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RESEARCH REPORT

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Decreased sympathetic cardiovascular influences and hormone-physiological changes in response to Covid-19-related adaptations under different learning environments

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Abstract

To examine the implications of the transition from face-to-face to online learning from a psychobiological perspective, this study investigated potential differences in physiological stress parameters of students engaged in online or face-to-face learning and determined whether these can be identified as possible mediators between learning experience and achievement emotions. In a randomized experimental field study, medical students ($n = 82$) attended either regular face-to-face classes of the microscopic anatomy course or the same practical course online using Zoom videoconferencing platform. The present study investigated Heart Rate Variability (HRV) and salivary cortisol concentration as stress correlates, within the contexts of online and face-to-face learning and compared these parameters with a control group that was measured at rest. Additionally, participants completed a standardized questionnaire about their experienced emotions in relation to task achievement and subjective stress levels. A significant reduction in HRV was found in face-to-face learning, suggesting stronger stress responses in the face-to-face learning environment ($\eta^2 = 0.421$, $p < 0.001$). Furthermore, participants engaged in face-to-face learning showed significantly higher cortisol concentrations ($\eta^2 = 0.115$, $p = 0.032$). Additionally, increased sympathetic activation correlated with the discrete positive emotion of enjoyment exclusively within the face-to-face condition ($r = 0.365$, $p = 0.043$). These results indicate that the transfer of a face-to-face practical course in microscopic anatomy to an online learning environment is associated with decreased sympathetic and enhanced vagal cardiovascular influences, together with lower cortisol concentrations in healthy medical students.

KEYWORDS

cortisol, Covid-19, face-to-face teaching, heart rate variability, histology education, learning environments, medical education, microscopic anatomy education, online teaching, stress, undergraduate education

INTRODUCTION

A high-quality and stable medical education system is essential for the society, especially during a pandemic that consumes significant resources. Covid-19 infection risk reduction required widespread adaptations, including significant restructuring of university teaching approaches (Daumiller et al., 2021). Field reports on the transfer of anatomical learning content to digitally accessible learning units are highly heterogeneous: each one focuses on different aspects of digital learning. Virtual microscopy offerings, for instance, are considered a productive tool to transfer microscopic anatomy or pathology courses to a digital learning environment (Lee et al., 2020; Chang et al., 2021; Somera Dos Santos et al., 2021). A few advantages of virtual microscopy include simple and flexible access to high-resolution histological specimens as well as unaltered examination results, comparing offers of digitally supported microscopy to conventional light microscopy (Amer & Nemenqani, 2020). Darici et al. (2021) reported a consistently positive overall evaluation of a newly established digital version of a histology course compared to the previous in-person teaching method. Anatomy teaching involves substantial in-person transfer of theoretical and practical knowledge; therefore, several studies have identified limited interaction, poor Internet connectivity, technical problems etc., as limiting factors (Al-Alami et al., 2022; Nikas et al., 2022). Additionally, there are the social and mental challenges. Although many students showed positive academic outcomes, several others reported increased anxiety and poor concentration in fully online learning environments (Lemay et al., 2021). Another evaluation of an online-based anatomy course during the Covid-19 pandemic reflected a 20% improvement of examination grades, but lower self-reported confidence and engagement with the course materials when compared to face-to-face learning (Wilhelm et al., 2022).

To appropriately classify the experiences gained in this context, a distinction must be made between planned, systematically developed, long-term online lessons and the rapid adjustments necessitated by Covid-19. The term emergency remote teaching was coined to indicate this distinction (Hodges et al., 2020). Evaluation of students' perspectives and experiences related to emergency remote teaching in preclinical medical education revealed their concerns about their education quality, examination performance, academic progression, and emotional and mental well-being (Cuschieri & Calleja Agius, 2020; Loda et al., 2020). Other shortcomings of emergency remote teaching in medical education included unsatisfactory content, technical issues, engagement difficulties, poor organization, and lack of social life (Pokryszko-Dragan et al., 2021). Singal et al. (2021) suggested that relevant and timely modifications in digital anatomy education would be required after data corroborate the observation that students prefer traditional anatomy learning—dissection courses, face-to-face lectures, interactions with mentors, etc.

Evidence-based modifications in professional online learning must be preceded by identification of dynamic variables through comparison of online and face-to-face learning modes. Many studies have investigated the subjectively perceived stress level of students with online learning during the Covid-19 pandemic (Attarabeen et al., 2021; Chinna et al., 2021; Fitzgerald & Konrad, 2021; Wang et al., 2021; Chen & Lucock, 2022). However, no physiological data are available for the state of arousal of students in the associated learning environments.

Stress is a complex concept and is variously defined. Individual experiences of stress depend on initial subjective account (primary appraisal) and available coping mechanisms (secondary appraisal) (Lazarus, 1993). A well-established psychological model of stress development is based on the assumption that stress arises when "demands exceed the personal and social resources the individual is able to mobilize" (Lazarus, 1966). Definitions of stress based on psychology should include references to its relation with the field of emotions, which can be toned either negatively (anxiety, anger, fright, guilt, shame, etc.) or positively (happiness, pride, gratitude, etc.) (Lazarus, 2006). In research orientated toward physiology, stress is considered an organism's adaptive response to stressors aimed at restoring homeostasis (de Kloet et al., 2005). Hence, stress is a deviation from a physiological ideal, triggering adaptive mechanisms to efficiently handle the stressor. These allostatic processes involve the hypothalamic–pituitary–adrenal and the sympathetic–adrenal–medullary axes, regulated through the central nervous system (McEwen, 1998).

Effects of stress on cognitive functions have been frequently investigated. It should be noted that the intensity of a stress response can correlate with the modulation of cognitive processes. According to the Yerkes–Dodson Law and other well-documented studies, stress effects on cognitive performance follow an inverse U-function with improvements at moderate stress levels (Yerkes & Dodson, 1908; Hanoch & Vitouch, 2004; Sapolsky, 2015). Beneficial effects on memory consolidation typically arise when the stressor occurs close to the learning event and is related to the learning material, emphasizing the importance of the timing component related to the associated learning phase (Wolf, 2019). Stress results in memory performance deterioration in terms of retrieval of stored information (Roozendaal, 2002; Wingenfeld & Wolf, 2014) as well as improvements in memory consolidation (Joëls et al., 2006; Diamond et al., 2007; Roozendaal et al., 2009; Roozendaal & McGaugh, 2011). Comprehensive models of stress effects on learning and memory processes emphasize the importance of respective memory phases (consolidation versus retrieval), which are subject to opposing stress-associated modulations (Shields et al., 2017; Wolf, 2017; Bierbrauer et al., 2021).

Cortisol—a major glucocorticoid—is a well-established stress marker; it is released after a stressful event from the zona fasciculata layer of the adrenal cortex regulated by the hypothalamus–pituitary–adrenal axis. The hormone provides energy during stressful situations by activating processes such as catabolic metabolism, which increase blood glucose level (Rensing et al., 2006). Due to the proportional correlation between serum unbound cortisol and salivary cortisol concentration, non-invasive saliva analyses can be used to determine cortisol concentration (Vining et al., 1983). The hypothalamus–pituitary–adrenal axis-driven peripheral glucocorticoid release occurs with a delay of 10–20 min and exerts its action via mineralocorticoid and glucocorticoid receptors (Kudielka et al., 2004; Schwabe et al. 2008; Schwabe & Wolf, 2009). In addition to genomic effects, cortisol exerts its impact via non-genomic effects mediated via membrane-bound versions of mineralocorticoid and glucocorticoid receptors (Joëls et al., 2008; Roozendaal et al., 2010). Genomic and non-genomic cortisol effects typically cause an impaired memory retrieval (de Quervain et al., 1998; Atsak et al., 2016), while the initial encoding process as well as memory consolidation of learning material perceived around the time of the stressor are enhanced (Joëls et al., 2006; Wiemers et al., 2013; Wolf, 2017).

In addition to glucocorticoid-associated memory modulation, activation of the sympathetic nervous system and the consequential release of (nor)adrenaline also contribute to the memory function. Regarding memory modulations, activation of the sympathetic nervous system is associated with hypervigilance at the expense of selective attention (Arnsten, 2009; Hermans et al., 2014). Furthermore, content classified as relevant was learned more effectively with stronger activation of the sympathetic nervous system (Joëls et al., 2006; Wiemers et al., 2013).

Thus, stress occurs when an organism's physiological demands are no longer fulfilled via regular functioning of the parasympathetic nervous system; measurements of parasympathetic tone also indicate stress and stress vulnerability (Kim et al., 2018). A sensitive marker for evaluating the parasympathetic–sympathetic interplay is the Heart Rate Variability (HRV). It reflects variation in time between successive heartbeats (RR intervals) reciprocal to the time elapsed between two successive R-waves of the QRS signal on the electrocardiogram. The HRV is an established non-invasive quantitative marker for autonomic cardiac regulation—that is, balance between vagal and sympathetic cardiac modulations (Shaffer & Ginsberg, 2017). Well-established HRV-associated markers of parasympathetic activity include the root mean square of successive differences between normal heartbeats (RMSSD) and the percentage of interval differences of successive RR intervals greater than 50 ms (pNN50) (Shaffer & Ginsberg, 2017; Kim et al., 2018). Additionally, a spectral analysis of the Heart Rate Variability data helps examine how much of the signal lies in certain frequency bands. The two main frequency bands used in spectral analysis are the high frequency power (HF), which reflects vagal influences, and the low frequency power (LF) reflecting both sympathetic and vagal influences (Li et al., 2019). On this basis, it could be demonstrated that—in response to a stressful event—the frequency-domain measure LF/HF (ratio of the two frequency bands LF; ranging from 0.04 to 0.15 Hz and HF; ranging from 0.15 to 0.40 Hz) increases, while the time-domain measures RMSSD and pNN50 decrease (Shaffer & Ginsberg, 2017; Kim et al., 2018).

In addition to the physiological measurable parameters, emotions experienced during academic settings are also relevant for this study, as it has already been shown that they are closely linked to achievement and task-related problem-solving (Fredrickson, 2001; Pekrun et al., 2002a, 2002b; Clore & Huntsinger, 2009). The control-value theory of achievement emotions (CVT) has provided a framework that describes the emergence of stress-inducing characteristics in learning environments using two appraisal dimensions: (1) subjective control over achievement activities and outcomes, and (2) the value attached to them (Pekrun, 2006). Here, the achievement component is associated with the self-assessed ability to deliver academic performance, whereas the value appraisal indicates how personally relevant associated activity is rated. Consequently, the emergence of either discrete positive (e.g., enjoyment or hope) or negative (e.g., boredom or anxiety) emotions depends on the expression of both appraisal dimensions (Pekrun et al., 2006). In an activating, practical, and factual designed learning environment, mainly activity-related achievement emotions, such as enjoyment (positive, high activation) and boredom (negative, low activation), occur consistently (Pekrun & Stephens, 2012; Itzek-Greulich et al., 2017).

Given the documented effects of physiological arousal on learning and memory processes in correlation with achievement emotions, a deeper understanding of the potential influence of a digital learning environment on these variables is of substantial interest. Therefore, the present study aimed to determine whether online and face-to-face learning modes are associated with differing physiological states of arousal and whether they are related to achievement emotions, subjective stress perception, or academic performance.

In contrast to other German universities, where—due to the Covid-19 pandemic—practical histology classes were delivered entirely online (Böckers et al., 2021), the Anatomical Institute at the Ruhr University relied on a hybrid learning approach (Eringfeld, 2021; Singh et al., 2021), simultaneously offering the same histology practical course in both modes: face-to-face and online, to reduce group size. The regular practical histology course for first semester medical students commenced in the winter term and was accompanied by a series of lectures that conveyed the theoretical basics of the course (Lu et al., 2016). In the first semester, medical students complete 12 lecture hours and 18 laboratory hours in the field of histology. The practical course is divided into nine main topics, from basics of histological staining methods to neurocytology. In the face-to-face practical course, each student was equipped with a slidebox containing over 100 histological specimens in the form of glass slides and a microscope that, connected to a computer, enabled both analog microscopy via the eyepiece and digital microscopy via the screen to make use of the didactic advantages of digital microscopy in face-to-face classes as well (Mills et al., 2007; McCready & Jham, 2013; Tian et al., 2014; Kuo & Leo, 2019; Caruso, 2021; Saverino & Zarcone, 2022). Due to Covid-19-associated restrictions on face-to-face teaching (Evans et al., 2020; Böckers et al., 2021), classes were halved in strength so that students attended either the face-to-face course or its simultaneous live online transmission, on a weekly basis.

Salivary cortisol measures, HRV recordings, subjectively perceived stress levels, performance tests, as well as achievement emotions were assessed under both conditions, online and face-to-face learning. To better interpret the data collected—and considering the circadian rhythm of cortisol levels—the same physiological measurements were performed by a control group at the same time on a weekend day (Posener et al., 1996).

MATERIAL AND METHODS

Design and procedures

The present study—a randomized field experiment with two experimental groups and one control group—was conducted within the framework of the practical course of microscopic anatomy at the Ruhr University in Bochum, Germany. The first semester medical students participated either in regular face-to-face learning at the histology lecture hall at the Ruhr University, Bochum, or attended the same course online via Zoom videoconferencing platform, version 5.8.3 (Zoom Video Communications, Inc., San Jose, CA). Data were collected on the third day of the course, which addressed basics of surface epithelia using various histological specimens (renal papilla; azane staining, jejunum; Masson's trichrome staining, testis and epididymis; Masson's trichrome staining, trachea; toluidine blue staining, ureter; Masson's trichrome staining, esophagus; hematoxylin and eosin staining, finger; hematoxylin and eosin staining) along with transmission electron microscopic images. For the online transmission of the course, all histological specimens were digitized and made permanently available via a virtual microscopy platform. This virtual microscopy platform enables high-resolution microscopy of the same histological specimens on a web-based application, MyMi.mobile (Ulm University, Ulm, Germany). Additionally, the virtual microscope enables a holistic overview of all histological specimens relevant to the course as well as navigation and continuous zooming in the virtual slides. To digitize the histological glass slides for the virtual microscope, the histological specimens were scanned with the Zeiss Axio Scan. Z1 Slide Scanner (Carl Zeiss AG, Oberkochen, Germany) and further processed with ZEN Blue software, version 2.3 (Carl Zeiss AG, Oberkochen, Germany).

Participants in the online learning condition received the materials for all measurements in advance and brought the completed questionnaires, HRV sensors, and saliva samples taken back to the institute after their participation in the microscopic anatomy practical course. For all participants, the collection phase of the physiological data lasted from 2:00 to 4:00 p.m. Immediately before the commencement of the course, participants completed questionnaires requesting demographic information (sex, age, height, weight, etc.) and reported their self-perceived stress level on a visual analog scale (VAS; Luria, 1975). Prior to the experiment, the students received a detailed, intelligible manual with instructions for completing the questionnaire, applying the HRV sensor, and collecting saliva samples. One hour prior to the experiment, participants were instructed to refrain from eating, smoking, or drinking anything except water to eliminate possible contamination of saliva cortisol measures (Foley & Kirschbaum, 2010).

Performance measurements were performed online via Moodle—a learning management system—version 3.11.3–3.11.5 (eLeDia eLearning im Dialog GmbH, Berlin, Germany), in the week after the course and consisted of a content- and an attention-related task. The content test comprised three multiple-choice questions on the course topic (e.g., which specimen contains pseudostratified columnar epithelia with goblet cells?—there were four to five answer options per question). For the attention task, the lecturer was instructed to mention five course-independent anatomical facts during the course that were neither part of the previous lecture nor found in the course script (e.g., which protein provides mechanical and chemical protection for the urinary tract?—five answer options per question). This attention task—decoupled from the core objectives of the learning unit—measured the general attention toward anatomical facts in the respective learning environment.

The control measurements for HRV, salivary cortisol concentrations, and subjectively perceived stress were performed on one day of the weekend. Therefore, the participants of the control measurement received the materials for all measurements in advance and took them home. To prevent measurement errors due to the circadian rhythm of cortisol (Dahlgren et al., 2009; Kumari et al., 2009), the participants were asked to wake up at the same time as during the week and perform the control measurement between 2:00 and 4:00 p.m.

Structurally, the outline of the course could be divided into three parts: (1) The first half hour was about reactivating previous knowledge to tie in with the following learning unit. (2) During the following 60 min, time was allotted for microscopy and drawing of the histological specimens. (3) During the last 30 min, questions were discussed and special features of selected course specimens were highlighted. Each student participating in face-to-face teaching was equipped with a Leica DM500 microscope (Leica Microsystems GmbH, Wetzlar, Germany) that, via the camera module Leica ICC50 W (Leica Microsystems GmbH, Wetzlar, Germany), enabled studying the histological specimens both on a monitor and through the eyepiece. The students who participated in the simultaneous online transmission of the course examined the same histological specimens using the virtual microscope. The Zeiss Axioscope microscope (Carl Zeiss AG, Oberkochen, Germany) with the Olympus UC90 camera module (Olympus Deutschland GmbH, Hamburg, Germany) was used to transmit the image of the lecturer's microscope on both the projectors in the histology lecture hall and a computer from which the image was streamed via Zoom for online students. Using the camera and audio system of the histology lecture hall, the lecturer's video and audio were transmitted online, to ensure complete online transmission of the face-to-face course.

The heart rate variability was measured continuously during the course (120 min). Saliva samples for determining cortisol concentration and subjective stress ratings were recorded at the beginning of the course, after 60 min, and at the end of the course. After the course, a standardized questionnaire was used to assess the students' achievement emotions.

Participants

Eighty-two first semester medical students (30 males: mean age = 20.8 ± 0.37 years; 52 females: mean age = 19.63 ± 0.22 years [mean ± SEM]) participated in this study. The average body mass index (BMI) was 22.15 ± 0.47 kg/m^2 for the females and 23.57 ± 0.53 kg/m^2 for the males. The participants did not differ significantly in terms of age (F[2, 79] = 0.72, p = 0.49, partial η^2 = 0.02) or BMI (F[2, 79] = 1.31, p = 0.28, partial η^2 = 0.03). Eleven female participants used oral contraceptives (cf. Table 1). The students were randomized into groups by the Dean's Office of the Medical Faculty and assigned to the different study cohorts. During the recruitment process, the predefined exclusion criteria were checked, restricting study inclusion to participants without any chronic or acute mental illnesses or disorders, endocrine disorders known to affect endogenous hormone levels, a history of or current dependence or abuse of alcohol or medication as well as previous experiences of attending the microscopic anatomy course. If the criteria were met, the participants were included and assigned to the different cohorts. The control group comprised participants from the experimental groups and 10 additional participants who did not participate in either of these two experimental groups (cf. Table 1). The same inclusion and exclusion criteria were applied for the experimental and control groups. Participants were recruited at the Ruhr University Bochum; they provided written informed consent 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 (protocol number 20–7135).

Measurements

Heart rate variability

Variations in time between successive heartbeats were recorded with the movisens ECG Move 3 sensor (Movisens, Karlsruhe, Germany)—an ambulatory monitoring system to collect high-quality raw electrocardiogram (ECG) data. The sensor was attached with two adhesive electrodes below the left lateral chest, and ECG data were sampled continuously at 1024 Hz. To extract the relevant data frame from the raw data, the entire data set was inspected and cut at the respective timestamps using the UnisensViewer software, version 3.0 (Movisens GmbH, Karlsruhe, Germany). Additionally, recorded data were inspected and—in case of abnormal or biologically implausible beats—corrected with a threshold-based medium artifact correction algorithm using Kubios HRV, version 3.4.3 (Kubios Oy, Kuopio, Finland). Furthermore, the DataAnalyzer, version 1.13.5 (Movisens GmbH, Karlsruhe, Germany) was used to calculate the time-domain measures RMSSD and pNN50 as well as the frequency-domain measures LF and HF according to the guidelines of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). The root mean square of successive differences between heartbeats—a commonly used measure derived from interval difference—was calculated to estimate the vagally mediated changes reflected in HRV (Shaffer et al., 2014). The pNN50 was calculated to provide information about the percentage of successive RR intervals that differ by more than 50 milliseconds. To reflect sympathetic modulations, the ratio of the main spectral components low frequency (0.04–0.15 Hz) and high frequency (0.15–0.4 Hz) was calculated using the fast Fourier transform (FFT) algorithm (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Six heart rate variability data sets were missing because of measurement errors.

Cortisol

Salivary cortisol concentrations as markers of the hypothalamus–pituitary–adrenal axis activity were sampled at three time points: (1) at the beginning of the course, (2) after 60 min, and (3) at the end of the course (after 120 min). The participants engaged in either face-to-face or online learning were reminded to collect saliva samples by means of an announcement by the lecturer at the respective time points during the course. Saliva samples were collected using Salivette® Cortisol sampling devices, catalog # 51.1534.500 (Sarstedt AG & Co. KG., Nümbrecht, Germany) and stored at −20°C

TABLE 1 Demographic characteristics

Characteristics	Face-to-face learning	Regular online learning	Control group	Total
Number of total participants	35	37	32	82
Male, *n* (%)	11 (31.43)	12 (32.43)	13 (40.625)	30 (36.59)
Female, *n* (%)	24 (68.57)	25 (67.56)	19 (59,38)	52 (63.41)
Age, mean (±SD)	20.31 (±1.98)	19.94 (±1.76)	19.65 (±1.76)	20.15 (±1.90)
Complete HRV data set, *n* (%)	33 (94.28)	35 (94.59)	30 (93.75)	98 (94.23)
Complete cortisol data set, *n* (%)	33 (94.28)	33 (89.18)	32 (100)	98 (94.23)
Complete questionnaire data set, *n* (%)	35 (100)	37 (100)	–	72 (100)
Regular use of oral contraceptives, *n* (%)	7 (20)	4 (10.81)	4 (12.5)	11 (13.25)

Abbreviation: HRV, heart rate variability.

until analyses. Saliva samples were analyzed in the laboratory of the Departments of Genetic Psychology and Cognitive Psychology at the Ruhr University Bochum using a cortisol enzyme-linked immunosorbent assay (Cortisol Saliva ELISA, catalog # SA E-6000R IBL International Corp., Hamburg, Germany). Intra- and inter-assay variability were both less than 10%. Prior to statistical analyses, data were log-transformed to obtain a normal distribution. 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.

Subjective stress perception and emotions

During the experiment, the subjectively perceived stress levels of the participants were recorded at three time points: (1) right before course commencement, (2) during the course, and (3) after the course. Therefore, standardized Visual Analog Scales (VAS) were used (Luria, 1975). Participants placed a cross on a 100 mm-long horizontal line, labeled from "no stress" to "maximum stress." To evaluate the data, the distances between the left end of the VAS and the cross were measured. Previous validation studies of the visual analog scale highlighted its discriminative sensitivity, interconcept, and external validity (Lesage et al., 2011; Lesage et al., 2012), and specifically referred to its good concordance with the Perceived Stress Scale (Lin's concordance correlation coefficient = 0.66)—a classic stress assessment instrument developed by Cohen et al. (1983) (Lesage & Berjot, 2011).

To explicitly obtain the emotions relevant to the current study, the Achievement Emotions Questionnaire (AEQ) was used, which was constructed based on a multi-component definition of achievement emotion and tested in a study using a sample of university students (n = 389) (Pekrun, 2006; Pekrun et al., 2011). A confirmatory factor analysis and measures of internal consistency were performed indicating internal validity of the AEQ scales along with a high reliability as indicated by Cronbach's alpha ranging from $\alpha = 0.85$ to 0.93 for the emotion factors of enjoyment, anxiety, and boredom (Pekrun et al., 2011).

Parallel item wordings were used to assess enjoyment, boredom, and anxiety with four items per scale. The wording of the original items was 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 five-point Likert scale (1 = completely disagree, 5 = completely agree) was used to record item responses.

Statistical analysis

All statistical analyses were performed in R statistical software (R Foundation for Statistical Computing, Vienna, Austria). Data were checked for normality using the Shapiro–Wilk test and log-transformed, if necessary. The heart rate variability data were recorded continuously over the course of the experiment and aggregated to five-minute intervals, resulting in 24 discrete time points per measurement. Differences in means for RMSSD, pNN50, and LF/HF aggregated to the total time period were calculated using analyses of variance (ANOVAs) always including the condition (control vs. online learning vs. face-to-face learning) as a between-subjects factor. Furthermore, for heart rate variability parameters, mixed factorial ANOVAs were performed with the between-subject factor condition and the repeated measurement within subject factor time (t_1, t_2, t_3, [...], t_{24}), analyzing time-related changes in HRV between and within all conditions.

Changes in activation of the neuroendocrine stress system were analyzed by calculating the area under the curve with respect to ground (AUCg) reflecting the total hormonal output (Pruessner et al., 2003). Differences in salivary cortisol concentration over the three measurement points (t_0, t_{12}, t_{25}) were analyzed using a mixed factorial ANOVA with the between-subject factor condition and the repeated measurement within subject factor time. Presuming that the assumption of sphericity was violated, Greenhouse–Geisser-adjusted p-values were reported. Analyses were performed with the significance level set to $\alpha = 0.05$ and Bonferroni–Holm corrected for multiple comparisons when necessary. As estimations of effect sizes, partial eta square (η^2) were reported.

For performance analysis (content- and attention-related assessments) as well as achievement emotions and subjective stress levels, Welch independent two samples t-tests were performed to examine differences between the two learning environments.

To investigate linear relationships between achievement emotions, perceived stress, and physiological measures, a bivariate correlation matrix was specified calculating the Pearson correlation coefficient.

RESULTS

Heart rate variability analyses

Neither the sex nor the intake of oral contraceptives influenced the HRV parameter expression or cortisol concentration of the participants (all p-values >0.13).

For both the face-to-face and online learning conditions, a decreased HRV was found compared to the control group. For the root mean square of successive differences between normal heartbeats, a significantly large-sized effect of the condition was found, aggregating the associated values to the total time period of 120 min ($t_1 - t_{24}$) ($F[2, 95] = 34.54$, $p < 0.001$, partial $\eta^2 = 0.421$; see Figure 1A). Students in the online learning condition displayed significantly lower parasympathetic activity compared to the control group, indicated by lower RMSSD values ($p = 0.01$). The face-to-face group exhibited the lowest proportion of parasympathetic activity, as indicated by the lowest RMSSD values; × control group ($p < 0.001$), × online learning group ($p < 0.001$). In line with these findings, a significantly large-sized effect of the condition was found for the HRV parameter pNN50

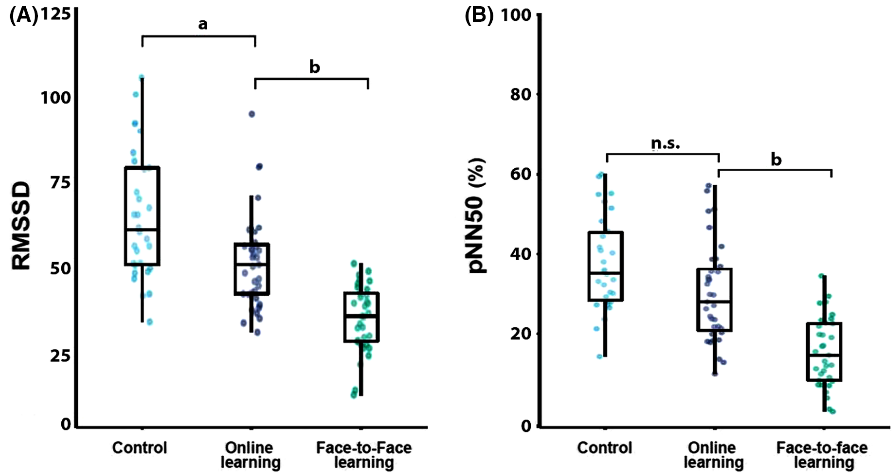

FIGURE 1 Boxplots of the root mean square of successive differences between heartbeats (RMSSD) in graph A and the percentage of interval differences of successive RR intervals greater than 50 ms (pNN50) in graph B while participating in online –, face-to-face learning, or while conducting the control measurement. Control ($n = 30$), online learning ($n = 35$), face-to-face learning ($n = 33$). $^{a}p<0.01$; $^{b}p<0.001$; n.s., not statistically significant. Each boxplot displays the minimum, first quartile, median, third quartile, and maximum of the underlying data.

($F[2, 95] = 27.75$, $p<0.001$, partial $\eta^2 = 0.369$; see Figure 1B). While no significant difference could be determined for the parameter pNN50 in the comparison between the control and online groups ($p = 0.1$), the face-to-face group deviated significantly from the other conditions; × control-group ($p<0.001$), × online learning group ($p<0.001$).

For both the face-to-face and online learning groups, an increased sympathetic activity was found compared to the control group. For the ratio of the two frequency bands LF and HF, a large-sized effect of the condition was found, as indicated by significant differences in the quotient of the low- and high-frequency bands ($F[2, 95] = 12.54$, $p<0.001$, partial $\eta^2 = 0.209$; see Figure 2). Participants in the face-to-face group exhibited the highest values for LF/HF; × control group ($p<0.001$), × online learning group ($p = 0.02$). The online learning group showed significantly lower LF/HF values compared to the face-to-face group, but displayed significantly higher values than the control group ($p = 0.02$).

For the face-to-face group, parasympathetic activity increased toward the end of the course, as indicated by higher percentages of interval differences of successive RR intervals greater than 50 ms and higher values of the root mean square of successive differences between heartbeats. For the root mean square of successive differences between heartbeats, both a significant interaction effect between condition and time were found ($F[16.17, 767.98] = 3.33$, $p<0.001$, partial $\eta^2 = 0.019$) along with significant time-dependent differences within the condition ($F[8.08, 767.98] = 15.04$, $p<0.001$, partial $\eta^2 = 0.042$). While no time-dependent significant differences in RMSSD were found within the control group ($t_1 \times t_2$-t_{24}; all p-values >0.174) and the online group ($t_1 \times t_2$-t_{24}; all p-values >0.836), clear time-dependent differences were detected within the face-to-face group. During the first 75 min, RMSSD did not change significantly within the face-to-face group ($t_1 \times t_2$-t_{15}; with p-values ranging between 1.0 and 0.228 in a time-dependent manner), but then increased significantly from minute 80 onward ($t_1 \times t_{16}$-t_{24}; with p-values ranging between 0.046 and <0.001) reflecting higher

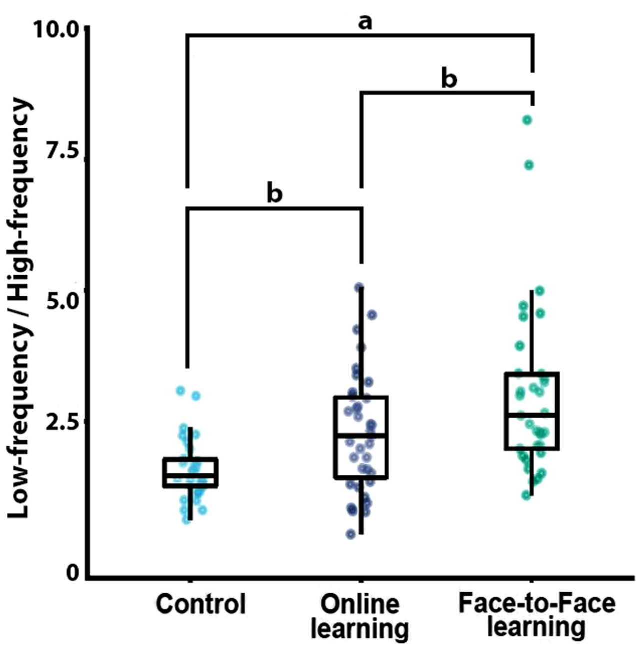

FIGURE 2 Boxplot of the ratio of the two frequency bands, low frequency: 0.04–0.15 Hz and high frequency: 0.15–0.4 Hz, while participating in online–, face-to-face learning or while conducting the control measurement. Control ($n = 30$), online learning ($n = 35$), face-to-face learning ($n = 33$). $^{a}p<0.001$, $^{b}p<0.05$. Each boxplot displays the minimum, first quartile, median, third quartile, and maximum of the underlying data.

parasympathetic activity toward the end of the course. For the percentage of interval differences of successive RR intervals greater than 50 ms, both a significant interaction between condition and time ($F[16.24, 754.94] = 5.35$, $p<0.001$, partial $\eta^2 = 0.040$) as well as significant time-dependent differences within a condition were found ($F[8.12, 754.94] = 16.52$, $p<0.001$, partial $\eta^2 = 0.060$). No time-dependent significant differences in pNN50 were found within the control group ($t_1 \times t_2$-t_{24}; all p-values >0.991) or within the online group ($t_1 \times t_2$-t_{24}; all p-values >0.868). During the first 50 min, pNN50 did not change significantly within the face-to-face group ($t_1 \times t_2$-t_{10}; with p-values ranging from 1.0 to 0.153) but then increased significantly from minute 55 onward ($t_1 \times t_{11\text{-}24}$; with p-values ranging from 0.032 to <0.001, except for t_{13}; $p = 0.227$).

While the ratio of the two frequency bands LF and HF of the face-to-face group was significantly higher than the values of the control group for a long period of time, no significant fluctuations could be found within the three conditions. Regarding the ratio of the two frequency bands LF and HF, a significant interaction effect between condition and time was found (F[29.60, 1405.89] = 1.65, $p = 0.015$, partial $\eta^2 = 0.013$) with main effects for the comparison of the control group to the face-to-face group ($t_{2\text{-}15} \times t_{2\text{-}15}$; with p-values ranging between 0.048 and <0.001, except for t_6, t_{10}, t_{13}, t_{14}; p-values >0.089). However, no effect of the condition and the within-subjects variable of time (F[14.80, 1405.89] = 0.77, $p = 0.712$, partial $\eta^2 = 0.003$) could be shown for LF/HF.

Cortisol analyses

While cortisol concentrations of participants in the face-to-face group were significantly higher than those of participants in the online and control groups, no significantly higher cortisol concentrations were found in the online learning group compared to the control group. For salivary cortisol concentrations, a significant effect was found for the between-subject factor condition (AUCg; F[2, 95] = 6.17, $p = 0.003$, partial $\eta^2 = 0.115$; see Figure 3A). While salivary cortisol concentrations expressed by participants of the face-to-face group were significantly higher compared to those of the control group ($p = 0.003$) and the online group ($p = 0.032$), the cortisol concentrations of the online group did not differ significantly from the values of the control group ($p = 0.335$). Additionally, significant time-dependent differences within a condition were determined for salivary cortisol concentrations (F[1.63, 155.23] = 33.88, $p < 0.001$, partial $\eta^2 = 0.122$; see Figure 3B). The cortisol concentration in the control group remained constant over the duration of the three times of measurement ($t_1 \times t_2$; $p = 0.640$, $t_1 \times t_3$; $p = 0.110$, $t_2 \times t_3$; $p = 0.467$). After initially elevated cortisol levels consistent with expectations of an anticipatory response, a significant decline in cortisol concentration across the first 60 min of the course was found for both the face-to-face group ($t_1 \times t_2$; $p = 0.004$) and the online group ($t_1 \times t_2$; $p < 0.001$). Although from minute 60 to the end of the course, no significant decline in cortisol concentration could be determined for either of the two conditions (face-to-face: $t_2 \times t_3$; $p = 0.492$, online: $t_2 \times t_3$; $p = 0.961$), the decline in cortisol concentration from t_1 to t_3 was significant for both conditions (face-to-face: $t_2 \times t_3$; $p < 0.001$, online: $t_2 \times t_3$; $p = 0.002$).

Performance, subjectively perceived stress, and achievement-related emotions

With regard to performance analysis, constant results have been achieved in both content assessment (t[69.538] = 0.082, $p = 0.533$) and attention assessment (t[67.183] = 0.0148, $p = 0.442$) in both learning conditions. While the face-to-face group achieved 94.27% ± 15.09 in content assessment and 47.43% ± 21.19 in attention assessment, the online group achieved 94.59% ± 14.73 in content assessment and 50.81% ± 26.50 in attention assessment.

Furthermore, no significantly different expressions of the achievement-related emotions enjoyment (t[68.206] = 0.054, $p = 0.522$), anxiety (t[67.118] = 1.023, $p = 0.155$), and boredom (t[62.707] = 1.173, $p = 0.877$) were found across the different learning environments. Significant differences in subjectively perceived stress levels were found across the three conditions (F[2, 101] = 28.581, $p < 0.001$, partial $\eta^2 = 0.361$). Compared to the

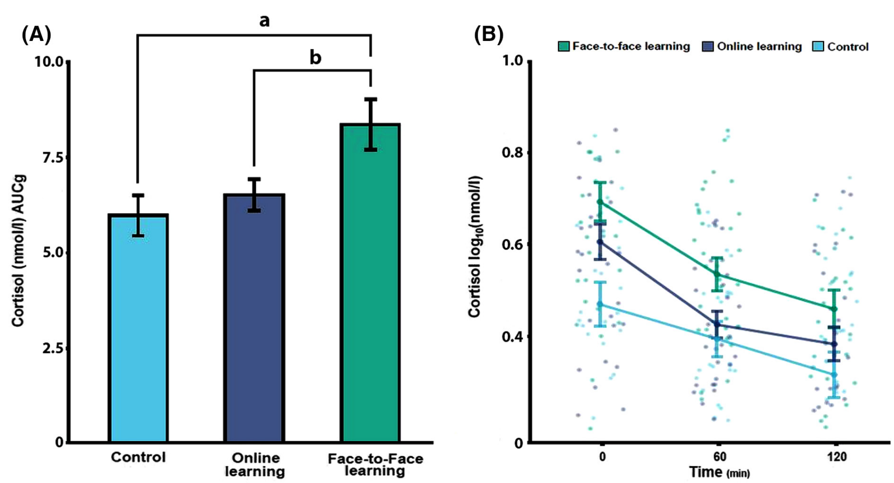

FIGURE 3 Participants' salivary cortisol concentration as displayed by (A) area under the curve with respect to ground (AUCg) reflecting the total hormonal output, [a]$p < 0.01$, [b]$p < 0.05$; and (B) progressive graph demonstrating the course of the cortisol concentration, log-transformed for normalization, over the three measurement points. Data represent means ± SEM of the control ($n = 32$), the online learning ($n = 33$), and the face-to-face learning ($n = 33$) group.

control group, the subjective perception of stress was higher in the face-to-face group ($p < 0.001$) and the online group ($p < 0.001$), but did not diverge significantly when comparing the two learning environments ($p = 0.27$).

Correlation patterns

Correlations could be shown within the measured physiological parameters, within the questionnaire constructs as well as between questionnaire constructs and physiological parameters. Investigating associations between achievement emotions and physiological stress measurements (cf. Table 2), the LF/HF ratio of the face-to-face group exhibited a significant positive medium-sized association with enjoyment ($r = 0.365$, $p = 0.043$). Values of the root mean square of successive differences between normal heartbeats of the face-to-face group exhibited a significant positive correlation with boredom ($r = 0.361$, $p = 0.046$), while boredom was strongly negatively correlated with enjoyment ($r = -0.503$, $p = 0.004$). Further, subjective stress levels within the face-to-face group were negatively correlated with enjoyment ($r = -0.445$, $p = 0.043$) and LF/HF ratio ($r = -0.431$, $p = 0.016$), but exhibited a positive large-sized strength of association with anxiety ($r = 0.592$, $p < 0.001$) (cf. Table 2). Salivary cortisol concentrations within the face-to-face group were negatively correlated with RMSSD ($r = -0.495$, $p = 0.005$) and pNN50 ($r = -0.492$, $p = 0.005$), while RMSSD was strongly positively correlated with pNN50 ($r = 0.976$, $p < 0.001$) (cf. Table 2). In line with H3, differing correlation patterns were found within the online group (cf. Table 2). While no significant associations between achievement emotions and physiological measurements were found within the online group, the strong negative correlation between boredom and enjoyment ($r = -0.568$, $p = 0.001$) and the positive correlation between subjective stress levels and anxiety ($r = -0.520$, $p = 0.003$) remained constant. The ratio of the two frequency bands LF and HF within the online group exhibited a negative large-sized strength of association with both RMSSD ($r = -0.764$, $p < 0.001$) and pNN50 ($r = -0.787$, $p < 0.001$).

DISCUSSION

The aim of the present study was to investigate physiological differences in students when participating in online or face-to-face learning and to determine whether these correlated with achievement emotions, subjectively perceived stress levels, or academic performance. Therefore, the authors used: (1) heart rate variability measurements as a marker for autonomic nervous system activity, (2) salivary cortisol measurements as an indicator of the hypothalamus–pituitary–adrenal axis activation, and (3) standardized questionnaires to assess experienced achievement emotions and subjectively perceived stress levels.

For both the face-to-face and the online learning conditions, the authors hypothesized a decreased HRV and an increased salivary cortisol concentration compared to the control group (H1). For the online learning condition, the authors hypothesized higher HRV values in RMSSD and pNN50 indicating stronger parasympathetic activation, lower frequency-related values in LF/HF indicating a weaker sympathetic activation, and an attenuated hypothalamus–pituitary–adrenal axis activity reflected by a lower salivary cortisol concentration compared to the face-to-face group (H2). Moreover, the authors hypothesized differing correlation patterns between activity-related achievement emotions/low activation emotions, and physiological measurements of arousal, comparing the face-to-face group with the online group (H3).

In this study, heart rate variability measures were performed, calculating the fluctuation in time intervals between subsequent heartbeats, and, thus, providing information regarding neurocardiac function generated by heart–brain interactions and dynamic non-linear autonomic nervous system processes (McCraty & Shaffer, 2015). Thus, physiological stress—for example, sympathetic nervous system activation and cortisol release—influences memory in various ways (Roozendaal, 2002; Atsak et al., 2016; Gagnon & Wagner, 2016); the academic environment is a reasonable target for associated stress research (Lester et al., 2010; Preuss et al., 2010; Minkley et al., 2017; Myint et al., 2021; Tammayan et al., 2021). To evaluate a timed and well-defined stressor in academic settings, previous research has mainly focused on acute stress before and during examinations, which consistently resulted in increased cortisol concentrations (Evans et al., 1994; Lacey et al., 2000; Spangler et al., 2002; Weekes et al., 2006; Takatsuji et al., 2008; Preuss et al., 2010). Although a recent study reported a negative correlation between HRV and examination scores (Yoo et al., 2021), little is known about the connection between cardiac modulations—quantified via HRV measurements—in relation to different learning environments, such as the comparison between online and face-to-face learning environments.

Here, the authors report significant reductions in HRV for the face-to-face group compared to the control group, as indicated by the decreased parasympathetic markers RMSSD and PNN50, together with the significantly increased sympathetic marker LF/HF. Similarly, the online learning condition differed significantly from the control group regarding the parameters RMSSD and LF/HF. Although participants of the face-to-face learning condition expressed significantly elevated cortisol levels compared to the control group, participants of the online learning group did not differ significantly in this regard. Previous studies on autonomic cardiac regulation in real-life situations have already indicated strong correlations between mental workload and decreased HRV (Sloan et al., 1994; Myrtek et al., 1996). Thus, the decreased heart rate variability in both experimental learning conditions compared to the control group is consistent with previous research that provided clear associations between HRV parameter modulations and mental engagement during cognitive tasks (Hjortskov et al., 2004; Pendleton et al., 2016).

As outlined in Hypothesis 2, for face-to-face learning compared to online learning, the results revealed a significantly higher

TABLE 2 Correlations between physiological parameters, subjectively perceived stress measures, and achievement emotions

Parameters	RMSSD	pNN50	LF/HF	Cortisol	VAS stress	Enjoyment	Anxiety
Face-to-face learning							
RMSSD							
pNN50	**0.976 (<0.001)**						
LF/HF	−0.351 (0.053)	−0.328 (0.072)					
Cortisol	**−0.495 (0.005)**	**−0.492 (0.005)**	−0.088 (0.638)				
VAS Stress	0.271 (0.140)	0.214 (0.248)	**−0.431 (0.016)**	−0.063 (0.735)			
Enjoyment	−0.297 (0.105)	−0.231 (0.211)	**0.365 (0.043)**	−0.087 (0.642)	**−0.445 (0.012)**		
Anxiety	−0.043 (0.817)	−0.083 (0.659)	−0.192 (0.300)	0.030 (0.873)	**0.592 (<0.001)**	−0.264 (0.151)	
Boredom	**0.361 (0.046)**	0.315 (0.084)	−0.162 (0.383)	−0.128 (0.492)	0.199 (0.284)	**−0.503 (0.004)**	0.354 (0.050)
Online learning							
RMSSD							
pNN50	**0.912 (<0.001)**						
LF/HF	**−0.764 (<0.001)**	**−0.787 (<0.001)**					
Cortisol	0.055 (0.769)	0.137 (0.461)	−0.025 (0.892)				
VAS Stress	−0.129 (0.488)	0.031 (0.869)	0.143 (0.443)	−0.031 (0.867)			
Enjoyment	−0.009 (0.962)	−0.056 (0.765)	0.005 (0.979)	0.199 (0.283)	−0.265 (0.150)		
Anxiety	0.066 (0.725)	0.195 (0.294)	−0.150 (0.419)	−0.113 (0.546)	**0.520 (0.003)**	−0.223 (0.229)	
Boredom	−0.104 (0.578)	−0.053 (0.777)	0.175 (0.345)	−0.011 (0.955)	0.179 (0.336)	**−0.568 (0.001)**	−0.148 (0.426)

Note: The values reported show the Pearson coefficient and *p*-values in brackets. Statistically significant correlations are marked in bold font.

Abbreviations: AUCg, area under the curve with respect to ground; LF/HF, ratio of the two frequency bands low frequency: 0.04–0.15 Hz and high frequency: 0.15–0.4 Hz; pNN50, the percentage of interval differences of successive RR intervals greater than 50 ms; RMSSD, root mean square of successive differences between normal heartbeats; VAS, visual analog scale.

physiological arousal among the participants in the face-to-face condition indicated by reduced parasympathetic markers (RMSSD, pNN50), a higher expressed sympathetic marker (LF/HF), and higher cortisol concentrations. These findings indicated a stronger cardiovascular adjustment—for example, enhanced sympathetic and reduced vagal cardiovascular influences—in the face-to-face learning condition, which—in line with classical psychophysiological concepts (Thayer et al., 2009) and laboratory studies (Hansen et al., 2003; Duschek et al., 2009)—might be attributable to stronger mental activity during the face-to-face condition. Neuroimaging studies provided further insight into the underlying mechanisms and interactions between the sympathetic and parasympathetic branches of the autonomic nervous system and higher brain functions that are relevant for attention and emotion regulation (Thayer et al., 2012; Ruiz Vargas et al., 2016; Valenza et al., 2017). These pathways, described in the central autonomic network (CAN) model by Benarroch (1993), include projections from the prefrontal cortex to subcortical nuclei that modulate cardiovascular activity and, thus, provide the basis for associating HRV measurements with task-related cognitive processes and mental engagement (Thayer et al., 2009; Riganello et al., 2012; Pendleton et al., 2016). In conformity with the CAN model and other associated studies (Hjortskov et al., 2004; Taelman et al., 2011; Pendleton et al., 2016), the increased sympathetic and decreased parasympathetic activities of participants engaged in face-to-face learning can be explained by a higher mental engagement compared to the online learning condition. Moreover, the significantly higher cortisol levels of participants engaged in face-to-face learning suggested that within the online learning condition, factors such as uncontrollability and social-evaluative threat do not come into effect equally strong; previous studies have already shown such connections between these stressors and increased cortisol expression (Henry & Grim, 1990; Kirschbaum & Hellhammer, 1994; Dickerson & Kemeny, 2004). Since Dickerson and Kemeny (2004) showed in their landmark meta-analysis that tasks containing uncontrollability, social-evaluative threat, as well as a performance component were associated with the largest cortisol increases, it can be assumed that these stressors are severely diminished within the online learning condition. However, as the pure transfer of face-to-face learning to an online-supported learning environment was evaluated here, it should be considered that the results could have been different if more interactive and activating components had been incorporated into the learning experience.

In contrast to the students in the online condition, the learning process of students in the face-to-face condition was potentially under the observation and evaluation of the lecturers during the microscopy task, the drawing tasks, and throughout the general participation in the course. According to the self-preservation theory (Kemeny et al., 2004), the resulting impression of constant evaluation—and, thus, the possible threat to the social self—during face-to-face learning could be a distinctive characteristic of the face-to-face teaching format, resulting in the documented elevated psychobiological responses.

In line with Hypothesis 3, comparing the face-to-face group with the online group, differing correlation patterns between activity-related achievement emotions/low activation emotions and physiological measurements of arousal could be shown. An interesting finding was that the sympathetic-related HRV parameter LF/HF only correlated with enjoyment within the face-to-face condition, whereas increased sympathetic values were not associated with enjoyment within the online learning condition. In a review of 134 publications that reported experimental investigations of emotional effects on peripheral physiological responding, joy—in contrast to all other positive emotions—could be characterized by increased β-adrenergic sympathetic activation and, therefore, is in line with the findings of the face-to-face condition (Kreibig, 2010). Enjoyment was shown to be systematically related to students' cognitive and motivational engagements in learning material on the one hand, and appraisals of mastery and success, on the other (Wright, 1996; Pekrun et al., 2002b). The correlation of sympathetic activity with positive affect during face-to-face learning was additionally confirmed by the outcome that an increased LF/HF value during face-to-face learning correlated negatively with subjectively perceived stress. Within the online group, subjective stress levels correlated exclusively with anxiety. The finding that LF/HF was not only significantly reduced during online learning compared to face-to-face learning, but also—in contrast to face-to-face learning—exhibited no correlation with enjoyment, should be emphasized in the comparison of both learning environments. However, pointing to the validity of the data—and in line with the results—previous research found significant associations between cortisol levels and HRV during periods of increased stress, whereas during low stress periods, these associations were attenuated and became nonsignificant (Looser et al., 2010). Moreover, correlations between increased RMSSD values and more passive learning conditions could be shown in a randomized experimental field study with high school students (Minkley et al., 2017). Additionally, RMSSD exhibited strong positive associations with pNN50 scores across all conditions (Shaffer & Ginsberg, 2017).

Identifying reduced parasympathetic and increased sympathetic activities as well as increased cortisol concentrations of participants engaged in face-to-face learning indicates a relevant factor in the context of the transition from face-to-face to digital learning environments, which has been rarely discussed or researched to date.

Limitations of the study

The present study has several limitations. First, although approximately a quarter of the total population (first semester medical students) was recruited for this study, one limitation is the relatively small sample size. As the data indicated intra-individual differences in the physiological and emotional experiences during the course, a larger number of participants would have been desirable to better identify relevant correlations. However, as the collection of physiological data in the academic context described was costly and particularly complex because of Covid-19 restrictions, the sample size can be considered adequate—and even bigger than those of similar studies investigating stress parameters in academic settings (Weekes et al., 2006; Melillo et al., 2011; Stetler & Guinn, 2020).

Another methodological limitation was the composition of the control group. Although the control group partly consisted of participants from the experimental group, it would have been beneficial to assess the control measurement with each participant from the experimental group. However, such a study design requires an extremely high level of compliance, which is difficult to achieve during a running semester and, therefore, explains the comparatively small number of control measurements. A further limitation was performance assessment. The content-related assessment was part of the regular course schedule and contained questions that could be answered after reading the course script in detail, so that a high score did not necessarily have to correlate with the type of teaching. To counter this fact, the authors performed another performance test, including questions that were neither part of the previous lecture nor found in the course script. However, this performance test could have been biased because of varying levels of prior knowledge. Moreover, as the performance tests were conducted online via Moodle, it cannot be ruled out that additional sources were used for processing the tasks. In fact, no significant between-group differences in terms of performance were found, although previous laboratory experiments suggested clear associations between stress and memory processes (Cahill & McGaugh, 1998; Hoskin et al., 2014; Wolf, 2017).

Therefore, future research approaches should assess physiological data in different learning environments with a focus on performance differences that should be investigated as individually as possible. Future studies designed in this way should set a special methodological focus on assessing performance regarding long-term memory, because a large body of previous research has already indicated clear connections between the state of physiological arousal and long-term memory modulations (McGaugh & Roozendaal, 2002; McGaugh, 2003; Roozendaal & McGaugh, 2011; Finsterwald & Alberini, 2014). The aim of this study was to evaluate the pure transfer of face-to-face teaching to an online-supported learning environment without incorporating didactic modifications. Therefore, future studies should examine whether the physiological arousal of students in online learning can be modulated—for example, through activating teaching methods (Gläser-Zikuda et al., 2005). If the results in this regard will show that an increase in the physiological state of arousal is possible within the framework of online learning, it will be necessary to examine whether similar correlation patterns compared to face-to-face teaching can be identified.

CONCLUSION

In contrast to studies on learning-related stress, which typically rely on self-report data, this study reports objectively measured psychobiological responses of healthy medical students when participating in online or face-to-face learning. Taken together, this study introduces novel insights into varying activating characteristics of online and face-to-face learning modes and provides further evidence of differing correlation patterns across the learning conditions regarding the hypothalamus–pituitary–adrenal axis activation, autonomic cardiac regulation, subjectively perceived stress, and achievement-related emotions. The transfer of a face-to-face practical course in microscopic anatomy to an online learning environment was associated with a significantly reduced physiological arousal during online learning, as indicated by increased HRV and reduced salivary cortisol concentrations. The results of this study indicate decreased sympathetic and enhanced vagal cardiovascular influences during online learning, indicating a weakened mental activity, contextualizing, and supplementing previous self-reported findings regarding difficulties in engaging—for example, concentration issues, loss of motivation, and emerging frustration during online learning (Cuschieri & Calleja Agius, 2020; Pokryszko-Dragan et al., 2021). Additionally, as sympathetic activity correlated with the achievement-related emotion of enjoyment exclusively within face-to-face learning, this study adds a physiological component to previous findings, reporting a lack of positive achievement-related emotions and mental well-being during online learning. As a basis for further research on the influence of physiological parameters—and their possible modulation—in different learning environments, this study provides a starting point for evaluating associated implications in detail.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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