke acute stress. The Paced Auditory Serial Addition Task (PASAT) is known as an effective mental arithmetic task to evoke acute stress [8]. In the task, the subjects are
continuously given auditory presentation of random numerals from one to nine with a constant rate and asked to add the last two presented numerals and answer verbally, as soon as possible, throughout the task period. However, few studies have provided detailed reports regarding the effects of the PASAT in parallel with self-reported levels of stress and the physiological aspects based on ANS responses with multiple variables. At present, it is not known what the optimal rate of the PASAT should be to induce acute stress as assessed by self-reports and trigger robust changes in ANS response. Thus, this methodological study in healthy subjects aimed to compare the PASATs with different rates in a randomized and controlled manner. We hypothesized that the faster PASAT would be associated with less correct responses, higher self-reported levels of stress, and bigger changes in the ANS responses compared with the slower PASAT.
Materials and methods

Participants

Sixteen healthy volunteers were recruited from advertising at Aarhus University campus and screened to participate in the current study. None of the participants took any medication or suffered from neurological, cardiovascular, or psychiatric disorders (self-reported). All participants had a twelve-lead electrocardiogram (ECG) taken at a screening visit. One participant was excluded due to abnormal ECG and was referred to private physician for further examination. Consequently, twelve men (mean age ± SD, 28.4 ± 4.4 years) and three women (29.0 ± 5.3 years) were included. All participants received written and oral information about the experiment before they signed an informed consent document.

The study was approved by the Central Denmark Region Committees on Biomedical Research Ethics and conducted in accordance with the guidelines of the Helsinki Declaration.

Experimental protocol

All participants took part in one experimental session consisting of five sequences. The session was conducted between 9 a.m. and 1 p.m. in a quiet room with controlled temperature (23.0 ± 1.0 °C). Participants were positioned supine on a portable bed during experimental procedures and repeatedly performed the PASAT with different rates with simultaneous recording of heart rate variability and cardio-haemodynamic changes. Correct responses and self-reported levels of stress to the tasks were also collected (Fig. 1).
The PASAT was used as the experimental task. The numeral presentation rate was set at intervals of 1.2 s, 1.6 s, 2.4 s, which were chosen from standard rates [9], and 3.6 s. That is, four PASATs with different rates were used. The participants also carried out an additional task, where they were given the same auditory presentation as the 2.4 s-PASAT and asked to repeat verbally the last presented numeral. This task was named the Paced Auditory Numeral Repetition Task (PANRT) after the PASAT.

The 2.4 s-PANRT was used as a control test against the 2.4 s-PASAT, since an influence of respiration pattern and speech on ANS changes could be ignored by comparing these results. One sequence consisted of a five-minute baseline, a five-minute task, and a five-minute recovery period, and the order of in total five sequences was randomized between participants (Fig. 1). The percentage correct responses were calculated for each task.

Self-reported measures

The participants were asked to give a score on a 0-10 numeric rating scale (NRS) of their “stress level” in response to the PASAT. The instructions were that 0 represented “no stress” and 10 “most imaginable stress”. No further explanations were provided to operationalize the concept of stress. In addition to the self-reported NRS scores of stress, the participants were asked to score “annoyance”, “difficulty” and “motivation” of the task on 0-10 NRSs with 0 meaning “not at all” and 10 meaning “most
Heart rate variability and cardio-haemodynamic measures

Heart rate and cardio-haemodynamic measures were recorded simultaneously and non-invasively with the use of a Task Force® Monitor (CNSystems, Medizintechnik AG, Austria) consisting of blood pressure meters, 3-lead ECG, and impedance cardiography. From these recordings, mean values of systolic and diastolic blood pressure (sBP, dBP), heart rate, stroke volume (SV) [10], cardiac output (CO) [11], total peripheral resistance (TPR), and respiration rate (RESP) were estimated.

For estimation of heart rate variability, raw data from the ECG was used. In order to detect and correct a false signal such as a missing beat or an ectopic beat, a custom-made software was employed [12]. The detected missing beats were corrected with interpolation. RR-intervals were defined as the distance in ms between consecutive normal R waves from the QRS complexes. The time domain analysis included the mean of all normal RR-intervals (mean RR) (ms), the standard deviation of all normal RR-intervals (SDNN) (ms), and the square root of the mean squared differences of successive normal RR-intervals (RMSSD) (ms). The frequency domain analysis derived from power spectrum included the low-frequency (0.04-0.15 Hz) power (LF-power) (ms²/Hz), the coefficient of LF component variance (CCV-LF) (%), the high-frequency (0.15-0.40 Hz) power (HF-power) (ms²/Hz), the coefficient of HF component variance (CCV-HF) (%), and the total power (ms²/Hz) [13]. The auto-regressive method was used for five-minute with a model order of 20 for the power spectrum.
Statistical analysis

The PASAT correct responses and subject-based NRS scores were analyzed with the use of one-way repeated measures analysis of variance (ANOVA) followed by Tukey HSD post-hoc test, when appropriate. In heart rate variability and cardio-haemodynamic measures, paired t-tests were used to compare each task value and the respective baseline value and to compare the values of 2.4 s-PANRT and the 2.4 s-PASAT. Also, one-way repeated measures ANOVA followed by Tukey HSD post-hoc test, when appropriate, was used for comparison of four PASATs in the task values and the relative changes from the respective baseline. Data are presented as means ± standard deviation (SD). Values of $P < 0.05$ were considered statistically significant.
Results

PASAT correct responses and subject-based NRS scores

The percentage correct responses of the PASAT decreased significantly step-by-step depending on the increases in the PASAT rate (95 ± 8, 82 ± 14, 66 ± 17, and 50 ± 14, respectively, ANOVA: $F = 122; P < 0.001$, Fig. 2). In contrast, the faster PASAT induced higher self-reported levels of stress (3.3 ± 1.4 to 7.3 ± 1.6, ANOVA: $F = 32.4; P < 0.001$), difficulty (3.3 ± 2.0 to 9.0 ± 1.1, ANOVA: $F = 82.2; P < 0.001$), and annoyance levels (2.7 ± 1.8 to 6.9 ± 2.3, ANOVA: $F = 20.0; P < 0.001$), while the motivation levels were equally high without relation to the PASAT rate (ANOVA: $P = 0.986$, Fig. 2). The 2.4 s-PANRT as a control induced significantly lower NRS scores for stress (1.2 ± 1.3, paired t-test: $P < 0.001$), difficulty (0.6 ± 0.9, paired t-test: $P < 0.001$), and annoyance level (2.2 ± 2.5, paired t-test: $P = 0.010$) compared with the 2.4 s-PASAT (5.0 ± 2.3, 5.9 ± 1.7, and 4.9 ± 2.5, respectively), and a slight difference was found in the motivation level (6.5 ± 2.0 and 7.3 ± 1.7, respectively, paired t-test: $P = 0.048$, Fig. 2).

Heart rate variability and cardio-hemodynamic measures

There was a significant difference in some variables between the five baseline values ordered in time, but no significant difference was seen between those classified based on the respective task. This result showed that a randomized manner within-subject was necessary to eliminate the time-course effects on ANS responses.

Regarding the comparison of heart rate variability measures between each task and the respective baseline, mean RR decreased significantly during the four PASATs (paired t-test: $P < 0.012$)
but did not change during the 2.4 s-PANRT. HF-power and CCV-HF increased slightly during the 3.6 s-PASAT, but there were significant decreases during the other tasks (paired t-test: $P < 0.019$ and $P < 0.023$, respectively). Total-power decreased significantly during all tasks (paired t-test: $P < 0.033$, Table 1a). For the cardio-hemodynamic measures, sBP and dBP increased significantly during the four PASATs (paired t-test: $P < 0.027$ and $P < 0.042$, respectively) but did not change during the 2.4 s-PANRT. SV and TPR did not change during any tasks. CO increased significantly during the four PASATs (paired t-test: $P < 0.009$) but decreased during the 2.4 s-PANRT (paired t-test: $P = 0.013$). RESP increased significantly during the 2.4 s-PANRT and the 2.4 s-PASAT (paired t-test: $P = 0.012$ and $P = 0.002$, respectively, Table 1b).

Regarding the comparison of heart rate variability measures, the mean RR during the 2.4 s-PASAT was significantly lower than the 2.4 s-PANRT (paired t-test: $P < 0.001$) and the same for the relatives change from baseline (paired t-test: $P < 0.001$). For RMSSD, HF-power, and total-power there were no significant differences in either absolute or relative values. For cardio-hemodynamic measures, sBP and dBP during the 2.4 s-PASAT were slightly higher than the 2.4 s-PANRT, but the relative changes from baseline were significantly larger in the 2.4 s-PASAT (paired t-test: $P = 0.002$ and $P = 0.006$, respectively). CO during the 2.4 s-PASAT was significantly higher than the 2.4 s-PANRT (paired t-test: $P < 0.001$) and the same for the relative changes (paired t-test: $P < 0.001$). For RESP, there were no differences in either absolute or relative values.

Finally, regarding the comparison of heart rate variability measures between the four PASATs,
for mean RR, the relative changes from baseline in the 2.4 s-, 1.6 s- and 1.2 s-PASAT were significantly larger than the 3.6 s-PASAT (Tukey test: $P = 0.004$, $P < 0.001$, and $P = 0.004$, respectively).

For RMSSD, the relative changes in the 1.6 s- and 1.2 s-PASAT were significantly larger than the 3.6 s-PASAT (Tukey test: $P = 0.02$ and $P = 0.011$, respectively). For HF-power and CCV-HF, the relative changes in the 2.4 s-, 1.6 s-, and 1.2 s-PASAT were significantly different compared to the 3.6 s-PASAT (Tukey test: $P < 0.012$, $P < 0.007$, and $P < 0.005$, respectively) and furthermore, only the 3.6 s-PASAT was associated with positive changes. For the total-power, there were no significant differences in either absolute or relative values (Table 1a). Cardio-hemodynamic measures, sBP, dBP, and SV showed no significant differences in either absolute or relative values. For CO, the relative changes in the 3.6 s-PASAT were significantly smaller than the 1.6 s-PASAT (Tukey test: $P = 0.033$). RESP in the 2.4 s-PASAT was significantly higher than the other PASATs (Tukey test: $P < 0.001$) and the relative changes revealed that only the 3.6 s-PASAT was associated with a negative change (Table 1b).
Discussion

The main findings in the present study were that the rate of the PASAT differentially influenced the correct responses of the tasks and the subject-based scores of stress, as hypothesized, but did not affect the ANS responses substantially. The faster PASAT resulted in less correct responses and higher levels of self-reported stress, however, there were few differences in the ANS changes between the 2.4 s- and 1.6 s- and 1.2 s-PASAT even though the slowest 3.6 s-PASAT evoked the least substantial changes compared to the faster PASATs. It can be speculated that because the self-reports were done immediately after each task, the participants set the NRS scores by comparing with the previous tasks, unlike with the physiological reaction of ANS responses measured in real time during the task. Overall, it could be argued that our results demonstrated a discordance between self-reported measures of stress and physiological measures of the ANS. At least, these findings demonstrate that the PASAT rate is important to standardize when the dynamics of the ANS is tested in relation to self-reported measures of stress.

Our results are in accordance with a previous finding that heart rate and blood pressure values were relatively constant across the four different rates of the PASAT (2.4, 2.0, 1.6, or 1.2 s intervals) [16]. The present study, however, gave important new information by using the 2.4 s-PANRT and the 3.6 s-PASAT in addition. It is suggested that the significant differences between the 2.4 s-PANRT and the 2.4 s-PASAT, seen for mean RR, BP, and CO in terms of the relative changes from baseline, were caused by a pure task difference, that is, the latter requires significant cognitive processing which affects psychological state (i.e. higher NRS scores of stress) which then is associated
with significant ANS responses. Importantly, the applied study design and inclusion of the control task (PANRT) leaves out the possible the observed changes in ANS responses simply could be related to speech and/or respiratory effects. It is also suggested that there may be some correlations between RESP and HF-power, CCV-HF, and total-power, since there were significant differences in the variables during both the 2.4 s-PANRT and 2.4 s-PASAT, but no difference was seen in the relative changes. This point is supported by a previous study [17], and the same effect is also indicated in other studies using mental stress tasks without speech [18]. The 3.6 s-PASAT may be useful as a less stressful experimental task than the other faster PASATs judged from the results of both the self-reported levels and ANS responses. Meanwhile, we acknowledge that the individual math ability or IQ level could represent a selection bias in PASAT studies. It could be important to control for such factors in future studies in addition to the presentation rate of the PASAT when self-reported levels of stress and ANS responses are examined [8]. Finally, a small increase in HF-power and CCV-HF during the 3.6 s-PASAT may be attributed to a decreased RESP which was also likely caused by the regularly paced speech [19].

Limitations and strengths

Some studies have shown a high prevalence of sympathetic nervous system disturbances in chronic pain patients [20]. Moreover, studies have indicated complex interactions between the altered or enhanced sympathetic responses to life-relevant physical or psychological stressors and altered pain
perception in patients with chronic pain [21]. The present study was designed to investigate the direct impact of different rates of the PASAT on self-reported levels of stress and ANS function in healthy subjects. However, there are a few limitations in the present study. First, while the RESP was measured, ventilation volume which is also known to affect HF-power [17] was not measured. Second, selection bias of the participants has to be considered (see above) and may limit the generalizability of the findings. In the current study, the participants reported high levels of motivation to perform the PASAT, even though they also reported relatively high levels of stress at the same moment, suggesting that the PASAT produced a “fight” instinct rather than a “flight” instinct. It should also be noted that self-reports of stress may, to some extent, be reflected in the self-reports of difficulty (Fig. 2).

The strengths of the present study were first of all that we included the 2.4 s-PANRT as a control task and used highly standardized dependent variables and a custom-made program to eliminate any artifacts on the heart rate variability measures. The current statistical method using comparison of the relative changes can be considered as another strength because the slight differences between baselines were entirely avoided. The subtle changes in baseline ANS responses are important to control for in the study design. Furthermore, a within-subject design is better suited for the detection of changes induced by the rate difference of the PASAT than a between-subjects design because of higher between-subject variability than within-subject variability. Overall, we believe that the current study has provided valid and important new data in the field of ANS responses and self-reported measures of stress.
Conclusions

Standardization of the PASAT rate may be important for studies on autonomic nervous system function and self-reported measures of stress. Future studies may test more complex interactions between stress, autonomic responses and pain responses, for example, in TMD pain patients.

Acknowledgements

Informed consent was obtained from each participant and the experimental protocol followed the Helsinki Declaration and had been approved by the local ethics committees. None of the authors have any conflicts of interest associated with this study. No research fund has supported this study.
References


[16] Mathias CW, Stanford MS, Houston RJ. The physiological experience of the Paced


### Table 1

#### a. Heart rate variability

<table>
<thead>
<tr>
<th></th>
<th>2.4 s-PANRT</th>
<th>2.4 s-PASAT</th>
<th>P-value¹</th>
<th>3.6 s-PASAT</th>
<th>2.4 s-PASAT</th>
<th>1.6 s-PASAT</th>
<th>1.2 s-PASAT</th>
<th>P-value²</th>
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</thead>
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<td>Mean RR</td>
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<tr>
<td>Absolute value (ms)</td>
<td>933 (100)</td>
<td>830 (102)</td>
<td>&lt;0.001</td>
<td>875 (90)</td>
<td>830 (102)*</td>
<td>828 (124)*</td>
<td>842 (99)</td>
<td>0.024</td>
</tr>
<tr>
<td>Relative change (%)</td>
<td>0.6 (2.7)</td>
<td>-10.1 (7.2)</td>
<td>&lt;0.001</td>
<td>-5.1 (7.1)</td>
<td>-10.1 (7.2)*</td>
<td>-11.3 (8.6)**</td>
<td>-10.1 (8.6)*</td>
<td>&lt;0.001</td>
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<tr>
<td>P-value¹</td>
<td>n.s.</td>
<td>&lt;0.001</td>
<td></td>
<td>0.012</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>SDNN</td>
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<tr>
<td>Absolute value (ms)</td>
<td>54 (15)</td>
<td>58 (17)</td>
<td>n.s.</td>
<td>57 (15)</td>
<td>58 (17)</td>
<td>53 (14)</td>
<td>53 (11)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Relative change (%)</td>
<td>-17.4 (24.3)</td>
<td>-5.7 (29.6)</td>
<td>n.s.</td>
<td>-3.9 (28.1)</td>
<td>-5.7 (29.6)</td>
<td>-15.8 (26.8)</td>
<td>-22.0 (21.6)</td>
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<td>P-value¹</td>
<td>0.017</td>
<td>n.s.</td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.031</td>
<td>0.003</td>
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<td>RMSSD</td>
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<tr>
<td>measured value (ms²Hz⁻¹)</td>
<td>41 (15)</td>
<td>37 (13)</td>
<td>n.s.</td>
<td>40 (12)</td>
<td>37 (13)</td>
<td>33 (12)</td>
<td>36 (13)</td>
<td>n.s.</td>
</tr>
<tr>
<td>change ratio from baseline (%)</td>
<td>-10.9 (25.4)</td>
<td>-15.9 (32.1)</td>
<td>n.s.</td>
<td>-4.8 (23.2)</td>
<td>-15.9 (32.1)</td>
<td>-21.7 (28.0)*</td>
<td>-22.9 (26.6)*</td>
<td>0.008</td>
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<tr>
<td>P-value¹</td>
<td>n.s.</td>
<td>0.038</td>
<td></td>
<td>n.s.</td>
<td>0.038</td>
<td>0.009</td>
<td>0.005</td>
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<td>LF-power</td>
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<tr>
<td>measured value (ms²Hz⁻¹)</td>
<td>1015 (672)</td>
<td>722 (333)</td>
<td>0.045</td>
<td>760 (429)</td>
<td>722 (333)</td>
<td>778 (406)</td>
<td>812 (241)</td>
<td>n.s.</td>
</tr>
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<td>change ratio from baseline (%)</td>
<td>-25.3 (50.1)</td>
<td>-5.6 (92.2)</td>
<td>n.s.</td>
<td>-20.2 (46.2)</td>
<td>-5.6 (92.2)</td>
<td>1.3 (85.3)</td>
<td>-5.5 (96.6)</td>
<td>n.s.</td>
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<tr>
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<td>0.017</td>
<td>0.03</td>
<td></td>
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<td>0.03</td>
<td>n.s.</td>
<td>0.02</td>
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<tr>
<td>measured value (%)</td>
<td>3.3 (1.2)</td>
<td>3.2 (0.8)</td>
<td>n.s.</td>
<td>3.1 (0.9)</td>
<td>3.2 (0.8)</td>
<td>3.3 (0.7)</td>
<td>3.4 (0.5)</td>
<td>n.s.</td>
</tr>
<tr>
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<td>-17.9 (26.4)</td>
<td>-2.3 (41.9)</td>
<td>n.s.</td>
<td>-9.7 (29.8)</td>
<td>-2.3 (41.9)</td>
<td>4.3 (44.3)</td>
<td>-1.1 (46.7)</td>
<td>n.s.</td>
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<tr>
<td>P-value¹</td>
<td>0.014</td>
<td>n.s.</td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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<tr>
<td>measured value (ms²Hz⁻¹)</td>
<td>457 (398)</td>
<td>450 (331)</td>
<td>n.s.</td>
<td>663 (450)</td>
<td>450 (331)*</td>
<td>400 (261)*</td>
<td>455 (316)</td>
<td>0.009</td>
</tr>
<tr>
<td>change ratio from baseline (%)</td>
<td>-38.6 (46.7)</td>
<td>-33.9 (52.0)</td>
<td>n.s.</td>
<td>4.3 (72.7)</td>
<td>-33.9 (52.0)*</td>
<td>-38.0 (40.6)*</td>
<td>-37.0 (50.5)*</td>
<td>&lt;0.001</td>
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<tr>
<td>P-value¹</td>
<td>0.019</td>
<td>0.017</td>
<td></td>
<td>n.s.</td>
<td>0.017</td>
<td>0.005</td>
<td>0.004</td>
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<tr>
<td>CCV-HF</td>
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<tr>
<td>measured value (%)</td>
<td>2.2 (0.9)</td>
<td>2.4 (0.8)</td>
<td>0.034</td>
<td>2.8 (1.0)</td>
<td>2.4 (0.8)</td>
<td>2.3 (0.7)*</td>
<td>2.4 (0.7)</td>
<td>0.009</td>
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<tr>
<td>change ratio from baseline (%)</td>
<td>-26.5 (27.8)</td>
<td>-16.3 (30.6)</td>
<td>n.s.</td>
<td>2.2 (28.3)</td>
<td>-16.3 (30.6)*</td>
<td>-17.3 (25.4)*</td>
<td>-18.0 (27.0)*</td>
<td>0.002</td>
</tr>
<tr>
<td>P-value¹</td>
<td>0.004</td>
<td>0.023</td>
<td></td>
<td>n.s.</td>
<td>0.023</td>
<td>0.011</td>
<td>0.01</td>
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</tr>
<tr>
<td>Total-power</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>measured value (ms²Hz⁻¹)</td>
<td>1650 (1040)</td>
<td>1333 (679)</td>
<td>n.s.</td>
<td>1536 (760)</td>
<td>1333 (679)</td>
<td>1287 (668)</td>
<td>1398 (496)</td>
<td>n.s.</td>
</tr>
<tr>
<td>change ratio from baseline (%)</td>
<td>-30.0 (41.9)</td>
<td>-26.7 (48.9)</td>
<td>n.s.</td>
<td>-21.0 (31.2)</td>
<td>-26.7 (48.9)</td>
<td>-24.8 (48.8)</td>
<td>-33.8 (38.5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>P-value¹</td>
<td>0.009</td>
<td>0.019</td>
<td></td>
<td>0.017</td>
<td>0.019</td>
<td>0.033</td>
<td>0.008</td>
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b. Cardio-haemodynamics

<table>
<thead>
<tr>
<th>n = 15</th>
<th>2.4 s-PANRT</th>
<th>2.4 s-PASAT</th>
<th>P-value&lt;sup&gt;2&lt;/sup&gt;</th>
<th>3.6 s-PASAT</th>
<th>2.4 s-PASAT</th>
<th>1.6 s-PASAT</th>
<th>1.2 s-PASAT</th>
<th>P-value&lt;sup&gt;3&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>sBP</td>
<td>measured value (mmHg)</td>
<td>113 (15)</td>
<td>116 (14)</td>
<td>n.s.</td>
<td>114 (16)</td>
<td>116 (14)</td>
<td>116 (12)</td>
<td>118 (14)</td>
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<tr>
<td></td>
<td>change ratio from baseline (%)</td>
<td>0.1 (2.9)</td>
<td>7.2 (7.5)</td>
<td>0.002</td>
<td>3.5 (5.5)</td>
<td>7.2 (7.5)</td>
<td>5.7 (8.1)</td>
<td>4.5 (5.8)</td>
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<tr>
<td></td>
<td>P-value&lt;sup&gt;1&lt;/sup&gt;</td>
<td>n.s.</td>
<td>0.002</td>
<td>0.027</td>
<td>0.002</td>
<td>0.006</td>
<td>0.017</td>
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<tr>
<td>dBP</td>
<td>measured value (mmHg)</td>
<td>70 (10)</td>
<td>73 (10)</td>
<td>n.s.</td>
<td>73 (12)</td>
<td>73 (10)</td>
<td>75 (10)</td>
<td>77 (11)</td>
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<tr>
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<td>change ratio from baseline (%)</td>
<td>-0.5 (3.7)</td>
<td>7.6 (8.9)</td>
<td>0.006</td>
<td>4.5 (7.6)</td>
<td>7.6 (8.9)</td>
<td>8.7 (10.7)</td>
<td>7.9 (7.9)</td>
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<tr>
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<td>P-value&lt;sup&gt;1&lt;/sup&gt;</td>
<td>n.s.</td>
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<td>0.042</td>
<td>0.004</td>
<td>0.002</td>
<td>0.002</td>
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<tr>
<td>SV</td>
<td>measured value (ml)</td>
<td>100 (18)</td>
<td>103 (20)</td>
<td>n.s.</td>
<td>100 (18)</td>
<td>103 (20)</td>
<td>102 (20)</td>
<td>98 (17)</td>
</tr>
<tr>
<td></td>
<td>change ratio from baseline (%)</td>
<td>-1.5 (4.7)</td>
<td>2.2 (8.8)</td>
<td>n.s.</td>
<td>0.7 (5.7)</td>
<td>2.2 (8.8)</td>
<td>0.8 (7.5)</td>
<td>-0.4 (6.7)</td>
</tr>
<tr>
<td></td>
<td>P-value&lt;sup&gt;1&lt;/sup&gt;</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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<tr>
<td>CO</td>
<td>measured value (l/min)</td>
<td>6.5 (1.3)</td>
<td>7.6 (1.8)</td>
<td>&lt;0.001</td>
<td>6.9 (1.3)</td>
<td>7.6 (1.8)</td>
<td>7.6 (2.1)</td>
<td>7.1 (1.7)</td>
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<tr>
<td></td>
<td>change ratio from baseline (%)</td>
<td>-2.3 (3.4)</td>
<td>13.8 (7.8)</td>
<td>&lt;0.001</td>
<td>6.4 (6.8)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>13.8 (7.8)</td>
<td>14.5 (13.3)</td>
<td>11.5 (13.0)</td>
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<tr>
<td></td>
<td>P-value&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.013</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>0.009</td>
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<tr>
<td>TPR</td>
<td>measured value (dyne*s/cm&lt;sup&gt;5&lt;/sup&gt;)</td>
<td>1081 (275)</td>
<td>970 (239)</td>
<td>0.004</td>
<td>1045 (265)</td>
<td>970 (239)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>992 (238)</td>
<td>1062 (218)</td>
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<tr>
<td></td>
<td>change ratio from baseline (%)</td>
<td>1.9 (5.4)</td>
<td>-4.1 (8.2)</td>
<td>0.018</td>
<td>-1.3 (5.2)</td>
<td>-4.1 (8.2)</td>
<td>-4.1 (13.1)</td>
<td>-2.7 (10.7)</td>
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<tr>
<td></td>
<td>P-value&lt;sup&gt;1&lt;/sup&gt;</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
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<tr>
<td>RESP</td>
<td>measured value (tpm)</td>
<td>20.0 (1.8)</td>
<td>20.2 (1.3)</td>
<td>n.s.</td>
<td>17.2 (0.9)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>20.2 (1.3)</td>
<td>18.5 (0.8)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>18.4 (0.8)&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>change ratio from baseline (%)</td>
<td>13.1 (18.6)</td>
<td>13.3 (13.6)</td>
<td>n.s.</td>
<td>-4.9 (10.0)&lt;sup&gt;8&lt;/sup&gt;</td>
<td>13.3 (13.6)</td>
<td>4.9 (14.4)</td>
<td>5.7 (17.5)</td>
</tr>
<tr>
<td></td>
<td>P-value&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.012</td>
<td>0.002</td>
<td>n.s.</td>
<td>0.002</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
</tbody>
</table>
Legends

Table 1
ANS responses
a. Heart rate variability
b. Cardio-haemodynamics
Values are expressed as means ± SD (n = 15).
P-value¹: Paired t-test (each measured value was compared to the respective baseline).
P-value²: Paired t-test.
P-value³: One-way repeated measures ANOVA with Tukey HSD post hoc test.
*: compared with 3.6 s-PASAT
†: compared with 2.4 s-PASAT
¶: compared with 1.6 s-PASAT
§: compared with 1.2 s-PASAT
Single sign: P < 0.050, Double sign: P < 0.001.

Fig. 1
Experimental design
¹) Numeric rating scale (NRS): stress, difficulty, annoyance, and motivation level.
²) The order of the tasks was randomized.

Fig. 2
Means ± SD of the PASAT correct responses and subject-based scores (n = 15)
NRS: 0 = not at all, 10 = most imaginable.
*P < 0.050, **P < 0.001.