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Cognitive Stress Responses During an N-Back Task: Pupillary Dynamics and Salivary Alpha-Amylase Activity

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Abstract

Pupil area reflects not only the light reflex but also cognitive activity and arousal, and has been proposed as a non-invasive indicator of stress. However, the temporal characteristics of pupillary responses during cognitive stress tasks and their associations with other physiological and psychological measures remain unclear. This study examined cognitive stress responses during an N-back task using pupil area and changes in salivary alpha-amylase (sAA). Twenty-nine university and graduate students (mean age = 21.07 ± 0.91 years; 4 males, 25 females) participated. Pupil area was continuously recorded during a 2-back task. Salivary alpha-amylase was measured before (pre) and immediately after the task (post), and the change in sAA (post–pre) was calculated. Subjective stress responses and mood states were assessed using the SRS-18 and TDMS. A notable gender imbalance (predominantly female) limits the generalizability of findings, and future studies should aim for more balanced samples. Pupil area significantly decreased in the latter half of the task, consistent with a time-on-task effect. Although sAA showed no significant group-level change (BF_{10} = 0.25, indicating evidence for the null hypothesis), individual differences in the change in sAA (post–pre) were evident. Task accuracy and the change in sAA were associated with subjective measures of mood and stress. These findings suggest that cognitive stress is reflected differently across pupil area, changes in sAA, and subjective measures, highlighting the value of integrating multiple physiological and psychological indicators.

Keywords: pupil area; cognitive stress; N-back task; salivary alpha-amylase; autonomic nervous activity; non-invasive stress assessment

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Academic Editor: Marianna Mazza

Received: 31 March 2026
Revised: 6 May 2026
Accepted: 13 May 2026
Published: 14 May 2026

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1. Introduction

1.1. Cognitive Stress as a Multidimensional Process

Stress is widely recognized as a critical factor influencing both mental and physical health (Lazarus & Folkman, 1984). In contemporary academic and occupational environments, individuals are frequently exposed to sustained cognitive demands, such as learning, problem-solving, and information processing, which may give rise to cognitive stress: a form of stress that emerges when task requirements continuously tax or exceed available cognitive resources.

To understand cognitive stress, it is essential to distinguish it from related constructs. Cognitive load refers to the amount of information processing required for task performance, particularly within working memory systems (Baddeley, 1992). When cognitive

load is sustained or exceeds processing capacity, it may be appraised as stressful, resulting in cognitive stress responses. Over time, prolonged cognitive stress may lead to mental fatigue, characterized by reduced arousal, diminished attentional resources, and decreased task engagement (Hopstaken et al., 2015). In addition, individuals may experience anticipatory stress prior to task onset, reflecting expectations and perceived demands associated with an upcoming task.

These constructs can be conceptualized as a dynamic sequence: anticipatory stress (pre-task) → cognitive load (task demand) → cognitive stress (response) → mental fatigue (time-dependent outcome). A key implication of this model is that different physiological indicators may be sensitive to different phases of this process, underscoring the need for a multimodal measurement approach.

1.2. Limitations of Single-Indicator Approaches

A major challenge in stress research lies in accurately capturing this multidimensional process. Traditional self-report measures provide valuable insights into subjective experience but are susceptible to social desirability bias and response distortions (Bradley et al., 2008). Common physiological indicators, such as heart rate, heart rate variability, electrodermal activity, and cortisol, reflect autonomic and endocrine activity but present limitations related to invasiveness, temporal resolution, or ecological validity. As a result, relying on a single indicator may provide an incomplete or misleading picture of cognitive stress.

1.3. Pupil Area as an Indicator of Cognitive Load and Mental Fatigue

Pupil area has emerged as a promising non-invasive physiological indicator with high temporal resolution. The regulation of pupil area is strongly influenced by the locus coeruleus–norepinephrine (LC-NE) system, which reflects fluctuations in arousal and attentional control (Aston-Jones & Cohen, 2005; Joshi et al., 2016). Previous research has consistently demonstrated that pupil dilation increases with cognitive load, reflecting cognitive effort during working memory tasks, including the N-back task (Beatty, 1982; van der Wel & van Steenbergen, 2018). However, during prolonged task engagement, pupillary responses attenuate over time, a phenomenon known as the time-on-task effect, thought to reflect the emergence of mental fatigue and reduced task engagement (Hopstaken et al., 2015; McLaughlin et al., 2023). Thus, pupil area captures not only task demand but also temporal dynamics, making it uniquely informative in cognitive stress research.

1.4. Salivary Alpha-Amylase and Anticipatory Stress

Salivary alpha-amylase (sAA) reflects activity of the sympathetic–adrenal–medullary (SAM) system and responds rapidly to psychological stressors (Nater & Rohleder, 2009). sAA has been widely used as a biomarker of psychological stress, particularly in socially evaluative or emotionally salient contexts. However, findings regarding sAA responses to cognitive tasks have been inconsistent (Petrakova et al., 2015; Duchemin et al., 2015), suggesting that sAA may not directly reflect ongoing cognitive load but may instead be sensitive to anticipatory stress and individual differences in stress reactivity. Accordingly, sAA and pupil area may capture distinct components of cognitive stress: sAA indexing anticipatory and individual-difference aspects, and pupil area indexing dynamic changes during task performance.

1.5. Research Gap

Despite growing interest in cognitive stress, several important gaps remain in the literature. First, few studies have simultaneously examined physiological indicators with different temporal sensitivities within the same experimental framework. Second, the relationship between anticipatory stress, task-related cognitive load, and mental fatigue

remains unclear. Third, the extent to which these processes are reflected in subjective psychological measures and task performance has not been sufficiently investigated. The present study addresses these gaps by integrating pupillometry, sAA measurement, and self-report instruments within a single N-back task paradigm.

1.6. Purpose and Hypotheses

To address these gaps, the present study employed a 2-back task to manipulate cognitive load and examined the relationships among pupil area, sAA, subjective psychological measures, and task performance. Based on the proposed conceptual framework, the following directional hypotheses were formulated:

H1. *Pupil area will decrease over time during task performance, reflecting mental fatigue (time-on-task effect).*

H2. *sAA levels will be elevated prior to task onset and decrease thereafter, reflecting anticipatory stress rather than task-induced stress.*

H3. *Task performance will be associated with subjective mood states, such that higher performance is linked to lower emotional tension and greater emotional stability.*

H4. *Changes in sAA will be positively associated with subjective stress indicators, reflecting individual differences in physiological stress reactivity.*

2. Materials and Methods

2.1. Stimuli and Apparatus

The N-back task was employed as a cognitive-demand manipulation. Both physiological indicators and questionnaire measures were used to assess responses. The physiological measures included salivary alpha-amylase (sAA) and pupil area. Salivary alpha-amylase was measured using a portable salivary amylase monitor (cocorometer; Nipro Corporation, Osaka, Japan). Pupil area was recorded using an eye-tracking system (Talk Eye Lite; Takei Scientific Instruments Co., Ltd., Niigata, Japan). The output values represented relative pupil area in arbitrary units, which is a standard approach in studies using this device (Kret & Sjak-Shie, 2019); absolute calibration was not available, but the within-session relative changes are considered interpretable. Two self-report questionnaires were administered. Mood states were assessed using the Two-Dimensional Mood Scale (TDMS), and psychological stress responses were evaluated using the Stress Response Scale-18 (SRS-18). The SRS-18 was administered once prior to the experiment, whereas the TDMS was administered at the pre-, post-, and follow-up time points.

2.2. Participants

Participants consisted of 29 university and graduate students (mean age = 21.07 $\pm$ 0.91 years; 4 males, 25 females). The sample was predominantly female, which represents a notable limitation for generalizability (see Section 4.6). Sample size was determined based on practical constraints and prior studies using pupillometry in similar cognitive tasks (e.g., Hopstaken et al., 2015; McLaughlin et al., 2023), which have employed comparable sample sizes. A formal a priori power analysis was not conducted, which is acknowledged as a methodological limitation. The study protocol was approved by the Ethics Committee of the Graduate School of Humanities and Social Sciences, Kumamoto University (Approval No. 45).

2.3. Experimental Procedure

Upon entering the laboratory, participants first completed the SRS-18 to assess baseline psychological stress responses, followed by the TDMS and the first sAA measurement. The eye tracker was then fitted and calibrated. To minimize the influence of potential confounding variables, the following controls were implemented: (a) all sessions were conducted in a temperature-controlled room under standardized artificial lighting conditions to reduce pupillary light-reflex variability; (b) participants were asked to refrain from caffeine intake for at least two hours prior to the session; and (c) sessions were scheduled within a consistent time window (9:00–12:00 a.m.) to minimize circadian variation in both pupil size and sAA. Participants were seated in a stable position with viewing distance fixed at approximately 42 cm using a chin rest to minimize head movement artifacts during pupillometry.

The N-back task was conducted under the 2-back condition. Stimuli consisted of six English color words (black, white, red, blue, green, yellow), presented sequentially at 0.5 s intervals on a 15.6-inch monitor. Participants pressed the space bar whenever the current word was identical to the one presented two trials earlier. The task comprised 300 trials, with target stimuli occurring at approximately 30% of trials. After completing the N-back task, the eye tracker was removed and the second sAA measurement was obtained, followed by TDMS administration. After a 20 min rest period, the final sAA measurement and TDMS were administered.

2.4. Statistical Analysis

For sAA, a one-way repeated-measures analysis of variance (ANOVA) was performed with measurement time point (pre, post, follow-up) as the within-subject factor. For pupil area, task duration was divided into two segments (first half and second half), and mean pupil area was compared using a two-tailed paired-samples *t*-test. Participants with missing pupil data were excluded from pupillometry analysis (final $n = 19$). Although more fine-grained time-series approaches (e.g., trial-level modeling or growth curve modeling) would be optimal, the temporal resolution of the present data and the exploratory nature of this study did not allow for such analyses; this is acknowledged as a limitation, and future research should adopt these methods. To examine relationships among behavioral performance, psychological measures, and physiological indicators, Spearman's rank correlation coefficients (ρ) were used, as Shapiro–Wilk tests indicated violations of normality. Effect sizes are reported as Cohen's *d* for *t*-tests and partial η^2 for ANOVAs. Ninety-five percent confidence intervals (95% CI) are reported alongside effect size estimates. To supplement frequentist analyses, Bayes factors (BF_{10}) were computed using default priors (Cauchy prior, r = 0.707) to quantify the evidence for or against the null hypothesis for primary group-difference tests (i.e., the repeated-measures ANOVA for sAA and the paired *t*-test for pupil area). BF_{10} was not computed for Spearman correlations, given the exploratory nature of these analyses. The significance level was set at 5% for all analyses.

3. Results

3.1. Physiological Results

3.1.1. Salivary Alpha-Amylase (sAA)

A one-way repeated-measures ANOVA was conducted with time (pre, post, follow-up) as a within-subject factor. The main effect of time was not significant, $F(2, 56) = 0.653$, $p = 0.524$, partial $\eta^2 = 0.023$, 95% CI [0.000, 0.091], $BF_{10} = 0.25$. The BF_{10} of 0.25 indicates moderate evidence in favor of the null hypothesis, suggesting that the absence of a significant sAA change is more consistent with no effect than with insufficient statistical power alone. Mean sAA values (in arbitrary units) were 11.7 (SD = 6.3, pre), 10.6 (SD = 5.8, post),

and 9.7 (SD = 5.4, follow). Although not significant, means exhibited a gradual decrease, with the highest level at the pre-task measurement (Figure 1). This pattern is directionally consistent with H2, though statistical significance was not achieved.

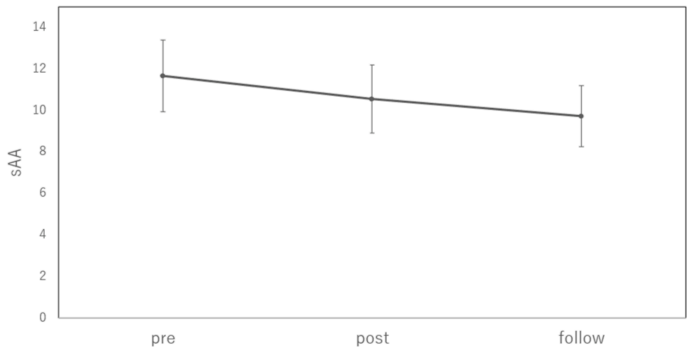

Figure 1. Changes in salivary alpha-amylase across measurement time points (error bars represent standard errors).

3.1.2. Pupil Area

Mean pupil area in the first half of the task was compared with the second half using a paired-samples *t*-test. The results revealed a significant reduction in pupil area in the second half relative to the first, $t(18) = 5.717$, $p < 0.001$, Cohen's $d = 1.311$, 95% CI [0.773, 1.836], $BF_{10} > 100$, indicating very strong evidence for the alternative hypothesis (Figure 2). This result is consistent with H1.

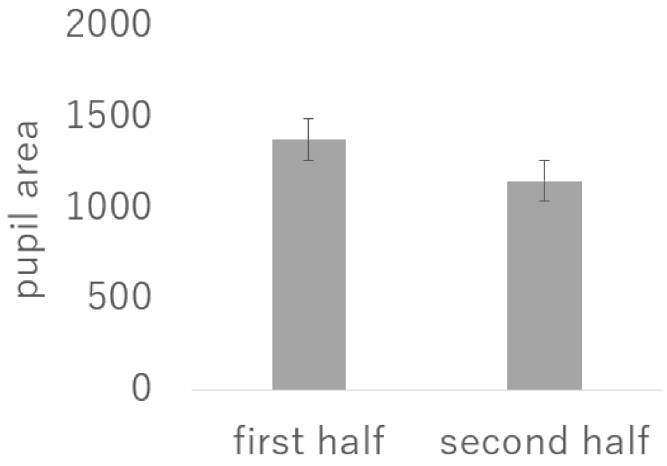

Figure 2. Changes in pupil area during the N-back task (first half vs. second half; error bars represent standard errors).

3.2. Behavioral Results

Task Performance

Mean task accuracy was 56.3% (SD = 8.9%), with a maximum of 69.5% and a minimum of 35.8%, indicating substantial interindividual variability in performance.

3.3. Correlation Analyses

3.3.1. Preliminary Analysis

Shapiro–Wilk tests indicated that task accuracy, questionnaire scores, and sAA values deviated significantly from normality ($p < 0.05$). Therefore, nonparametric correlation analyses using Spearman's rank correlation coefficient (ρ) were conducted for all associational analyses.

3.3.2. Associations Between Task Performance and Subjective Measures

Task accuracy showed significant associations with post–pre changes in TDMS subscale scores, consistent with H3. Higher task accuracy was positively associated with greater increases in the "Calm" subscale ($\rho = 0.525$, $p = 0.003$; Figure 3) and the "Lethargic" subscale ($\rho = 0.397$, $p = 0.033$), and negatively associated with the "Irritated" subscale ($\rho = -0.595$, $p = 0.001$).

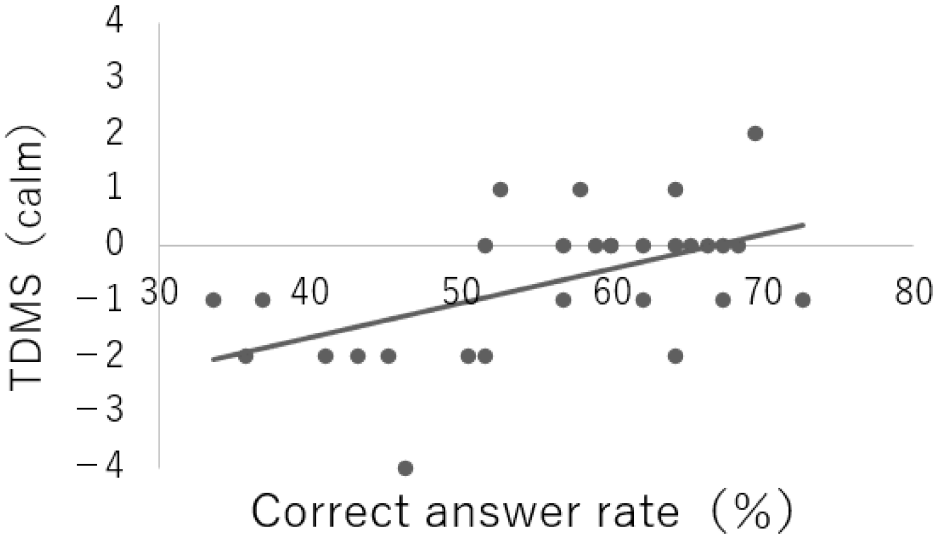

Figure 3. Scatterplot of the relationship between task accuracy and the TDMS "Calm" subscale change score (post–pre).

3.3.3. Associations Between Physiological and Psychological Measures

Changes in sAA (post–pre) were significantly associated with subjective stress responses, consistent with H4. A moderate positive correlation was found between sAA changes and the SRS-18 "Lethargy" subscale ($\rho = 0.464$, $p = 0.011$; Figure 4), and a weak positive correlation was observed with the SRS-18 standardized total score ($\rho = 0.382$, $p = 0.041$).

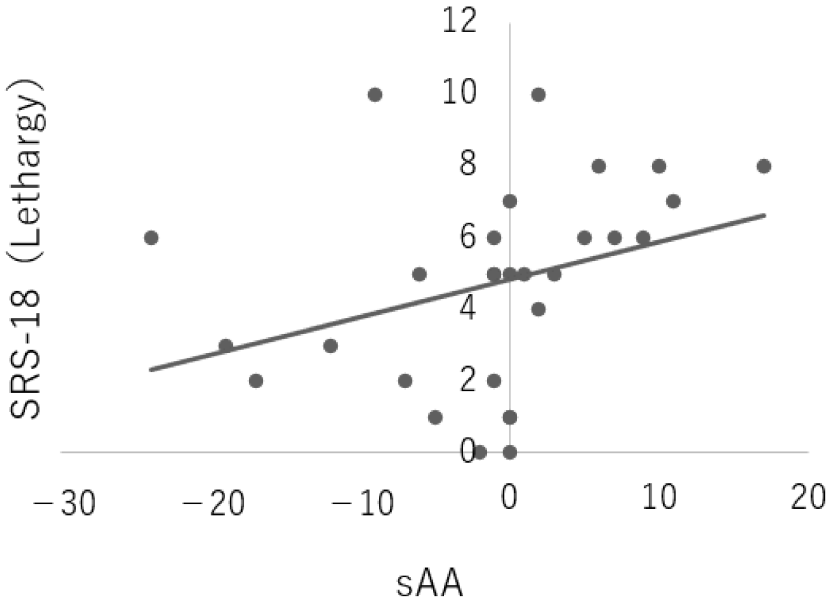

Figure 4. Scatterplot of the relationship between sAA change (post–pre) and the SRS-18 "Lethargy" subscale score.

4. Discussion

4.1. Pupil Area: Time-on-Task Effects and Mental Fatigue (H1)

The present study manipulated cognitive load using a 2-back task and examined physiological, behavioral, and subjective measures. Consistent with H1, pupil area decreased significantly in the second half of the task, suggesting the emergence of mental fatigue during sustained cognitive engagement. This finding aligns with the time-on-task effect reported in prior research (Hopstaken et al., 2015; McLaughlin et al., 2023), wherein prolonged task performance leads to attenuation of pupillary responses, reflecting reduced LC-NE activity and declining arousal (Aston-Jones & Cohen, 2005; van den Brink et al., 2016).

Importantly, the N-back task imposes substantial cognitive load but may not necessarily function as a strong stressor in the absence of additional evaluative or motivational pressure. Research on the conditions required to elicit reliable psychobiological stress responses indicates that tasks involving uncontrollability and social-evaluative threat are most effective in doing so (Dickerson & Kemeny, 2004). Accordingly, the observed pupillary changes should be interpreted primarily as reflecting cognitive load and time-dependent fatigue processes, rather than as direct evidence of acute stress induction. Future research should include explicit stress manipulations (e.g., evaluative pressure, performance feedback) to disentangle cognitive load from stress properly.

4.2. Temporal Dynamics of sAA and Anticipatory Stress (H2)

Contrary to H2, the ANOVA did not reveal a statistically significant main effect of time on sAA levels. Nevertheless, the pattern of means—highest at pre-task and gradually decreasing thereafter—is directionally consistent with the anticipatory stress hypothesis. The BF_{10} of 0.25 provides moderate evidence favoring the null hypothesis, suggesting that the absence of a significant effect is unlikely due solely to limited statistical power. Rather, the N-back task may have functioned primarily as a cognitive challenge rather than a conventional stressor. Alternative explanations include the following: (a) the task may not have induced sustained sympathetic activation; (b) participants may have experienced primarily anticipatory stress prior to task onset; and (c) sAA's limited temporal sensitivity—owing to discrete rather than continuous measurement—may have failed to capture transient peaks during task execution (Nater & Rohleder, 2009). These considerations align with prior work showing that sAA responses to cognitive tasks are inconsistent and context-dependent (Petrakova et al., 2015; Duchemin et al., 2015). The notion that elevated pre-task sAA reflects anticipatory stress is theoretically plausible but would benefit from stronger empirical support, such as measuring sAA at multiple within-task time points or incorporating additional stressor contexts.

4.3. Task Performance and Subjective Mood States (H3)

Consistent with H3, higher task accuracy was associated with greater post-task calmness and lower irritation, indicating that optimal performance co-occurs with emotional stability. These findings are consistent with attentional control theory, which posits that elevated anxiety and emotional arousal impair working memory and executive control (Eysenck et al., 2007; Kane & Engle, 2002). The positive association between accuracy and lethargy is counterintuitive but interpretable within the TDMS framework: participants who performed well may have engaged efficient, effort-conserving cognitive strategies during the task, leading to greater subjective fatigue afterward (Sakairi et al., 2013; Hopstaken et al., 2015). However, alternative explanations exist—for example, higher-performing participants may have been more invested in the task, leading to greater post-task depletion. These competing accounts warrant investigation in future research.

4.4. Individual Differences in sAA and Stress Reactivity (H4)

Consistent with H4, individual differences in sAA change were positively associated with SRS-18 lethargy scores and the standardized total SRS-18 score. These associations underscore that, despite the absence of a significant group-level change in sAA, meaningful individual variation exists in physiological stress reactivity. Participants exhibiting larger sAA changes may be more susceptible to stress-related fatigue and psychological strain, consistent with prior work suggesting that sAA reflects chronic stress exposure and individual sensitivity (Nater et al., 2007).

4.5. Implications for Multidimensional Stress Assessment

Taken together, the findings demonstrate that cognitive responses to the N-back task were not uniformly reflected across indicators. Pupil area captured fatigue-related temporal dynamics (cognitive load and mental fatigue), sAA reflected anticipatory stress and individual reactivity, and subjective measures captured emotional tension and post-task fatigue. These results reinforce the necessity of integrating multiple physiological, behavioral, and subjective indicators to achieve a comprehensive assessment of cognitive stress. Single-indicator models risk mischaracterizing fundamentally multidimensional processes.

4.6. Limitations

Several limitations should be acknowledged. First, the sample consisted exclusively of university and graduate students and showed a strong gender imbalance (4 males, 25 females), substantially limiting generalizability across gender and age groups. Findings may be particularly non-representative for male populations, and future studies should recruit samples with greater gender balance.

Second, the reduction in sample size for pupillometry (from 29 to 19) warrants careful consideration. Data loss may reflect systematic factors (e.g., poor calibration in participants with certain eye characteristics, or higher rates of blinking), which could introduce selection bias. The pupillometry subsample may thus differ systematically from the full sample, and this limitation should be kept in mind when interpreting pupillary findings.

Third, the 2-back task was used without systematic variation in cognitive load conditions (e.g., 0-back, 1-back, 3-back), limiting conclusions about dose–response relationships between cognitive demand and physiological stress indicators. Fourth, sAA measurements were taken at discrete time points, potentially missing transient within-task fluctuations. Fifth, subjective measures were obtained only at pre- and post-intervention time points, not during the task, leaving moment-to-moment affective dynamics uncharacterized. Sixth, a formal power analysis was not conducted, and the statistical power of the study is limited. The effect sizes and Bayesian analyses provided here may assist future researchers in planning adequately powered studies. Seventh, analysis of pupil area was limited to a coarse first-half vs. second-half comparison; more fine-grained time-series approaches (e.g., trial-level modeling, growth curve analysis) are recommended for future work.

4.7. Future Directions

Future studies should (a) employ explicit stress manipulations to distinguish cognitive load from stress, (b) include multiple cognitive load conditions, (c) incorporate more frequent sAA sampling or continuous physiological monitoring, (d) use time-series or trial-level approaches for pupillometry, (e) recruit more gender-balanced and diverse samples with formal a priori power analyses, and (f) explore longitudinal designs to examine stress vulnerability and resilience.

5. Conclusions

The present study examined cognitive stress responses using a multidimensional approach integrating physiological, behavioral, and subjective indicators. A 2-back task was used to manipulate cognitive load, and relationships among pupil area, sAA, subjective mood states, and task performance were investigated. Consistent with H1, pupil area decreased significantly in the second half of the task, reflecting time-on-task effects and emerging mental fatigue. Although sAA did not show statistically significant group-level changes (H2 directionally supported but not confirmed; $BF_{10} = 0.25$), the pattern of pre-task elevation is suggestive of anticipatory stress. Consistent with H3 and H4, task accuracy was associated with emotional stability, and individual differences in sAA changes were linked to subjective stress reactivity.

These findings suggest that cognitive stress responses are multidimensional and cannot be fully captured by a single indicator. Integrating physiological indicators with different temporal sensitivities and subjective measures provides a more comprehensive understanding of cognitive stress dynamics during demanding tasks. The present study contributes to this integrative framework while also identifying important methodological directions for future research, including time-series pupillometry, explicit stress manipulations, and more balanced sampling strategies.

Author Contributions: Conceptualization, T.K. and A.Y.; methodology, T.K.; formal analysis, T.K.; writing—original draft preparation, T.K.; writing—review and editing, T.K.; supervision, A.Y.; project administration, A.Y.; funding acquisition, A.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by JSPS KAKENHI Grant Numbers JP25H00572 and JP23K17293 to A.Y.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Review Committee of the Faculty of Humanities and Social Sciences, Kumamoto University (Approval number: 45, Approval date: 13 May 2024).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the participants to publish this paper.

Data Availability Statement: The data supporting the findings of this study are available upon reasonable request from the corresponding author.

Acknowledgments: The authors are extremely grateful to all the participants of this study.

Conflicts of Interest: The authors declare no conflicts of interest.

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