Interactive teaching enhances students' physiological arousal during online learning

Morris Gellisch a,⁎,1, Gabriela Morosan-Puopolo a,2, Oliver T. Wolf b,3, Dirk A. Moser c,4, Holm Zaeihes a,5, Beate Brand-Saberi a,6

⁎Department of Anatomy and Molecular Embryology, Institute of Anatomy, Medical Faculty, Ruhr University Bochum, Bochum, Germany
b Department of Cognitive Psychology, Faculty of Psychology, Ruhr University Bochum, Bochum, Germany
c Department of Genetic Psychology, Faculty of Psychology, Ruhr University Bochum, Bochum, Germany

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The pure transfer of face-to-face teaching to a digital learning environment can be accompanied by a significant reduction in the physiological arousal of students, which in turn can be associated with passivity during the learning process, often linked to insufficient levels of concentration and engagement in the course work. Therefore, the aim of this study was to investigate whether students' psychobiological stress responses can be enhanced in the context of anatomical online learning and how increased physiological parameters correlate with characteristics of learning experiences in a digital learning environment. Healthy first-year medical students (n = 104) experienced a regular practical course in Microscopic Anatomy either in face-to-face learning, in passive online learning or in an interaction-enhanced version of online learning. Compared to passive online learning, students engaged in the interaction-enhanced version of online learning displayed a significantly reduced Heart Rate Variability (P < 0.001, partial η² = 0.195) along with a strong increase in salivary cortisol (P < 0.001, partial η² = 0.381) and salivary alpha-amylase activity (P < 0.001, partial η² = 0.195). These results demonstrated that the physiological arousal of students engaged in online learning can be enhanced via interactive teaching methods and pointed towards clear correlations between higher physiological responses and elementary criteria of learning experience such as engagement and attention.

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1. Introduction

As necessary responses to the outbreak of the Covid-19 pandemic, global conversions from pre-clinical medical face-to-face teaching to digital learning approaches could be observed, initially described by the term emergency remote teaching (Nikas et al., 2022; Wilhelmin et al., 2022). Although the elaboration and evaluation of digital learning attempts in medical teaching was already of great interest before the Covid-19 pandemic (Choules, 2007; Guimarães et al., 2017; O’Doherty et al., 2018), the changes in teaching caused by restrictions regarding face-to-face events brought substantive insights with reference to advantages and disadvantages as well as indications of need for optimization of digital learning environments (Egarter et al., 2021; Attardi et al., 2022; Chang et al., 2022). There is no doubt that the possibility of switching to digital learning made a significant contribution to maintaining academic teaching activities during the essential restrictions on face-to-face teaching to limit interpersonal contact.

Evaluations of faculties and students’ perceptions of online learning during Covid-19 have further complemented concerns regarding data privacy and security (Almahasees et al., 2021). Further, holistic evaluations of digital learning environments indicated that the obstacles associated with online teaching are not only technological, but also include social and affective challenges that are associated with difficulties in concentrating and maintaining attention (Lemay et al., 2021). In addition, a quantitative and qualitative

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analysis of the shift to emergency remote teaching in preclinical medical education revealed concerns about students’ perceived progression and their emotional and mental wellbeing (Cuscheri and Calleja Agius, 2020). Another research work validated these findings and specified a limitation of online learning by emphasizing that students reported lower levels of engagement with the course materials compared to face-to-face learning (Wilhelm et al., 2022). Additionally substantiating this outcome, a questionnaire-based survey on online medical education highlighted difficulties engaging as a major complaint and disentangled the term into the sub-items: problems with concentration, loss of motivation and therefore emerging frustration (Pokryszko-Dragan et al., 2021).

At this point, the question arises as to the fundamental implications of online learning and to identify which of these may promote difficulties in concentrating, difficulties engaging as well as further affective components to counteract these negative accompanying phenomena in digital learning environments. Investigating these potential implications, the authors demonstrated that the pure transfer of a face-to-face practical course to an online learning environment was associated with decreased sympathetic and enhanced vagal cardiovascular influences together with lower cortisol concentrations in healthy medical students (Gellisch et al., 2022). Since the authors additionally could show that the increased physiological arousal in face-to-face learning correlated with positive affect, the research question arose whether the psychological stress response of students can be enhanced in the context of online learning and how increased physiological parameters correlate with characteristics of learning experiences. The present investigation thus offers a continuation of the study described with the added implementation of a new experimental group, consisting of n = 29 participants, for the purpose of evaluating stress-associated parameters of interaction-enhanced online teaching.

Stress can be regarded as a complex biological and psychobiological/psychosocial concept that has been described by a variety of different definitions. The psychologically based stress model by Lazarus and Folkman, (1984) describes that stress occurs when “demands exceed the personal and social resources the individual can mobilize”. The individual component of stress is specified by another psychologically-based model, strengthening the assumption that the stress perception depends on the initial subjective account (primary appraisal) and available coping mechanisms (secondary appraisal) (Lazarus, 2013). Rather physiologically-oriented definitions describe stress as an organism’s adaptive response to stressors aimed at restoring homeostasis (De Kloet et al., 2005). Hence, stress can be regarded as a deviation from a physiological set-point, adjustment mechanisms in the form of hypothalamic-pituitary-adrenal (HPA) – and sympathetic-adrenal-medullary (SAM) axes activity are required for maintaining homeostasis (McEwen, 1998). Emerging stress exerts its effect on every organ system (Yaribeygi et al., 2017) and influences cognition, especially regarding learning and memory processes (Wingenfeld and Wolf, 2014). These stress effects on cognitive performance – according to the Yerkes-Dodson Law and other well-documented research – follow an inverse U-function with improvements at moderate stress levels and memory performance deteriorations during high-stress periods (Yerkes and Dodson, 1908; Hanoch and Vitouch, 2004; Sapolsky, 2015). Under stress, both memory performance declines in retrieval of stored information (Roozenaal, 2002; Wingenfeld and Wolf, 2014; Wolf, 2017) and improvements in memory consolidation (Joëls et al., 2006; Diamond et al., 2007; Roozenaal and McGaugh, 2011) have been reported. Although the memory-modulating properties of stress are mainly attributed to circulating glucocorticoids, the activation of the autonomic nervous system (ANS) as well as the interaction between glucocorticoids and norepinephrine also contribute to modulations in memory function (Arnsten, 2009; Krugers et al., 2012; Hermans et al., 2014). When discussing the effects of stress on cognitive functions, reference should also be made to the complex interdependence with positive or negative emotions during a stressful event (Lazarus, 2006).

A frequently validated stress marker is the major glucocorticoid cortisol. The diverse actions of cortisol are mediated by the glucocorticoid receptor, a ligand-dependent transcription factor which modulates the transcription rates of numerous genes (Nicolaides et al., 2020). HPA axis-driven peripheral glucocorticoid release occurs with a delay of around 20–40 min after encountering an associated stimulus (Dickerson and Kemeny, 2004; Kudelka et al., 2004; Schwabe and Wolf, 2009; Hummel et al., 2018). Due to the well-established properties and the valid detectability of the lipophilic hormone cortisol, it has been used as a reliable biomarker for stress detection in many interdisciplinary research designs (Kirschbaum et al., 1993; Schwabe et al., 2008; Hellhammer et al., 2009; Oei et al., 2012; Pacharra et al., 2016; Goodman et al., 2017).

In addition to HPA activity, strong modulations of the physiological arousal also emanate from the autonomic self-regulatory system consisting of the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). A sensitive and well-established marker for assessing PNS and SNS involvement in autonomic cardiac modulation is the measurement of Heart Rate Variability (HRV) (Shaffer and Ginsberg, 2017; Pham et al., 2021). In terms of its information value, this neurophysiological phenomenon of variation in time intervals between consecutive heartbeats must be distinguished from heart rate measurements. By calculating the continuous antagonistic inputs from the PNS and SNS on the cardiac rhythm, neurocardiac function generated by heart-brain interactions and dynamic non-linear autonomic nervous system (ANS) processes become quantifiable (McCray and Shaffer, 2015; Massaro and Pecchia, 2019). Counterintuitively, a high HRV is associated with low levels of stress since the heart exhibits high variability in the homeostatic state. Therefore, time domain measures of standard deviation or mean successive difference of NN-intervals positively correlate with vagal tone at a resting state (Hayano et al., 1991; Massaro and Pecchia, 2019). Consequently, while experiencing a stressful event, the time-domain measures RMSSD (root mean square of successive differences between normal heartbeats) and pNN50 (percentage of interval differences of successive NN intervals greater than 50 ms) decrease while the frequency-domain measure LF/HF (ratio of the two frequency-bands low-frequency: 0.04 – 0.15 Hz and high-frequency: 0.15 – 0.4 Hz) increases (Castaldo et al., 2015; Kim et al., 2018; Shaffer and Ginsberg, 2017). This substantiated previous findings that reported an increased heart rate during demanding cognitive and semantic tasks (Carroll et al., 1986; Mulder, 1992; Massaro and Pecchia, 2019).

A candidate protein to report and validate autonomic activity is the salivary enzyme alpha-amylase (sAA) (Rohleder et al., 2004; Nater et al., 2006; Granger et al., 2007). Since secretion from human salivary glands, inter alia, occurs in response to sympathetic stimulation via norepinephrine, sAA-secretion patterns have been proposed to indicate catecholaminergic changes due to increased activation of the SAM axis and thus provide information regarding stress-reactive bodily changes (Nater et al., 2005; Petrakova et al., 2015; Ali and Nater, 2020). Although the HPA axis and its downstream hormone cortisol could be seen as the gold standard biomarker in the context of stress research in behavioral sciences, sAA has emerged as a reliable marker of ANS activity and thus contributed to the evaluation of stress-related interdisciplinary research designs (Chatterton et al., 1997; Skosnik et al., 2000; Takai et al., 2004; Stegeren et al., 2008; Nater and Rohleder, 2009; Becker et al., 2019).

Due to the strong interconnection between stress and emotions (Lazarus, 2006; Pekrun, 2006) and the well-described effects of emotions on cognition and learning (Dolan, 2002; Pessoa, 2008; Power and Dalgleish, 2015), academic emotions have been of
substantial interest within the field of educational psychology (Pekrun et al., 2002a; Ruthig et al., 2008; Pekrun and Stephens, 2011). On account of the multifaceted characteristics of academic emotions and their ability to influence learning processes in various ways, the Achievement Emotions Questionnaire (AEQ) was established as an integrated instrument to assess the emotions participants perceived during their learning experience (Pekrun et al., 2002b). Since it could be demonstrated that certain emotions are associated with academic achievement, the valence of the emotion is of particular importance (Pekrun et al., 2002b). Positive academic emotions are associated with high academic achievement, whereas deactivated negative academic emotions generally predict low academic achievement (Pekrun et al., 2002b).

Given the documented interdependence of physiological arousal, emotions and academic achievement, the aim of this study was to examine interactive teaching methods regarding their potential to induce higher psychobiological responses in the context of online learning and to highlight associated correlations regarding the learning experience. Therefore, two different blended-learning events of a two-hour practical course in Microscopic Anatomy were compared, offering the course content communicated both face-to-face and online: (i) a blended-learning histology course with a pure online transmission of the course events (passive online learning) and (ii) a blended-learning histology course with an implementation of interactive teaching methods for the participants engaged in online learning. The teaching methods applied in the intervention to increase the physiological arousal of students within the framework of online learning (ii) were derived from the theoretical framework of Learner-to-Instructor Engagement (Martin and Bolliger, 2018) and therefore focused on increased Learner-to-instructor interaction including subject-related discussions, testing of previous and acquired knowledge as well as providing immediate feedback (Gaytan and McEwen, 2007; Dixson, 2010; Tanis, 2020). Salivary cortisol measures, sAA activity, HRV recordings, subjectively perceived stress levels, performance tests along with achievement emotions were assessed in both online learning conditions and were further supplemented by a follow-up survey in which the students engaged in the interactive online course were supposed to compare their learning experience with that of passive online learning. To contextualize the collected data and to consider the circadian rhythmicity of glucocorticoid release, the same physiological measurements were performed at rest by a control group at the same time on a weekend day (Oster et al., 2017; Posener et al., 1996).

For the interactive learning condition, we hypothesized lower HRV values in RMSSD and pNN50 along with increased frequency related values of LF/HF indicating a stronger sympathetic activation, validated by increased sAA activity. Further, for the interactive learning condition, the authors hypothesized stronger cortisol responses compared to the passive online learning condition. Moreover, the authors hypothesized to find negative correlation-patterns between enhanced physiological parameters and deactivating negative academic emotions exclusively in the interactive learning condition. Along with the increased physiological arousal within the interactive learning condition, the authors conclusively hypothesized a self-reported increase in their ability to concentrate together with a higher engagement in the course work.

2. Material and methods

2.1. Design and procedures

This randomized field experiment was conducted within the framework of a two-hour hybrid-learning practical course of Microscopic Anatomy (for a detailed course description see Gellisch et al., 2022) and evaluated the data of three experimental groups and one control group. On each given teaching day, one group of students received conventional face-to-face teaching in the histology lecture hall while the second group simultaneously followed the same course online via Zoom (Zoom Video Communications, Inc.; version 5.8.3). The form of participation (online or face-to-face) for each group then rotated weekly. Students engaged in online learning were provided with an additional digital microscopy offer which enabled high-resolution microscopy of the same histological specimens on a web-based application (MyMi.mobile, developed by Professor S. Britsch, Chair of Molecular and Cellular Anatomy, Ulm University). Participants received HRV-sensors, salivettes for saliva collection, standardized questionnaires and a handout to extensively familiarize themselves with the proper way of collecting the saliva samples and the attachment of the HRV sensors in advance. To increase participant compliance, an additional Moodle (Modular Object-Oriented Dynamic Learning Environment, version 3.11.3 - 3.11.5) course was created in which all information on handling the saliva collection, attaching the HRV sensor and correctly completing the questionnaire were permanently accessible. One hour before data collection, participants were instructed to refrain from eating, dental or oral hygiene, regular or recent smoking or drinking anything except water to prevent possible contamination of saliva cortisol and sAA measures (Foley and Cooper et al., 2018). For the interactive learning condition, we hypothesized lower HRV values in RMSSD and pNN50 along with increased frequency related values of LF/HF indicating a stronger sympathetic activation, validated by increased sAA activity. Further, for the interactive learning condition, the authors hypothesized stronger cortisol responses compared to the passive online learning condition. Moreover, the authors hypothesized to find negative correlation-patterns between enhanced physiological parameters and deactivating negative academic emotions exclusively in the interactive learning condition. Along with the increased physiological arousal within the interactive learning condition, the authors conclusively hypothesized a self-reported increase in their ability to concentrate together with a higher engagement in the course work.

While the participants engaged in passive online learning followed the pure transmission of face-to-face teaching via Zoom, the participants in the interaction-enhanced online teaching condition experienced a considerably more activating online learning approach based on an increased Learner-to-instructor interaction including subject-related discussions, oral testing of previous knowledge as well as providing immediate feedback (Gaytan and McEwen, 2007; Dixson, 2010; Martin and Bolliger, 2018; Tanis, 2020). The theoretical and didactic basis for increasing interaction in the context of online teaching followed the evidence-based active learning strategy (Dallimore et al., 2013; Eddy et al., 2015) to increase students’ participation in class and therefore implemented cold call and random call elements (Cooper et al., 2018) in the online teaching process. In addition, as part of the interaction-enhanced online learning concept, the participants were asked to switch on their cameras to enhance the student–teacher relationship (Sederevičienė-Pacaiuskiene et al., 2022) and subsequently were informed that at the end of the course, 10 students will be drawn at random to participate in a multiple-choice test on the course topic provided on the online platform Moodle, to incorporate a typical active learning assessment-component into the online teaching concept (Freeman et al., 2014; Eddy et al., 2015).

HRV measures were performed continuously during the Microscopic Anatomy practical course, which lasted 120 min. Saliva samples for cortisol as well as sAA activity analyses and subjective stress ratings were collected at three points in time: at the start of the course, after 60 min, and at the end of the course. Performance assessment via Moodle took place the week following the course and consisted of a content- and an attention-related task. The content test consisted of three course related multiple-choice questions (e.g., which statement about myoepithelial cells is wrong?/four to five answer options per question). In addition, five course-day-independent anatomical facts that the lecturer mentioned during the two-hour course were queried in order to make general attention to subject-related information ascertainable. (e.g., how many taste...
buds does the papilla vallata contain?/five answer options per question).

To contextualize the collected data, control measurements for HRV, salivary cortisol concentrations, sAA and subjectively perceived stress were carried out on one day of the weekend. Considering the circadian rhythm of cortisol, participants were asked to get up at about the same time as during the week and to carry out the control measurement between 2:00 and 4:00 p.m. to always receive comparable measurement values (Dahlgren et al., 2009; Kumari et al., 2009).

### 2.2. Participants

One hundred and four first semester medical students (35 males: mean age = 20.6 ± 0.34 years; 69 females: mean age = 19.61 ± 0.18 years (mean ± SEM)) participated in this study (see Table 1). The average body mass index (BMI) was 21.80 ± 0.39 kg/m² for the females and 23.57 ± 0.45 kg/m² for the males. Since the inclusion criteria of this study prevented a large variance in the age, participants did not differ significantly from each other within the various conditions (F(3, 100) = 1.16, p = 0.33, partial η² = 0.03). Fourteen female participants used oral contraceptives. While the comparison of passive online teaching with face-to-face teaching has already been published (Gellisch et al., 2022), the interactive online learning group was added as a new experimental intervention group for comparison (see Table 1). All three experimental groups and the control group were recruited during the first week of the Microscopic Anatomy course. To ensure that associated previous study experience did not influence the measurement results, each test person was only allowed to participate in one of the three experimental conditions. During recruitment, the predefined exclusion criteria were checked restricting study inclusion to participants without any chronic or acute mental illnesses or disorders, a history of or current dependence or abuse of alcohol or medication, endocrine disorders known to affect endogenous hormone levels as well as previous experiences of attending the Microscopic Anatomy course. The participants were randomly assigned to one of the respective conditions by the Dean's Office of the Medical Faculty. The control group consisted of participants from the experimental groups and 3 additional participants who did not participate in either of the experimental conditions (see Table 1). Participants were recruited at Ruhr University Bochum, have granted written informed consent prior to inclusion, and were paid for participating. The study procedures were conducted in agreement with the Declaration of Helsinki and approved by the ethics committee of the Medical Faculty at the Ruhr University Bochum (20–7135).

### 2.3. Measurements

#### 2.3.1. Heart rate variability (HRV)

To collect high-quality raw electrocardiogram (ECG) data and to precisely measure RR-interval differences, the ambulatory monitoring systems ECG Move 3 (movisens, Karlsruhe, Germany) were used in this study. Participants were instructed to attach the sensor with two adhesive electrodes below the left lateral chest to continuously record ECG data, sampled at 1024 Hz. Data cleaning and processing was performed using the Unisens viewer software (http://unisens.org/index.php) to extract the relevant measurements from the raw data files stored in each sensor. The HRV data were inspected and – in case of measurement artifacts – corrected with a threshold based medium artifact correction algorithm using Kubios HRV 3.4.3 (Kubios, Kuopio, Finland). To calculate the frequency domain measures LF and HF as well as the time domain measures RMSSD and pNN50, the DataAnalyzer 1.13.5 (movisens, Karlsruhe, Germany) was used, whose algorithms follow the guidelines of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). The square root of the sum of all differences between successive R-R intervals was calculated to indicate the proportions of vagus nerve-mediated autonomic control of the heart and, therefore, was used as a parasympathetic marker (DeGiorgio et al., 2010; Shaffer et al., 2014). Further parasympathetic validation measurements sensitive to vagal cardiac control were obtained by calculating the percentage of successive RR intervals that differ by more than 50 ms (pNN50) (Mietus et al., 2002; Shaffer et al., 2014; Almeida-Santos et al., 2016). Since the HRV signal ranges within a bandwidth of 0–0.5 Hz, the properties of HRV measurements can be divided into different frequency components. While the high-frequency band ranges from 0.15 to 0.4 Hz and mainly reflects vagal activity, the low-frequency ranges from 0.04 to 0.15 Hz and displays, inter alia, power variations related to sympathetic activity (Acharya et al., 2006; Shaffer et al., 2014). Thus, the value of the ratio of LF to HF power (LF/HF ratio) provides information regarding the balance between SNS and PNS activity and increases with dominant sympathetic activity (Shaffer and Ginsberg, 2017). The ratio of the main spectral components was calculated using a fast Fourier transform (FFT) algorithm according to the Task Force of the European Society of Cardiology & the North American Society of Pacing & Electrophysiology (1996). Eight HRV data sets were missing due to measurement errors.

#### 2.3.2. Cortisol and α-amylase

The analyses were carried out on a Biotek Synergy 2 plate reader (Agilent Technologies, Santa Clara, USA) and evaluated using Gen5 software (Agilent Technologies, Santa Clara, USA). Saliva samples for the subsequent investigations of salivary cortisol and α-amylase concentrations were taken at three time points: (1) at course start, (2) 60 min after course start, and (3) at the end of the course (after 120 min). Saliva samples were collected using Salivette sampling devices (Sarstedt, Nümbrecht, Germany) and stored at −20 °C until analysis. Saliva samples were analyzed in the laboratory of the Departments of Genetic Psychology and Cognitive Psychology at Ruhr University Bochum using a cortisol enzyme-linked immunosorbent assay (Cortisol Saliva ELISA, IBL, Hamburg, Germany). Intra- and inter-assay variability were both less than 10 %. The cortisol data were log-transformed to achieve a normal distribution.
before being statistically analyzed. Six sets of saliva samples did not comply with the sample collection guidelines (saliva samples were either taken at the wrong time or food intake was not avoided beforehand) and therefore were not included. A colorimetric test using 2-chloro-4-nitrophenyl-α-maltrotriolide (CNP-G3) as a substrate reagent was applied to analyze the abundance of salivary alpha-amylase (sAA) activity as described elsewhere (Lorentz et al., 1999). The Assay had an intra- and interassay variability of less than 6% and 9%, respectively. Prior to statistical analyses, data were log-transformed to obtain a normal distribution followed by a calculation of the delta (Δ) of the first two measurement points to calculate the increase in the enzyme during the learning unit.

2.3.3. Subjective stress perception, achievement emotion and follow up survey

Standardized Visual Analog Scales (VAS) were used (Luria, 1975) to record the subjectively perceived stress levels of the participants at three points in time: (1) shortly before course start, (2) during the course, and (3) immediately after the course. Participants were asked to place a cross on a 100 mm-long horizontal line, labeled from “no stress” to “maximum stress”. Data were then quantified by measuring the distances between the left end of the VAS and the cross placed by the participants.

Data collection on the achievement-related emotions of anxiety, boredom and enjoyment was performed using the Achievement Emotions Questionnaire (AEQ) (Pekrun et al., 2011). Item-wordings were slightly adjusted to change the reference from class- to course-related emotions as suggested by the authors (e.g., “Thinking about class (the course) makes me feel uneasy”; Goetz et al., 2012). A 5-point Likert scale (1 = completely disagree, 5 = completely agree) was used to record item responses.

Since the participants who took part in the interaction-enhanced online learning also experienced the passive online learning in the following weeks, a follow-up survey was designed aiming to achieve a targeted comparison of both online learning formats regarding engagement with the learning material, self-assessment, anxiety, attention and engagement in the course work. To clarify that the follow-up survey was about comparing both online teaching formats, this information was integrated into the wording of each item (compared to the regular online learning course of Microscopic Anatomy, I felt more engaged in the course work while participating in the interaction-enhanced online course of Microscopic Anatomy). In order not to frame the participants, the word “passive” was not used in the follow-up survey, so that the word “regular” was used due to the higher frequency of passive online learning. A 3-point Likert scale (1 = agree, 2 = undecided, 3 = disagree) was used to record item responses. The response to the follow-up questionnaire was 96.55 % (n = 28).

2.4. Statistical analysis

The statistical software R in the development environment R-Studio (R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical analyses. Data were checked for normality using the Shapiro-Wilk-Test and log-transformed, where necessary. Since the Heart Rate Variability was measured continuously during the Microscopic Anatomy practical course, the raw data were subsequently aggregated to five-minute intervals. Analyses of variance (ANOVA) were performed to calculate the differences in means for all HRV parameters, always including the condition (control vs. online learning vs. interactive online learning vs. face-to-face learning) as a between-subjects factor. Mixed factorial ANOVAs were performed with the between-subject factor condition and the repeated measurement within subject factor time (t1, t2, t3, [...], t24).

Differences in cortisol concentrations were analyzed calculating the area under the curve with respect to ground (AUCg) providing a measure of the total hormonal output. Mixed factorial ANOVAs with the between-subject factor condition and the repeated measurement within subject factor time were performed, to investigate differences in salivary cortisol concentrations over the three measurement points (t1, t2, t3). To analyze the increase in α-amylase from the start of the course, individual delta scores were calculated.

To validate the repeated measures analysis of variance, the equality of variances of the differences between treatment levels were tested and if the assumption of sphericity was violated, Greenhouse-Geisser-adjusted P-values were reported. The significance level was set to α = 0.05 and the analyses were Bonferroni–Holm corrected for multiple comparisons, to control the α error when performing multiple significance tests. The partial eta square ($\eta^2$) was reported as estimation of effect size.

For performance analyses (content- and attention-related assessments) as well as for the achievement emotions factors and subjective stress ratings, ANOVAs were performed to calculate statistical differences between the means of the three conditions. Linear relationships between subjectively perceived factors and the physiological measures were investigated, performing bivariate correlation matrices calculating the Pearson Correlation coefficient.

3. Results

3.1. Heart rate variability and α-amylase analyses

For the interactive online learning group, a significantly reduced Heart Rate Variability was found compared to the control and the passive online learning group, while the reduction in Heart Rate Variability of the interactive online learning group was similar to the cardiac modulation of the face-to-face learning group and thus did not differ significantly from it (see Figs. 1, 2 and 3). For the root mean square of successive differences between normal heartbeats, a significant large-sized effect of the condition was found, aggregating the five-minute HRV intervals recorded to the total time period of 120 min (t1 - t24) (F(3, 121) = 24.80, P < 0.001, partial $\eta^2$ = 0.381; see Fig. 1). The parasympathetic activity of participants engaged in the interactive online learning condition was significantly decreased compared to the control group (P < 0.001) and the passive online learning group (P < 0.001), while no significant difference compared to face-to-face learning group could be found (P = 0.88). Additionally, a significant interaction effect between the conditions and the within subject factor time was found for the HRV parameter RMSSD (F(24.25, 978.06) = 2.47, P < 0.01, partial $\eta^2$ = 0.015). The strongest time-related differences in the comparison between the interactive online learning group and the passive online learning group regarding the HRV parameter RMSSD extended from t9-t12 with P-values ranging between P < 0.001 and P = 0.041 and from t20-t23 with P-values ranging between P = 0.004 and P = 0.027 except for t19 (P = 0.703). No time-related differences in the comparison between the interactive online learning condition and the face-to-face learning condition could be found regarding the HRV parameter RMSSD (P-values ranging between P = 0.997 and P = 1.000).

In congruence with these results, a significant large-sized effect of the condition was found for the HRV parameter pNN50 (F(3, 121) = 20.76, P < 0.001, partial $\eta^2$ = 0.340; see Fig. 2). While for the interactive online learning group compared to the face-to-face learning group no significant divergence could be found (P = 0.37), significantly reduced pNN50 values of the interactive online learning group could be shown compared to the control group (P < 0.001) and the passive online learning group (P < 0.001). Additionally, a significant interaction effect between the conditions and the within subject factor time was found for the HRV parameter pNN50 (F(25.05, 968.56) = 3.80, P < 0.001, partial $\eta^2$ = 0.026). The strongest time-related differences in the comparison between the interactive online learning group and the passive online learning group...
regarding the HRV parameter pNN50 extended from t_{24} to t_{24} with \( P \)-values ranging between \( P < 0.001 \) and \( P = 0.049 \) apart from \( t_{14}, t_{19} \) and \( t_{21} \) (\( P \)-values ranging between \( P = 0.107 \) and \( P = 0.495 \)). No time-related differences in the comparison between the interactive online learning condition and the face-to-face learning condition could be found regarding the HRV parameter pNN50 (\( P \)-values ranging between \( P = 0.947 \) and \( P = 1.000 \)).

For the sympathetic activity, determined via spectral analysis specifying the ratio of the frequency bands LF and HF, a significant increase could be found in the interactive online learning group compared to the control group, while – after adjusting probability (p) values because of the increased risk of a type I error – no significantly higher sympathetic activities for the passive online learning group were found in comparison with the control group (see Fig. 3). For sympathetic activity measures derived from the HRV signal, a large-sized effect of the condition was found, as indicated by significant differences in the quotient of the low- and high-frequency bands (\( F(3, 121) = 9.03, P < 0.001 \), partial \( \eta^2 = 0.183 \); see Fig. 3). For the interactive online learning group, LF/HF was significantly increased compared to the control group (\( P < 0.001 \)), whereas no significant differences could be determined compared to the passive online (\( P = 0.20 \)) – and the face-to-face learning group (\( P = 0.52 \)).

In line with the strong cortisol responses of the participants engaged in the interactive online learning condition, a stronger SAM axis activity could also be detected via analyses of \( \alpha \)-amylase concentrations, which were significantly increased after 60 min of course time compared to the control group, the passive online learning condition and the face-to-face learning condition (see Fig. 5). For \( \Delta \alpha \)-amylase(t_2-t_1), a significant large-sized effect was found for the between-subject factor condition (\( F(3, 123) = 9.94, P < 0.001 \), partial \( \eta^2 = 0.195 \); see Fig. 5). The \( \Delta \alpha \)-amylase(t_2-t_1) of participants engaged in the interactive online learning condition was significantly increased compared to the control condition (\( P = 0.008 \)), the passive online learning condition (\( P < 0.001 \)) and the face-to-face learning condition (\( P < 0.001 \)). Participants engaged in the passive online learning condition neither diverged significantly from the control group (\( P = 0.244 \)) nor from the face-to-face learning condition (\( P = 0.876 \)) regarding their \( \Delta \alpha \)-amylase(t_2-t_1) concentrations. Participants in the face-to-face group also did not differ

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**Fig. 1.** Continuous boxplots of the root mean square of successive differences between heartbeats (RMSSD) over the entire course time and aggregated to 5-minute intervals while participating in interactive online learning, face-to-face learning, passive online learning or while conducting the control measurement. Control (n = 30), passive online learning (n = 35), face-to-face learning (n = 33), interactive online learning (n = 27). Each boxplot displays the minimum, first quartile, median, third quartile, and maximum of the underlying data.

**Fig. 2.** Continuous boxplots of the percentage of interval differences of successive RR intervals greater than 50 ms (pNN50) over the entire course time and aggregated to 5-minute intervals while participating in interactive online learning, face-to-face learning, passive online learning or while conducting the control measurement. Control (n = 30), passive online learning (n = 35), face-to-face learning (n = 33), interactive online learning (n = 27). Each boxplot displays the minimum, first quartile, median, third quartile, and maximum of the underlying data.
significantly from the control group regarding their Δα-amylase(t₂-t₁) concentrations (P = 0.244).

3.2. Cortisol analyses

Participants engaged in the interactive online learning condition showed significantly higher cortisol values than the participants of the control and the passive online learning condition, but no significant differences compared to the participants engaged in face-to-face learning. For salivary cortisol concentrations with regard to the total hormonal output (AUCg), a significant large-sized effect was found for the between-subject factor condition (AUCg: F(3, 123) = 8.91, P < 0.001, partial η² = 0.179). The cortisol concentrations of the participants engaged in the interactive online learning condition were significantly increased compared to the control group (P < 0.001), the passive online learning group (P < 0.001) and compared to the face-to-face group (P = 0.036). Additionally, for salivary cortisol concentrations both a significant interaction effect between condition and time were found (F(5.05, 207.24) = 2.70, P = 0.021, partial η² = 0.025) along with significant time-dependent differences within the condition (F(1.68, 207.24) = 31.56, P < 0.001, partial η² = 0.090; see Fig. 4). While the cortisol concentration of the interactive online group did not differ significantly from that of the face-to-face group at any time point of measurement (t₁:t₂,t₃,t₄; all P-values > 0.181), the cortisol concentration of the participants engaged in the interactive online learning condition after 60 min was significantly higher than the cortisol concentrations of the control (P < 0.001) and the passive online learning condition (P < 0.001). After a strong increase in the cortisol concentration within the first 60 min of the interactive online learning condition, a significant drop in cortisol concentration could be measured towards the end of the course (P < 0.001).

Fig. 3. Continuous boxplots of the ratio of the two frequency-bands, low-frequency: 0.04–0.15 Hz and high-frequency: 0.15–0.4 Hz (LF/HF) over the entire course time and aggregated to 5-minute intervals while participating in interactive online learning, face-to-face learning, passive online learning or while conducting the control measurement. Control (n = 30), passive online learning (n = 35), face-to-face learning (n = 33), interactive online learning (n = 27). Each boxplot displays the minimum, first quartile, median, third quartile, and maximum of the underlying data.

Fig. 4. Salivary cortisol concentration of participants engaged in the control (baseline) -, the passive online learning -, the face-to-face learning - and the interactive online learning condition, as displayed by bar charts demonstrating the course of the cortisol concentration, log-transformed for normalization, over the three measurement points for each condition. Data represent means ± SEM of the control condition (n = 32), the passive online learning condition (n = 33), the face-to-face learning condition (n = 33), and the interactive online learning condition (n = 29), T = 0; first saliva measurement at the beginning of the course, t = 1; second saliva measurement after 60 min, t = 2; third saliva measurement after 120 min.
learning condition. For subjectively perceived stress ratings, a significant large-sized effect of the condition was found (F(2, 98) = 8.217, P < 0.001, partial $\eta^2 = 0.144$). Participants engaged in the interactive online learning condition rated their subjectively perceived stress levels significantly higher than those engaged in either passive online learning (P < 0.001) or face-to-face learning (P = 0.009).

3.4. Correlation-patterns interactive online learning

For the interactive online learning condition, correlations could be shown within the physiological parameters, within the questionnaire constructs as well as between questionnaire constructs and physiological parameters (see Fig. 6). Investigating associations within the physiological parameters, it could be demonstrated that RMSSD exhibited a significant positive large-sized association with pNN50 ($r = 0.958$, $P < 0.001$). The ratio of the two frequency-bands low-frequency and high-frequency exhibited significant negative large-sized association with both RMSSD ($r = -0.729$, $P < 0.001$) and pNN50 ($r = -0.692$, $P < 0.001$). In line with these findings, the second marker used to evaluate sympathetic activity – salivary alpha-amylase – displayed a significant negative moderate-sized association with both RMSSD ($r = -0.434$, $P = 0.024$) and pNN50 ($r = -0.406$, $P = 0.035$). Regarding the correlations between physiological parameters and questionnaire constructs, it could be shown that subjectively perceived stress levels exhibited a positive moderate-sized association with participants cortisol concentrations within the interactive online learning condition ($r = 0.408$, $P = 0.035$). Additionally, subjectively perceived stress levels exhibited a significant large-sized association with the achievement-related emotion of anxiety ($r = 0.635$, $P < 0.001$) and a significant negative moderate-sized association with enjoyment ($r = -0.385$, $P = 0.047$). Enjoyment, on the contrary, exhibited a significant negative moderate-sized association with boredom ($r = -0.397$, $P = 0.040$).

3.5. Follow-up questionnaire

Analysis of the follow-up survey showed that – at the expense of higher levels of the achievement-related emotion anxiety – participants engaged in the interactive online learning condition reported better engagement with the learning material, a higher level of attention and an increased level of engagement with the course work compared to passive online learning (see Fig. 7). 60.71 % of the participants engaged in the interactive online learning condition reported better engagement with the learning material compared to the passive online learning condition (21.43 % undecided, 14.29 disagree). In line with this result, 82.14 % of the participants engaged in the interactive online learning condition reported a higher level of attention compared to the passive online learning condition (71.4 % undecided, 71.4 % disagree). Additionally, 60.71 % of the participants engaged in the interactive online learning condition reported a better engagement with the course work compared to the passive online learning condition (25.0 % undecided, 10.71 % disagree). 78.57 % of the participants engaged in the interactive online learning condition reported higher levels of the achievement-related emotion anxiety compared to participation in passive online learning (71.4 % undecided, 10.71 % disagree).

4. Discussion

Since the authors have already shown that the pure transfer from face-to-face to online teaching was accompanied by a significant reduction in the physiological arousal of the attending students (Gellisch et al., 2022), the aim of this study was to explore whether and to what extent the physiological arousal of students within online learning can be modulated by means of activating teaching
methods. The analyses of the sympathetic and parasympathetic HRV markers (see Figs. 1, 2 and 3), validated by the additional sympathetic marker of alpha-amylase (see Fig. 5), and the comparison of the cortisol concentrations (see Fig. 4) indicated a strong increase in the participants' physiological arousal as a result of the implementation of activating teaching methods in the context of online learning.

4.1. Decreased HRV and increased α-amylase in the interactive online learning condition

Here, the authors report significant reductions in HRV for the interactive online learning condition compared to the passive online learning condition, as indicated by the significantly decreased parasympathetic markers RMSSD and PNN50, together with the increased sympathetic marker LF/HF. Since it has already been shown that high mental workload increases heart rate and blood pressure, this also offers differentiated approaches to interpret the increased physiological arousal in the interactive online learning condition in terms of enhanced cognitive engagement (Veltman and Gaillard, 1996; Hankins and Wilson, 1998; Hjortskov et al., 2004).

Supporting these results, it could be shown that increased mental effort and mental workload are negatively correlated with parasympathetic HRV markers (Mulder and Mulder, 1981; Hjortskov et al., 2004; Wang et al., 2005). Cardiac modulations triggered by mental activity are characterized by increased sympathetic activation combined with significantly reduced parasympathetic activation (Hatch et al., 1986; Hjortskov et al., 2004; Taelman et al., 2011; Delliaux et al., 2019). This pattern of modulation of physiological parameters could be found in the evaluation of the HRV and Δα-amylase data of the interactive online teaching condition, in which a significant parasympathetic reduction based on the parameters RMSSD and pNN50 and a significantly increased sympathetic activity based on increased α-amylase concentrations became evident. Since educational science based research consistently pointed
towards positive associations between interactive teaching methods and higher rates of attention, positive student outcomes along with a higher mental engagement (Crone, 1997; Sivan et al., 2000), this study highlights the autonomous correlates of interactive teaching methods in the context of online teaching and examines the interdependence between cardiac modulating processes and increased interaction within a digital learning environment.

4.2. Enhanced cortisol concentrations in the interactive online learning condition

In line with the expression of the autonomic markers, this study demonstrates a clear increase in cortisol concentrations of the interactive online learning group, while no significant activation of the HPA axis of participants engaged in the passive online learning condition occurred. The increase in cortisol concentrations of the participants engaged in the interactive online learning condition indicates that clearly defined stressors such as uncontrollability and social-evaluative threat were more strongly pronounced due to the increased interaction required by the teaching methodology described (Henry and Grim, 1990; Kirschbaum and Hellhammer, 1994; Dickerson and Kemeny, 2004). Special emphasis should be placed on the finding of the increased cortisol concentrations in the interactive online learning group, since the glucocorticoid cortisol has a strong modulatory effect on learning and memory processes and is therefore directly related to learning experience and performance (Roozendaal et al., 2009; Wiemers et al., 2013; Shields et al., 2017; Wolf, 2017). Since increased glucocorticoid concentrations can exert beneficial effects on memory consolidation (Roozendaal, 2002; Joëls et al., 2006; Diamond et al., 2007; Roozendaal and McGaugh, 2011), it should be noted that a moderate increase in cortisol concentration induced by interactive teaching methods can promote learning in a digital environment.

4.3. Correlation-pattern and follow-up questionnaire

About the correlation pattern of the physiological parameters within the interactive online learning condition, the parasympathetic markers consistently correlated negatively with the sympathetic markers, indicating the validity of the data collected. It was particularly noticeable that the high arousal within the interactive online learning group – characterized by both the subjectively perceived stress levels and the objectively measured cortisol concentrations – was strongly associated with the achievement-related emotion of anxiety. This result supports previous research findings, demonstrating that interactive teaching methods comprise an anxiety-inducing component, as they generate situations that potentially fuel fear of failure (Cooper et al., 2018). Further specifying this phenomenon, it was found that these anxiety-inducing factors are amplified when students felt their self-worth was threatened or their academic ability was tested (Stipek, 2002), aptly described by the term “achievement anxiety” (Covington, 1992). The increased anxiety scores of the interactive online learning condition can – due to reduced parasympathetic activity – also be discussed against a physiological background, evident from the decreased RMSSD and pNN50 values: High RMSSD and pNN50 values reflect a strong vagal activity which in turn is linked to emotion regulation and stress adaptability (Thayer et al., 2012; Park et al., 2014; Vanderhasselt et al., 2015). Since high vagal activity is strongly associated with increased activity in the prefrontal cortex (Thayer et al., 2012; Makovac et al., 2017) which in turn has an inhibitory effect on the amygdala (Davidson, 2002; Baeken et al., 2010; Motzkin et al., 2015), it becomes evident that the reduced parasympathetic markers RMSSD and pNN50 in the interactive online learning condition are associated with increased ratings of the achievement-related emotion of anxiety. This is also reflected in the results of the follow-up survey, in which the majority stated that interactive online learning was significantly more fear-inducing than passive online learning. Previous research results not only demonstrated that teaching methods that promote interaction could influence learning and attitudes towards learning (Chickering and Gamson, 1987; Armbruster et al., 2009), but also that interactive teaching methods are particularly relevant for engagement in online teaching (Khan et al., 2017). In line with these results, the participants of this study stated in a follow-up survey that they experienced a higher level of engagement with both the teaching material and the course events in the context of the interactive online learning condition compared to passive online learning. Supporting these findings, the participants reported increased attention during interactive online teaching compared to passive online teaching, which fits in well with the theoretical framework of previous research, in which interactive teaching methods have been closely linked to increased attention (Prince, 2004; Sentharamarai, 2018; Pamarthi et al., 2019).

In fact, this study was able to demonstrate that the implementation of interactive teaching strategies made it possible to adjust the physiological arousal of students in online learning to that of students engaged in face-to-face learning.

4.4. Limitations and areas for future research

The present study has several limitations. First, the limitation regarding the cohort of the control measurements should be mentioned. Due to the circadian rhythm of the cortisol curve, the control measurements at the weekend had to be carried out strictly in the same time window in which the course took place, which led to scheduling difficulties among the participants, so that only 32 students were able to complete the control measurement correctly. In subsequent studies with a similar research design, intra-individual differences between the participants should be collected holistically both on a physiological and on a psychological level.

A further limitation was performance assessment. Since the questions of the content test could also be answered by carefully studying the course materials, correlations between the points achieved do not necessarily have to correlate with the type of teaching. In addition, the performance assessment could have gained in information value by querying prior knowledge. Moreover, as because of Covid-19 related restrictions in in-person contact the performance tests were conducted online via Moodle, it was not entirely possible to ensure that the tasks had been completed without additional aids.

To build on the results of this study, future investigations should examine physiological parameters in activating digital learning environments with a clear focus on a performance component that is to be examined as isolated as possible, for instance by means of comprehensive tests of prior knowledge. This gives rise to another research question in which it should be investigated whether there is a direct connection between stress-associated physiological parameters or whether a causal relationship is only manifested via mediator variables, such as achievement emotions or motivational factors.

A limitation regarding the follow-up survey is that it was not psychometrically validated, as it had to be specially adapted to the comparison of passive online learning with interactive online learning within the framework of the hybrid course design that was only temporarily available. To further contextualize the research question of this study and to examine the external validity, comparable research designs of various disciplines and teaching units should be generated. It would also be valuable to examine and selectively evaluate how individual teaching methods and innovative pedagogical concepts affect the physiological arousal of students. Compared to face-to-face teaching, the higher arousal within interactive online learning did not correlate with positive achievement
emotions. Therefore, it would also be beneficial to investigate what extent teaching methods in the context of online teaching need to be adjusted to evoke a more positive affect in the context of an online learning experiences.

5. Conclusion

This study examined physiological correlates of activating teaching methods in a digital learning environment and demonstrated that autonomous modulation and increased HPA axis activity resulting in elevated cortisol concentrations can be induced through increased interaction in an online learning environment. It could be demonstrated that an interactive teaching strategy within an online learning format led to reduced parasympathetic activity, increased sympathetic activation, and elevated cortisol concentrations in the participating students. Further evaluations demonstrated that stronger psychobiological responses were associated with increased attention and higher levels of engagement with both the course work and the learning material. Considering the vast corpus of literature addressing the effects of physiological arousal on learning and memory processes, this study points to a strong relationship between increased physiological parameters and engagement along with an increased level of attention within a digital learning environment. In any case, the results of this study point to a previously undisputed, missing link between self-reported findings regarding difficulties in engaging ((Cuschieri and Calleja Agius, 2020; Pokryszko-Dragan et al., 2021) and expressions of physiological parameters as markers of an activating learning environment in a digital context. Since it has already been shown that the pure transfer of face-to-face to online learning is accompanied by a reduced physiological arousal (Gellisch et al., 2022), the results of this study should be used for elaborations of future didactic concepts to better classify the impact of interactive teaching methods in an online learning environment. Especially in these critical times, when the need for distance teaching is becoming more and more important, this study offers physiological evidence regarding the relevance of activating teaching methods in the context of online learning.

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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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aanat.2023.152050.

References


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