

# Heart rate variability and cortisol responses during attentional and working memory tasks in naval cadets

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## ABSTRACT

The aim of the paper was to study the relationship between heart rate variability (HRV) and cortisol release during cognitive challenging tasks. Forty-nine male naval cadets from the Royal Norwegian Naval Academy were administered computerised versions of attentional and working memory tests. The results from this study support the hypothesis of a negative correlation between HRV and cortisol secretion during cognitive tasks. Confirmation of the hypothesis with the low HRV group scoring higher on cortisol only during performance of cognitive tasks and recovery was also found. Furthermore, a replication of the previous findings of a negative association between cortisol levels and performance were supported when using uncorrected comparisons. None of the correlations survived Bonferroni corrections. The findings are discussed in relation to factors increasing HRV, thus improving tolerance to cognitive stress in onboard environments.

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**Key words:** heart rate variability, cortisol, cognitive tasks

## INTRODUCTION

The increased complexity of ship-handling has resulted in new challenges for modern seafarers. Advanced technology combined with a “lean manning principle” and high operational tempo results in vulnerability for safety violation. Although the technological development is fuelled by the need for tools in order to increase safety, operation with complex systems in acute stress situations often puts an additional demand on the sailors, resulting in human cognition as the most critical factor. The development of new technology provides the sailor with an increased amount of information, taxing the information processing capabilities such as vigilance and working memory. However, the human cognitive abilities are still the same. A consequence of

this paradox is the need for knowledge about individual factors involved in enhancing human performance in situations taxing attention and working memory, in order to improve safety and health in maritime operations.

Two stress response systems that have received increasing attention in the understanding of organism responses to stress are the cardiovascular and the endocrine systems. Insufficient information processing and poor stress tolerance can lead to different forms of stress reaction, such as overstimulation of neuroendocrinologically regulated hormones [1]. The glucocorticoid hormones are released into the bloodstream in order to counteract stressors to the organism. These hormones are thought of as being essential to survival during emergencies [2]. Cortisol, as one of these hormones, is thought of as a major human

stress response. Cortisol has consistently been shown to be released to the bloodstream, via the hypothalamopituitary-adrenal (HPA)-axis, as a result of external stressors that produce pain or discomfort, internal homeostatic disturbances and learned association (i.e. psychological stress [3]). The HPA response as a function of psychological stress has been tied in to forebrain pathways of both animal brains [4] and the human brain [3]. HPA responses are mediated by projections to the paraventricular nucleus of the hypothalamus. These projections are rooted in the central nucleus of the amygdala and relayed via the bed nucleus of the Stria Terminalis. It also requires the presence of projections from the midbrain dorsal raphe nucleus and brainstem catecholaminergic projections to the paraventricular nucleus.

The central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus are also thought of as being core structures in a prefrontal system involved in goal-directed behaviour and adaptation. This system is referred to as the Central Autonomic Network (CAN [5]). Structurally, this network includes the anterior cingulate; insular, orbitofrontal, and ventromedial prefrontal cortices (vmPFC); the central nucleus of amygdala; the paraventricular and related nuclei of the hypothalamus; the periaqueductal grey matter; the parabrachial nucleus; the nucleus of the solitary tract; the nucleus ambiguus; the ventrolateral medulla; the ventro medial medulla; and the medullary tegmental field. These components are reciprocally inter-connected. Functionally, the CAN is an integrated component of an internal regulation system through which the brain controls visceromotor, neuroendocrine, and behavioural responses that are critical for goal-directed behaviour, adaptability, and health [5]. In a series of studies, Thayer and co-workers (see [6] and [7] for an overview) tied the CAN to the autonomically mediated heart rate variability (HRV), which is viewed as an index of neurovisceral integration and the organism's self-regulatory ability. HRV has, in a series of studies, been tied to emotion and health [8] as well as prefrontal cognitive functions [9-11]. HRV has been related to activity of the prefrontal cortex [12], especially the vmPFC [13], and the prefrontal cortex has been inversely associated with activity in subcortical structures such as the amygdala [14]. Thus, previous research has shown that the activity of the prefrontal cortex can be indexed by measures of HRV. Furthermore, prefrontal cortical activity has been shown to be inversely related to activity of the amygdala particularly during stress.

Cortisol has also been linked to cognitive function. Studies have shown secretion of stress-related hormones during cognitive challenging tasks. Arnsten and Goldman-Rakic [15] showed that catecholamines were released into the bloodstream as a function of subjects responding to work-

ing memory tasks. Interestingly, these tasks were taxing prefrontal cortical functions. The conclusion of the Arnsten and Goldman-Rakic study [15] was that catecholamine modulated prefrontal functioning. However, fewer studies have focused on cortisol release as a function of both attentional and working memory thus increasing the cognitive load and challenge of the tasks. Some studies have shown an inverse relationship between cortisol levels and performance, i.e. higher levels of cortisol are associated with poorer performance on cognitive tests. Al'Absi et al. [16] examined the relationship between stress cortisol responses and performance during tasks that taxed working memory. They found that subjects with high cortisol response, compared to subjects with low cortisol response, made more errors and completed fewer mental arithmetic tasks than low reactors did. They argued that one possible explanation for their results could be the feedback hypothesis. There are abundant receptors for cortisol in the frontal and cingulate cortex, nucleus accumbens, bed nucleus of stria terminalis, and amygdala. All these areas may influence attention to external cues and memory performance. Long-term stress or consequent elevation of cortisol has an effect on these systems. One example of this is that the hippocampus is a major site of feedback regulation of cortisol secretion. Cortisol exposure reduces development of hippocampal cells and increases rates of cell death. Thus, there is evidence that stress levels of cortisol may impair working memory, as suggested by Lupien et al. [17].

Several studies have now reported inverse relationships between HRV and cortisol responses to stressors. For example, Looser et al. [1] examined the relationship between HRV and cortisol response in a sample of critical-care personnel. They found that during low levels of stress HRV and concomitant cortisol responses were largely uncorrelated. However, when the stress was more extreme, a significant inverse relationship was found between HRV and concomitant cortisol response. These results are consistent with the commonly reported inverse association between vmPFC activity and amygdala activity during stress (e.g. [14]).

Importantly, Weber et al. [18] found that resting HRV was associated with cortisol recovery from a mental stressor. Specifically, persons with high HRV showed cortisol levels had returned to baseline levels 20 min after the stressor whereas those with low HRV did not recover to baseline levels until one hour after the stressor. Thus, these findings suggest that HRV is associated with producing context-appropriate cortisol responses in the service of goal-directed behaviour.

The primary aim of the present study was to replicate and extend prior research on the relationship between individual differences in HRV and cortisol response. We hypoth-

esised that HRV would be inversely associated with cortisol response to mental challenge and subsequent recovery. In addition, we sought to replicate prior findings of a relationship between cortisol response and cognitive performance. Thus, we hypothesised that cortisol levels would be inversely associated with cognitive performance.

## METHODS

### SUBJECTS

Forty-nine male naval cadets, with a mean age of 23.3 years (range 18–34 years), from the Royal Norwegian Naval Academy participated in this study. Missing cortisol data caused variations in degrees of freedom.

### APPARATUS AND STIMULI

Three cognitive tests were presented using Micro Experimental Laboratory (second version [19]) installed on a Fujitsu Life Book with 10 × 7.5 inch screen. The tests were: a computerised version of a Working Memory Task (WMT), Continuous Performance Task (CPT), and a pop-up attentional task. As a WMT a modified version of a working memory test developed by Hugdahl et al. [20] based on Baddeley and Hitch's research [21] was chosen. The test consisted of a continuous flow of digits, and subjects were asked to detect identical digits to the one that was presented two trials previously (two-back task). The stimuli were numbers from 1 to 9. As a CPT, the California Computerized Assessment Package, Abbreviated version (CalCAP; Norland Software, Los Angeles, Calif.) was chosen. CalCAP is recognised as a test of sustained attention and consisted of four subtests, two with non-executive components (simple reaction time and response latencies to specific stimuli components) and two with executive components (detection of identical stimuli (Serial Pattern Matching 1; SPM1) and a simple addition task (Serial Pattern Matching 2; SPM2). The test was self-explanatory and needed only minimal supervision by the investigator. The pop-out attention test measured several shifts of attention and reaction time [22]. The test was based on actively searching for a target. During the pop-out attentional task, the subjects were asked to detect deviances from a background of the letter E scattered across the computer screen. The deviances were the letters F, L, I, and S [22].

Cardiovascular responses were registered using an Ambulatory Monitoring System (AMS [23]). The cardiac responses were measured with 8 mm Ag/AgCl ECG electrodes (Cleartrode, Disposable Pregelled Electrodes, 150, Standard Silver). One electrode was placed over the jugular notch of the sternum, between the collarbones, another was placed 4 cm under the left breast between the ribs, and the third electrode was placed at the right lateral side between the two lower ribs.

### PROCEDURE

All subjects were tested between 10.00 and 12.00 a.m. in groups of 4 to 6 subjects. All testing was performed in a classroom on a military base. This is a typical size of expert teams in the Royal Norwegian Navy. All groups arrived at the experimental room at the same time every day. Before the start of the experiment the subjects read and signed an informed consent statement. They were informed about their rights to leave the experiment at any time. No subject withdrew from the experiment.

After the AMS system was placed on the subjects, HRV was recorded during five minutes of baseline, cognitive tests, and five minutes of recovery. Heart rate variability was measured as the root mean of the squared successive differences (RMSSD) and averaged over the recording periods. Each R-wave-to-R-wave inter-beat interval in the selected period was used to calculate the average heart rate and the RMSSD.

The cognitive tests were administered in randomized order. Before presenting the tasks the subjects were instructed to focus on the computer and respond to the target stimuli by depressing the spacebar of the computer using their dominant hand.

Cortisol was sampled during the morning, before the subjects had breakfast, 15 min before the tests, immediately after each test, and 15 min after the last test. In addition, cortisol was sampled during the evening. Half of the subjects gave saliva samples during the evening before the test, and half the subjects during the evening after the test. Analyses of saliva cortisol was performed by using the Coat-a-Count radioimmunoassay kit from DPC (Diagnostic Products Corp. Los Angeles, CA) with an intra-assay variation of CV% = 7.8 and an inter-assay variation of CV% = 12.3. The cortisol data are presented as nmol/L.

The subjects were assigned into two groups; High HRV and Low HRV. This was based on the median split of the mean HRV over all conditions. The groups resulted in 24 subjects in the Low HRV group and 25 subjects in the High HRV group.

Performance on the cognitive tests was measured as manual reaction time (in milliseconds), number of correct responses, and number of errors.

### DESIGN AND STATISTICS

A 2 × 7 factorial design (high vs. low HRV × morning vs. baseline vs. pop-out vs. calcap vs. two-back vs. recovery vs. evening recordings) was used. The two-way ANOVA was followed up by Fisher LSD-tests. In addition, both correlational and ANOVA were performed collapsing cortisol measures from all test conditions, resulting in a 2 (Low vs. High HRV) × 5 (morning vs. baseline vs. cognitive tests vs. recovery vs. evening) design. Pearson product-moment correlations were

performed between the HRV and cortisol recordings. One-tailed tests were used because of the direct hypothesis of the negative relation between HRV and cortisol recordings [24, 25]. Two-tailed tests were used in other comparisons. Correlational analyses comparing cortisol levels with performance on cognitive tests were Bonferoni corrected due to the large number of comparisons. Both uncorrected and corrected results are presented.

## RESULTS

### GROUP DIFFERENCES

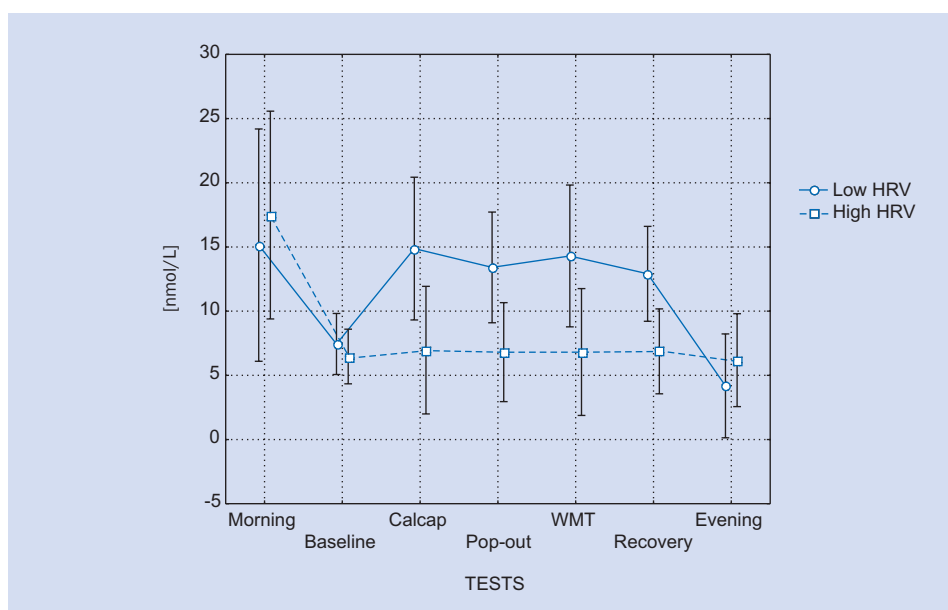
A main effect of conditions was found,  $F(1,6) = 6.58$ ,  $p < 0.01$ . Follow-up LSD test revealed higher cortisol levels in the morning compared to all other comparisons (all  $p$ 's  $< 0.01$ ). Furthermore, higher cortisol levels were found for all the tests as well as the recovery recordings, compared to the evening recording (all  $p$ 's  $< 0.03$ ). The interaction of group by condition was also significant,  $F(1,6) = 2.73$ ,  $p < 0.02$ . LSD test revealed higher cortisol levels in the Low compared to the High HRV group on all cognitive tests, as well as on recovery (all  $p$ 's  $< 0.03$ ). No other between-group differences were found (Fig. 1). An increase in salivary cortisol from baseline recordings to all tests (all  $p$ 's  $< 0.04$ ) was found only in the Low HRV group (Fig. 1).

An identical pattern emerged when collapsing the cortisol recordings from the three tests. A main effect of condition was found,  $F(1,4) = 8.88$ ,  $p < 0.01$ . LSD test showed that cortisol levels during the morning were higher than all

other conditions (all  $p$ 's  $< 0.01$ ). Both the test condition and recovery revealed higher cortisol levels compared to the evening recordings (both  $p$ 's  $< 0.04$ ). The interaction of group by condition was significant,  $F(1,4) = 2.44$ ,  $p < 0.05$ . A follow-up of this effect showed higher cortisol levels during test and recovery for the Low compared the High HRV group ( $p < 0.01$  and  $p < 0.04$ , respectively). No other differences were found.

### CORRELATIONAL ANALYSES

Baseline RMSSD correlated negatively with cortisol in response to the tasks ( $r = -0.35$ ,  $p < 0.04$ ,  $n = 35$ ). Baseline recordings of HRV correlated negatively with cortisol recordings after calcap ( $p < 0.04$ ), pop-out attention ( $p < 0.04$ , one-tailed), and recovery ( $p < 0.04$ , one-tailed) as well as the mean cortisol recordings over the tests ( $p < 0.04$ ). A borderline negative correlation was found between baseline HRV and cortisol registered after the two-back test ( $p < 0.07$ , one-tailed). Table 1 describes the details of the correlational analyses. The same pattern was revealed for correlation between HRV recorded during the calcap test and cortisol recordings. Significant negative correlations were found with cortisol recordings after the calcap test ( $p < 0.05$ , one-tailed), pop-out test ( $p < 0.03$ , one-tailed), recovery ( $p < 0.05$ , one-tailed), and the mean cortisol recordings after the tests ( $p < 0.03$ , one-tailed). In addition, a positive correlation was found on cortisol recordings during the evening ( $p < 0.03$ ). HRV during pop-out attention correlated negatively with cortisol recordings after the pop-out test ( $p < 0.04$ , one-tailed) and 15 min of recovery. HRV during the two-back



**Figure 1.** Cortisol release (in nmol/L) during morning, baseline, the continuous performance tests (calcap), pop-out attention (pop-out) and two-back working memory test (WMT), recovery and evening samples. The data is separated for the high (High HRV) and the low (Low HRV) heart rate variability group (Error bars = 95% confidence interval)

**Table 1.** Bivariate correlation between heart rate variability (HRV) recorded before cognitive testing (baseline), during the continuous performance test (calcap), during the attentional task (pop-out) and working memory task (two-back) and cortisol levels sampled during morning, baseline (15 min. before testing), after the cognitive tests as well as after 15 min of recovery and evening recordings

	Cortisol: morning	Cortisol: baseline	Cortisol: calcap	Cortisol: pop-out	Cortisol: two-back	Cortisol: average of tests	Cortisol: recovery	Cortisol: evening
HRV: baseline	0.00 < 0.99	-0.02 < 0.90	-0.33 < 0.04	-0.28 < 0.04*	-0.22 < 0.07*	-0.35 < 0.04	-0.31 < 0.04*	0.18 0.35
HRV: calcap	0.07 < 0.67	-0.05 < 0.75	-0.26 < 0.05*	-0.29 < 0.03*	-0.24 < 0.07*	-0.32 < 0.03*	-0.29 0.05*	0.35 < 0.05
HRV: pop-out	0.17 < 0.32	0.07 < 0.64	-0.19 < 0.26	-0.28 < 0.04*	-0.16 < 0.31	-0.25 < 0.07*	-0.30 < 0.05*	0.11 < 0.58
HRV: two-back	0.12 < 0.48	0.06 < 0.70	-0.30 < 0.04*	-0.31 < 0.05	-0.24 < 0.07*	-0.35 < 0.05	-0.27 < 0.06*	0.20 < 0.30
HRV: recovery	0.01 < 0.97	-0.12 < 0.43	-0.33 < 0.05	-0.35 < 0.02	-0.34 < 0.03	-0.47 < 0.01	-0.25 < 0.08*	0.21 < 0.27
HRV: mean	0.10 < 0.55	0.00 < 0.99	-0.30 < 0.04*	-0.35 < 0.03	-0.25 < 0.06	-0.39 < 0.03	-0.31 < 0.04*	0.30 < 0.22

\*one-tailed test

tests correlated negatively with cortisol sampled immediately after the calcap test ( $p < 0.04$ , one-tailed) and pop-out attention ( $p < 0.05$ ) together with the mean cortisol score on the 3 tests ( $p < 0.05$ ). HRV recordings during recovery correlated negatively with cortisol recordings after the calcap test ( $p < 0.05$ ), pop-out attention ( $p < 0.02$ ), and two-back test ( $p < 0.03$ ) as well as the mean of the 3 tests ( $p < 0.01$ ). The mean HRV recordings over all cognitive tests correlated negatively with cortisol recordings after the calcap test ( $p < 0.03$ , one-tailed), pop-out attention ( $p < 0.03$ ), and recovery ( $p < 0.03$ ). A borderline negative correlation was found with the cortisol recordings after the two-back test ( $p < 0.06$ , one-tailed).

## CORTISOL AND PERFORMANCE

Correlational analyses were performed separated for the High and the Low HRV groups between levels of cortisol and performance data on the cognitive tests. For the Low HRV group, positive correlations were found between evening levels of cortisol and different parameters of the attentional (calcap) and working memory task. Positive correlations were found on accuracy measures of number of errors of calcap – SPM1 ( $r = 0.75$ ,  $p < 0.01$ ) and SPM2 ( $r = 0.67$ ,  $p < 0.02$ ) as well as number of errors on the working memory task ( $r = 0.64$ ,  $p < 0.03$ ). Negative correlations were found for number of correct on SPM1 ( $r = -0.80$ ,  $p < 0.00$ ) as well as reaction time on SPM1 ( $r = -0.81$ ,  $p < 0.00$ ).

Correlational analyses of the High HRV group showed a negative correlation between morning measures of cortisol and number of correct responses ( $r = -0.61$ ,  $p < 0.01$ ), and a positive correlation with errors on the serial pattern matching 2 (executive functions;  $r = 0.51$ ,

$p < 0.03$ ). Furthermore, a borderline correlation ( $r = 0.46$ ,  $p < 0.058$ ) between morning levels of cortisol and the SPM2 was also found.

Baseline measures of cortisol were also negatively correlated to the number of correct responses on the SPM1 (executive functions;  $r = -0.52$ ,  $p < 0.02$ ).

Recovery levels of cortisol were positively related to choice reaction time on the calcap tests ( $r = 0.54$ ,  $p < 0.03$ ), as well as negatively correlated to the reaction time to correct responses on the calcap ( $r = -0.49$ ,  $p < 0.05$ ). When Bonferoni corrected, none of the correlations became significant.

## DISCUSSION

The results of this study replicate and extend previous research supporting the hypothesis of a negative association between HRV and cortisol secretion during cognitive tasks. Specifically, HRV measures were inversely associated with cortisol response during cognitive tasks as well as during recovery from such tasks. Importantly, when stratified on resting HRV levels, persons with low HRV had significantly higher cortisol levels during the performance of cognitive tasks and subsequent recovery. Furthermore, a replication of the previous findings of a negative association between cortisol levels and performance was supported.

The proposed inverse relation between cardiovascular and endocrine functions was supported and enhanced the knowledge of the role of the pre-frontal cortex in adaptive behaviour in cognitive challenging tasks. Both correlational analyses and the further investigation using ANOVA revealed a pattern of better adaptation to tasks taxing attention and working memory in subjects with high HRV compared to subjects showing lower HRV. The results revealed no dif-



ferences in cortisol levels on baseline and evening measures. However, group differences were found on all cognitive tasks as well as recovery. Furthermore, the High HRV group showed a low and stable cortisol level across testing, while the Low HRV group showed an increase in cortisol level from baseline to the first task, and across the three task conditions. They had a decrease in cortisol level from test conditions to recovery. The present findings replicate and extend the results of Weber et al. [18], which showed that persons with higher resting HRV showed context-appropriate cortisol responses, including faster recovery, compared to those with lower resting HRV. Taken together these results suggest that prefrontal activity as indexed by HRV modulates task-related amygdala activity as indexed by cortisol responses. Ahs et al. [26], in a neuroimaging study, showed that activity in the anterior cingulate was inversely associated with cortisol responses to a speech stressor in social phobics, further supporting the present findings indicating prefrontal regulation of stress responses.

In addition, a previous report from this sample showed that higher resting HRV levels were associated with superior cognitive performance, particularly on executive function tasks [10]. The present results extend these prior findings by suggesting that HRV may also influence cognitive performance via modulation of cortisol. Furthermore, previous studies have shown better performance on memory and attentional test on subjects with high HRV levels [10] as well as higher cognitive functions like situational awareness [27, 28]. However, the present study expands previous knowledge by showing increased stress-related cortisol levels in the Low HRV group. Increased stress-related cortisol levels are associated with decreased performance, and this was the case for both executive and non-executive function tasks in the present study. These findings should be interpreted with caution since they are based on non-corrected correlational analyses and did not survive Bonferroni corrections.

The inverse relationship between HRV and cortisol could have implications for the maritime industry. If subjects with high HRV perform better on attentional and working memory tasks, it raises the question of how to maintain or increase HRV in an onboard environment. HRV is a mechanism that has been shown to be sensitive to interventions such as physical exercise [29], cognitive behaviour treatment [30], and mindfulness training [31]. In all these studies HRV increased from pre- to post-test, and the higher the variability, the more adaptive is the organism, in addition to having a higher capacity to organize physiological resources to respond appropriately to environmental demands [32]. Interestingly, the Hansen et al. [29] study showed a decrease in HRV and non-optimal performance on cognitive tasks in a group of navy personnel on a four-week naval exercise on

board ship. This was compared to a group of naval personnel who stayed on shore and continued their physical training regime. Recent studies have also shown that nutrition may have an impact on HRV. For instance, fatty fish consumption caused a significant increase in HRV as well as a significant decrease in the low frequency/high frequency (LF/HF) of the HRV ratio [33]. The LF/HF ratio is an indicator of the organism's autonomic balance and is relevant to adaptive functioning during environmental demands. Fatty fish is one of the main sources of vitamin D, and vitamin D has also been shown to be associated with executive functioning, but not non-executive functioning [33]. In a recent study it was also found that fatty fish consumption caused an increase in executive functioning [34]. Thus, the beneficial effect of fatty fish consumption on both HRV and executive functioning may be attributed to other nutrients like selenium, iodine, vitamin B<sub>12</sub>, high quality proteins, omega-3, or salmon as whole food. Although, the mechanisms involved are not clearly mapped, the interesting finding is that fatty fish consumption had a beneficial effect. Thus, whereas we have shown that HRV may decrease aboard a ship due to decreased physical activity levels, fish consumption may be one approach to mitigate this decline in HRV and associated cognitive performance. One has to bear in mind that the goal of enhancing cognitive performance is complicated and depends on several not clearly understood factors, and recommendations should be made with caution.

## CONCLUSIONS

In summary, the present study replicates and extends previous research from our group supporting the hypothesis that HRV, as an index of the central-peripheral feedback loop, is inversely related to cortisol release during and after stressful, cognitive demanding tasks. Furthermore, we showed that cortisol levels were associated with cognitive performance. Given the decrease in physical activity levels, HRV, and cognitive performance associated with employment on ships, factors that might lessen these deleterious effects, such as fish consumption and physical activity, might have important implications for safety and performance in a naval environment.

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