



OPEN Effects of static and dynamic canine-assisted intervention in young adults: sex differences in hormonal, EEG, HRV, and emotional responses

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As mental health disorders like depression and anxiety rise, animal-assisted intervention (AAI) has emerged as a promising complementary approach, recognized for its psychological benefits. However, the underlying physiological mechanisms remain largely unexplored. The present study aims to investigate the impact of animal-assisted intervention on physiological, psychological changes and self-reported measurements. Thirteen young adults participated in three activities: meditation, static interaction with a dog, and dynamic interaction with a dog. Salivary hormone levels were measured by ELISA. Brain activity was recorded using electroencephalography (EEG). Cardiac activity was recorded using electrocardiography (ECG) for heart rate variability (HRV) analysis. Participants' emotional states were assessed using the semantic differential method (SDM). Results revealed sex-specific physiological and neurophysiological responses to AAI. In females, evidence for changes in oxytocin and cortisol was not consistent after multiplicity adjustment, and the point estimate for cortisol following dynamic activity suggested a decrease, although uncertainty remained. The EEG analyses indicated activity-related differences primarily in relative alpha power across multiple electrodes with some sex effects, whereas low-beta and high-beta effects were minimal after correction. Furthermore, SDM scores demonstrated emotional improvement following static and dynamic activities. These findings suggest that physiological and psychological responses after AAI are dependent on both sex and activity type.

Keywords Canine-assisted intervention, Hormones, Brainwaves, Heart rate variability, Sex difference, Emotional states

Mental health disorders, particularly depression and anxiety, are a critical global health challenge, with depression being the leading cause of disability worldwide^{1–3}. Animal-assisted intervention (AAI) has gained attention as a promising non-pharmacological approach for enhancing mental well-being^{4,5}. It can promote psychological stability and reduce stress, thereby improving treatment adherence and potentially mitigating the adverse effects associated with pharmacological treatments^{4,5}. As a complementary approach, AAI may enhance the effectiveness of mental health treatments and improve human quality of life^{6,7}.

Understanding the historical context and theoretical foundations of animal-assisted interventions (AAI) is essential for interpreting their effects. Before discussing the history of AAI, the term should be defined. AAI is defined as “any intervention that intentionally includes or incorporates animals as part of a therapeutic or ameliorative process or milieu⁸”. In the literature, AAI is often used as a collective term that includes animal-assisted therapy (AAT) and animal-assisted activities (AAA)⁸. AAT is a goal-directed intervention for

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an individual client⁸. In AAT, an animal plays a crucial role in the treatment process. AAT is delivered and supervised by a qualified professional within their scope of practice. Core features include individualized goals and standardized evaluation of progress. AAA is designed to provide motivational, educational, recreational, and/or therapeutic benefits⁸. The overall goal is to improve quality of life. AAA can be delivered in diverse settings by trained professionals or volunteers. AAA is a broad category and often lacks a standardized protocol. It can include one or more animals, either individually or in groups.

AAT has been studied for centuries, and animals began to be used in psychiatric facilities to improve patients' social skills in the late 18th century^{9,10}. This history reflects a long-standing recognition that human–animal interaction has therapeutic value. Within this tradition, several frameworks have been proposed to explain the effects of AAI, most notably the biophilia hypothesis¹¹. Biophilia posits that humans have an inherent affinity for nature and other living beings, offering a key framework for the positive effects of AAI. Dogs and certain other animals co-evolved with humans and developed human-like social skills^{12,13}. When calm, these animals can signal safety and comfort to people, which aligns with biophilia and may potentially enhance the effectiveness of AAI. Social support theory and attachment theory also help explain the effects of AAI. Animals can become part of a person's support network and promote emotional stability. They may reduce loneliness, facilitate social interaction, and support stable attachment through human–animal bonds^{14,15}. Collectively, this theoretical background suggests that AAI is based on biological and psychosocial mechanisms, and not solely on the presence of animals.

AAI encompasses activities with animals to promote physical, psychological, social, and cognitive health⁸. These activities are applied in healthcare, education, and social welfare settings and have been reported to provide a range of benefits to individuals. Previous studies have primarily focused on the psychological advantages of AAI, relying on subjective self-reported measures such as stress reduction and emotional improvement^{8,16–18}. However, these studies are limited by their dependence on subjective assessments and the absence of objective physiological validation. To objectively evaluate the impact of AAI, several physiological biomarkers are crucial. Oxytocin and cortisol are key hormones for understanding stress responses and social bonding^{19–22}. Oxytocin promotes parasympathetic nervous system (PNS) activity and emotional stability by enhancing trust and empathy, while suppressing cortisol secretion through inhibition of the hypothalamic–pituitary–adrenal (HPA) axis^{19,20,23}. Cortisol plays a fundamental role in the stress response by mobilizing energy reserves, regulating immune function, and influencing metabolic processes²⁴. Therefore, oxytocin and cortisol are essential biomarkers for evaluating the effects of AAI. Several studies have shown that AAI can increase oxytocin and decrease cortisol levels, thereby alleviating stress^{25–27}.

Additionally, electroencephalography (EEG) and heart rate variability (HRV) also provide additional physiological indicators that reflect stress levels and emotional states, providing a complementary perspective on the effects of AAI. In contrast to hormones, which reflect relatively slow and sustained physiological changes, EEG captures real-time neural activity and moment-to-moment changes in cognitive and emotional states during AAI^{28,29}. This real-time monitoring can clearly demonstrate the instantaneous relaxation or arousal effects induced by AAI. In this study, we selected three waves: relative alpha, low-beta, and high-beta power. Alpha waves indicate a state of relaxation and calmness, whereas beta waves are linked with attention and concentration^{30,31}. Beta waves are divided into low-beta and high-beta^{32,33}. Low-beta waves reflect focused attention and concentration³⁴. In contrast, high-beta waves are associated with nervousness, stress, and hyperarousal, although they also represent enhanced brain activation^{35–37}.

In conjunction with brainwaves, autonomic indices such as HRV provide pivotal information on stress regulation and emotional processes. HRV, defined as the fluctuation in time intervals between consecutive heartbeats, is a non-invasive and important indicator of neurocardiac physiology and overall health³⁸. It is particularly sensitive to emotional and stress responses, making it a valuable measure for examining physiological responses to both acute and chronic stress within the context of AAI^{39–41}.

The efficacy of AAI can be influenced by various factors, particularly the intensity of the intervention and the participant's sex. AAI intensity can be categorized according to the level of physical exertion. Static activities, such as petting or gentle interaction, are typically associated with relaxation and bonding^{6,26,41–45}. Conversely, dynamic activities, involving more vigorous engagement such as agility or disc play, may initially induce arousal but can subsequently lead to enhanced emotional regulation^{6,26,41–49}. Furthermore, participant sex is an important moderating factor. Previous studies have reported sex differences in oxytocin levels and brainwave responses during AAI^{25,50,51}. This highlights the importance of considering both activity intensity and sex when assessing the physiological effects of AAI.

This study aimed to (1) compare the hormonal, neurophysiological, and psychological effects of static and dynamic activities with dogs, and (2) investigate how participant sex modulates these responses. Time-series analyses for dynamic changes in oxytocin and cortisol were used to provide a more comprehensive understanding of the impact of AAI.

Materials and methods

Participants

A total of 13 undergraduate students from a college in Osan, South Korea, were recruited through voluntary enrollment. Participants ranged in age from 19 to 28 years (mean age \pm SD: 22.42 \pm 2.61 years) and included 7 females and 6 males. All participants were required to abstain from smoking and drinking for at least 30 min before the experiment began and until its completion.

This study was conducted as an exploratory/pilot study. A formal sample size calculation was not performed a priori. The sample size ($N=13$) was determined by feasibility, reflecting the number of volunteers who met the eligibility criteria and were available to complete all study procedures in a within-subject design, where each

participant participated in all three activity conditions. Therefore, results are presented as *p* values and effect estimates with 95% confidence intervals (CIs) to inform the design of future adequately powered studies.

All procedures involving human participants were conducted in accordance with relevant guidelines and regulations. The study protocol was reviewed and approved by the Institutional Review Board of Kyungpook National University (permit number: 2024–0489), and written informed consent was obtained from all participants prior to participation, including consent to saliva collection.

Animals

The experiment involved four dogs, consisting of two females and two males, all 2 years old (See Table S1 in the Supplementary Information). The dogs included two Border Collies, one Labrador Retriever, and one Flat-Coated Retriever. The mean body weight \pm SD was 20.79 ± 7.25 kg. All dogs were trained in basic obedience and socialization. To ensure consistency, participants were randomly paired with a dog for each activity.

All experimental procedures involving dogs were approved by the Animal Experimentation Ethics Committee of Kyungpook National University (permit number: 2025–0389) and were carried out in accordance with relevant guidelines and regulations for the care and use of animals in research.

Interventions

Over the 3 weeks, participants engaged in three types of activities: meditation, static activity, and dynamic activity (Fig. 1). The study was conducted at a college in Osan, South Korea. Due to constraints related to the institution's academic calendar and participant availability, the meditation session was conducted in the first study week, whereas the static and dynamic activities were conducted in randomized order during the following 2 weeks.

The activity used in this study was classified based on metabolic equivalent task (MET) values. In general, an activity with a MET value of 1.0 is defined as a resting state, 1.0–3.0 as mild activities, and 3.0–6.0 as moderate activities⁵². The MET values for meditation, static, and dynamic activities performed in this study were measured as 1.0, 2.8–3.0, and 4.0–5.0, respectively.

The meditation was conducted in an empty classroom, where participants engaged in a 10-min meditation. During the meditation, participants remained seated comfortably with their eyes closed. They were not instructed to suppress thoughts or to focus on a specific object (e.g., breathing). Instead, participants were instructed to stay quiet and still, letting thoughts arise naturally without trying to control or restrict them throughout the session. This activity aimed to induce relaxation and serve as a baseline comparison for the other two activities. No interactions with animals occurred during this activity.

The static activity was conducted at an outdoor pet training facility. Participants were introduced to a dog and allowed to freely interact, feed, and walk with the dog for 10 min under the supervision of a professional handler. The walking pace was self-selected, and participants could engage in light petting, verbal communication, and unstructured play with the dog. To differentiate this static activity from the dynamic activity, participants were instructed not to run and to avoid vigorous play (e.g., chasing or highly arousing activities) while remaining within the designated facility area. This condition was designed to simulate casual pet interactions commonly used in AAI.

The dynamic activity was also conducted at an outdoor pet training facility, where participants engaged in two structured dog sports: Disc dog and agility. Disc dog involves catching a frisbee mid-air and requires high-energy movement from both the dog and the handler. Agility involves guiding a dog through a structured

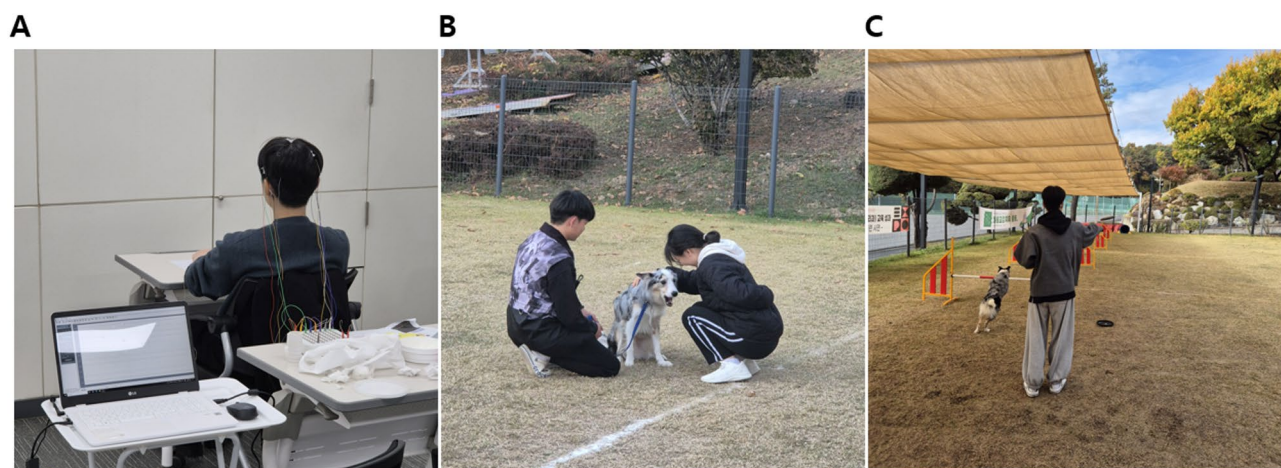


Fig. 1. Activities. All activities were conducted for 10 min. (A) Meditation was conducted in an empty room where participants were seated with their eyes closed, without animal interaction. (B) Static activity was conducted at an outdoor facility where participants freely interacted with a dog through activities such as feeding, petting, and walking. (C) Dynamic activity involved structured dog-sport tasks, including disc dog and agility, performed for 5 min each.

obstacle course, requiring precise coordination and communication between the participant and the dog. Each activity was performed for 5 min, and a brief description of the activity was provided before each session.

Saliva sampling

For saliva sampling, participants placed a Salivette tube (Sarstedt Salivette, blue cap, Article No. 51.1534.500, Nümbrecht-Rommelsdorf, Germany) under the tongue for 1 min before handing it to the experimenter. Saliva samples were collected at seven time points: 10 min before the activity (T-10), 5 min before the activity (T-5), immediately before the activity (T-1), immediately after the activity (T1), 10 min after the activity (T10), 20 min after the activity (T20), and 30 min after the activity (T30). The collected saliva samples were stored in an icebox at 4 °C until transport to the laboratory. Upon arrival, samples were centrifuged at 1000 × g for 2 min at 4 °C to separate the saliva from the Salivette tube. The separated saliva was then stored at –80 °C until hormone analysis.

EEG

EEG were categorized into five types based on their frequency bands: Delta (0.5–4 Hz), Theta (4–8 Hz), Alpha (8–13 Hz), Beta (13–30 Hz), and Gamma (above 30 Hz)^{53–55}. Among these, alpha and beta waves are commonly used to evaluate stress. Alpha waves indicate a state of relaxation and calmness, whereas beta waves are linked with attention and concentration. Beta waves are divided into low-beta (13–19 Hz) and high-beta (20–30 Hz)^{32,33}.

EEG data were acquired using the QEEG-64Fx (Laxtha Inc., Daejeon, South Korea) system and analyzed using TeleScan (CD-TS-3.1, Laxtha, South Korea). Resting-state EEG was recorded for 180 s while participants were seated and instructed to remain still with eyes closed. Data were sampled at 250 Hz and referenced online to A2 (device default), with no offline re-referencing applied. Online filtering consisted of a 0.5–50 Hz band-pass filter with a 50 Hz notch filter. Prior to each recording, electrode contact quality was checked using the device's impedance/quality-check function (impedance measured between each electrode and ground at AC 62.5 Hz), and channels were adjusted until the software-defined “Good” criterion was met (voltage measuring accuracy < 0.1%); per-session numeric impedance values (kΩ) were not exported/logged by the system. EEG signals were visually inspected, and segments showing gross transient spikes or obvious artifact contamination were removed before spectral computation. No additional automated artifact correction procedures and no independent component analysis were applied. Spectral power was estimated in TeleScan (CD-TS-3.1) using the built-in power spectrum estimation function. Spectra were computed from artifact-free continuous segments; no moving-window (overlap) procedures or user-defined windowing were applied. Relative band power was defined as band-limited power divided by total power across 0.5–50 Hz. Relative alpha power was calculated as 8–12 Hz power/total power (0.5–50 Hz), relative low-beta power as 13–19 Hz power/total power (0.5–50 Hz), and relative high-beta power as 20–30 Hz power/total power (0.5–50 Hz). Furthermore, spectral power was computed per electrode and averaged across artifact-free segments within each condition for each participant; no bilateral or region-based spatial averaging was performed.

During EEG and electrocardiography (ECG) recording, participants kept their eyes closed to minimize artifacts caused by eye blinks. Data were collected using Telescan software (CD-TS-3.2.9, Laxtha Inc., Daejeon, South Korea). For EEG, electrodes were placed according to the international 10–20 system, which assigns positions based on brain regions: prefrontal (Fp), frontal (F), temporal (T), and parietal (P). Eight channels were used: Fp1, Fp2, F3, F4, T3, T4, P3, and P4 (Fig. 2). Electrodes were attached with conductive paste (Laxtha Inc., Daejeon, South Korea), and reference electrodes were placed on the left (A1) and right auricular sites (A2) (Fig. 2). EEG signals were filtered between 0.5 and 50 Hz using a bandpass fast fourier transform (FFT) filtering.

ECG

ECG signals were recorded using the QEEG-64Fx system (Laxtha, South Korea) with electrodes attached to the inner side of both wrists for 3 min at pre- and post-activity, and the signals were exported for offline processing. RR intervals were derived in R (RStudio) by detecting R-peaks from the exported ECG time series, followed by visual inspection and manual adjustment when needed to correct missed or spurious detections. Segments showing evident double detections of R-peaks (i.e., two peaks detected within a single cardiac cycle) were removed; recordings shorter than 60 s after this cleaning step were excluded. The resulting RR series was exported as an ASCII text file (space-delimited; no header; time in column 2 in seconds; RR in column 3 in milliseconds) and imported into Kubios HRV Scientific Lite (ver 4.1.1; Kubios Oy, Kuopio, Finland) for HRV computation. Beat correction was not applied in Kubios. Frequency-domain indices were computed using FFT-based Welch's periodogram as implemented in Kubios, with bands defined as very low frequency (VLF) 0–0.04 Hz, low frequency (LF) 0.04–0.15 Hz, and high frequency (HF) 0.15–0.40 Hz. Respiration was not paced or directly monitored; participants breathed spontaneously.

HRV outcomes included PNS index, sympathetic nervous system (SNS) index, stress index, standard deviation of normal-to-normal intervals (SDNN), root mean square of successive RR interval differences (RMSSD), and LF/HF ratio. The PNS and SNS indices are composite metrics provided by Kubios that summarize autonomic modulation compared to normal resting values. In Kubios outputs, the PNS index relates to Mean RR/RMSSD/SD1, while the SNS index relates to Mean HR/stress index/SD2. Accordingly, these indices reflect autonomic modulation/arousal, not direct sympathetic nerve activity. The stress index in Kubios is the square root of Baevsky's stress index, serving as a geometric HRV measure of cardiovascular stress/strain; higher values indicate higher stress and reduced variability, but do not represent direct sympathetic nerve activity. The RMSSD indicates the variability in time intervals between successive heartbeats⁵⁶. It is the primary time-domain measure used to estimate parasympathetic activity in HRV, as RMSSD is influenced by PNS activity. SDNN reflects both PNS and SNS activity and is widely used to evaluate cardiovascular health^{56,57}. SDNN reflects overall HRV over the recording period and is widely used to evaluate cardiovascular health⁵⁶. Lower SDNN shows decreased overall HRV, commonly seen with stress or higher physiological demands. The LF/HF ratio was reported as

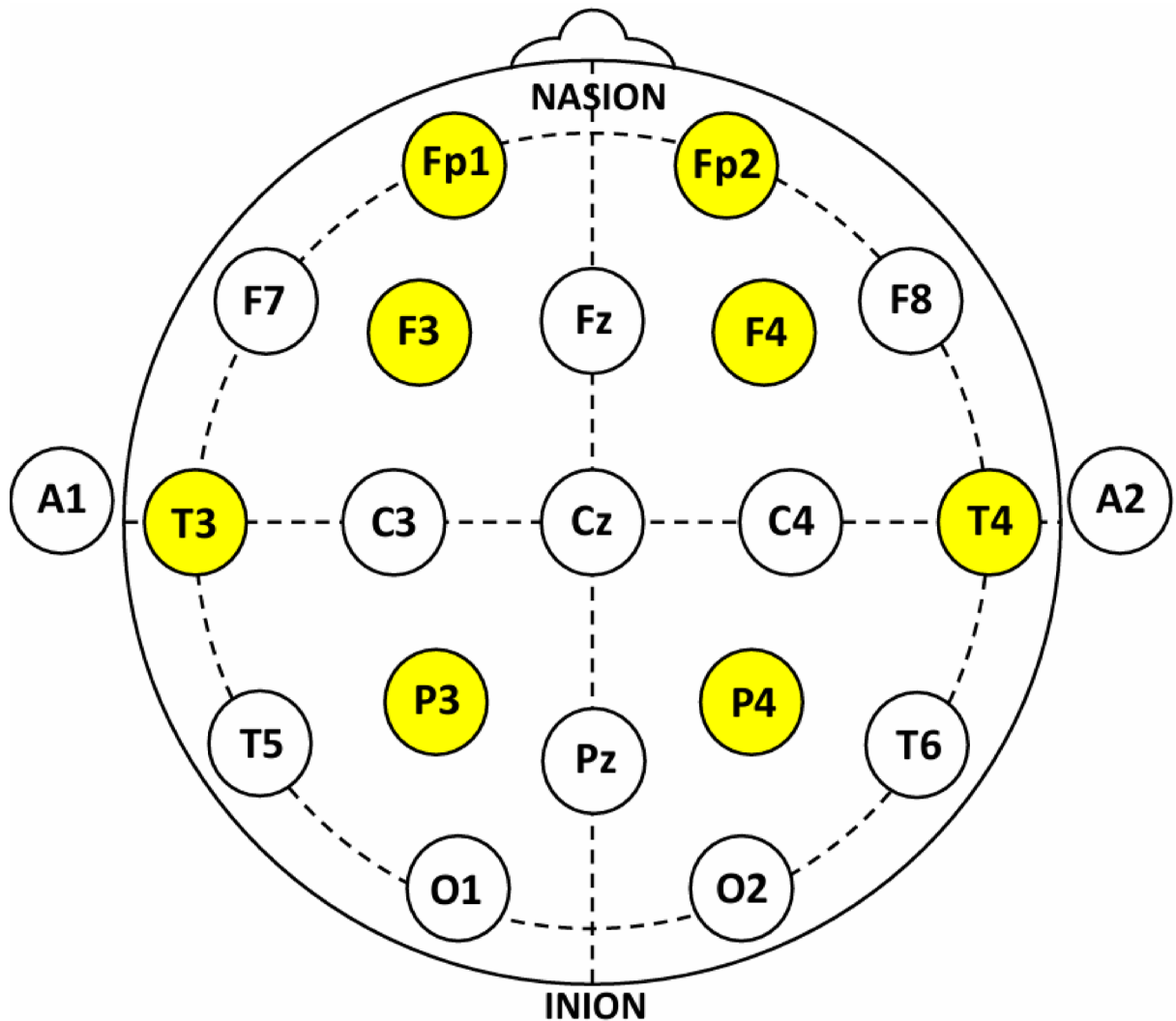


Fig. 2. The international 10–20 system. EEG recording channels were based on the international 10–20 system. Colored regions indicated the EEG channels used in this study. We collected ECG data in prefrontal (Fp1, Fp2), frontal (F3, F4), temporal (T3, T4), and parietal (P3, P4) lobes.

a descriptive frequency-domain metric, but it was not interpreted as “sympathovagal balance” due to ongoing methodological debate regarding this interpretation.

Self-reporting

The semantic differential method (SDM) was used before and after each activity to assess participants’ subjective emotional responses. The SDM evaluates the significance of each item related to participants’ emotional responses. SDM uses nine bipolar adjective pairs presented on a Likert scale to measure an individual’s subjective feelings. All items were selected based on established emotion models that represent basic emotions and their relationships^{58,59}. To enable subscale scoring, items were categorized via principal factor analysis (PFA). The final analyses used six pairs. The adjective pairs were ‘Pessimistic-Optimistic’, ‘Lethargic-Energetic’, ‘Depressed-Cheerful’, ‘Anxious-Calm’, ‘Nervous-Stable’, and ‘Feel pressured-Feel at ease’. Higher scores on each item indicate stronger positive emotional responses.

Additionally, the pet attitude scale (PAS) was used to assess participants’ attitudes toward pets⁶⁰. The PAS consists of 18 items (See Table S2 in the Supplementary Information). The total score ranges from 18 to 126, with higher scores indicating a more positive attitude toward pets. The original English version was translated using a forward–backward translation procedure^{61,62}.

Analysis of saliva samples

Saliva samples were analyzed by ELISA. Analyses were conducted using undiluted saliva samples in duplicate, and absorbance was measured using a Sunrise Absorbance Microplate Reader (Tecan, Mannedorf, Switzerland).

Oxytocin ELISA

An oxytocin ELISA kit (ADI-900-153 A, Enzo Life Sciences, NY, USA) was utilized to quantify oxytocin levels in saliva, following the manufacturer's instructions. The sensitivity of the kit was 15.6 pg/ml, and absorbance was measured at 405 nm. The intra-assay coefficient of variation (CV) was 8.26%, and the inter-assay CV was 18.99%.

Due to insufficient availability of saliva samples from one female participant, oxytocin ELISA analysis was performed using saliva samples from only six female participants for the meditation and static activity. Additionally, as one female participant did not participate in the dynamic activity, ELISA analysis for that condition included samples from only five female participants.

Cortisol ELISA

A cortisol ELISA kit (1-3002, Salimetrics, CA, USA) was used to quantify cortisol levels in saliva, following the manufacturer's instructions. The sensitivity of the kit was 0.018 µg/dL. Absorbance was measured at 450 nm. The intra-assay CV was 3.58%, while the inter-assay CV was 8.29%. As one female participant did not participate in the dynamic activity, cortisol ELISA analysis was performed using saliva samples from only six female participants for this condition.

Statistical analysis

SDM was categorized using SPSS software (version 29.0.2.0; IBM, Armonk, NY, USA). We used PFA to categorize items of the nine pair SDM. Sampling adequacy was assessed with Kaiser-Meyer-Olkin (KMO) and Bartlett's test (KMO = 0.831; Bartlett's $\chi^2(36) = 244.726, p < .001$). The number of factors was determined by the Kaiser criterion (eigenvalues > 1) and the scree plot. Varimax rotation was applied. Items were retained if the primary loading was ≥ 0.60 and, to ensure simple structure under varimax rotation, either all cross-loadings were ≤ 0.30 or the difference between the primary and the next-highest loading (Δ) was ≥ 0.20 . The final subscales comprised Factor 1 (Affective state) and Factor 2 (Vitality). Internal consistency was good (Cronbach's α of six pair SDM = 0.86; Affective state $\alpha = 0.94$; Vitality $\alpha = 0.80$).

SDM data were analyzed using a linear mixed-effects model (PROC MIXED) in SAS (version 9.4; SAS Institute, Cary, NC, USA). Sex (female and male), activity (meditation, static activity, and dynamic activity), time (pre and post), and all two- and three-way interactions were specified as fixed effects. Parameters were estimated using restricted maximum likelihood (REML), and the Kenward–Roger method was used to obtain denominator degrees of freedom and standard errors for fixed effects. Least-squares means (LSMeans) were estimated for activity×time and sex×activity×time, and planned pairwise contrasts were evaluated with multiplicity adjustment using the Sidak method, and adjusted p values and 95% CI are reported. Statistical significance was set at $\alpha = 0.05$, and effect sizes for Type III tests were reported as partial eta squared (η^2).

Hormone data were analyzed using PROC MIXED in SAS (version 9.4) with REML estimation. Fixed effects included sex, activity type (meditation, static activity, and dynamic activity), time (seven repeated measurements), and all interactions (sex×activity×time). Participant ID was treated as a subject-level random effect, and repeated measures over time within each activity were modeled with an unstructured covariance matrix at the ID×activity level. Degrees of freedom and standard errors were computed using the Kenward–Roger method. Type III tests were used to evaluate fixed effects. For planned post hoc comparisons, LSMeans and pairwise differences were obtained with multiplicity adjustment, and adjusted p values with adjusted 95% CIs were reported. η^2 was calculated from Type III F-tests.

EEG and HRV data were analyzed using PROC MIXED in SAS (version 9.4). For each outcome, PROC MIXED was fitted to test the fixed effects of sex (between-subject factor), activity (three levels), time (two levels; pre vs. post), and their interactions (sex×activity, sex×time, activity×time, and sex×activity×time). To account for within-subject dependence due to repeated measurements across activities and time points, participant-level clustering was modeled within the mixed-effects framework. For EEG, models were conducted separately for each electrode and frequency band. For HRV, the same modeling strategy was applied to each index. Fixed effects were evaluated using Type III tests of fixed effects, and results are reported as F statistics with numerator and denominator degrees of freedom and corresponding p values. Furthermore, multiplicity in pairwise testing was controlled using Sidak adjustment, and adjusted p values and 95% adjusted CIs were reported. Tests of effect slices were used to probe simple effects when interactions were examined. Effect sizes were presented as η^2 for Type III fixed-effect tests. All statistical tests were two-sided with a significance threshold of $\alpha = 0.05$.

Results

PAS

The PAS was used to categorize participants based on their attitudes toward pets. The mean PAS score \pm SEM was 102.39 ± 2.16 , with a maximum possible score of 126. No sex differences were found in PAS scores (females: 103 ± 1.23 ; males: 101.67 ± 4.67). Participants' scores ranged from 87 to 119, indicating that all participants had positive attitudes toward companion animals (See Table S3 in the Supplementary Information).

Changes in SDM subscales following different activity types

For affective state, across-activity contrasts at each time point did not provide clear evidence of differences in the total sample. The same pattern was observed in females, whereas the main effects of sex ($F(1, 11) = 0.09, p = .7658, \eta^2 = 0.008$) and activity ($F(2, 18.4) = 0.73, p = .4931, \eta^2 = 0.074$), as well as all interaction terms (all $p \geq .2359$), suggested minimal between-group variation as shown in Fig. 3.

For vitality, across-activity contrasts at pre did not indicate clear differences in the total sample. In females, across-activity contrasts did not indicate clear differences at either pre or post. In males, across-activity contrasts were also comparable at pre. At the model level, the main effects of time ($F(1, 32) = 18.06, p = .0002, \eta^2 = 0.361$)

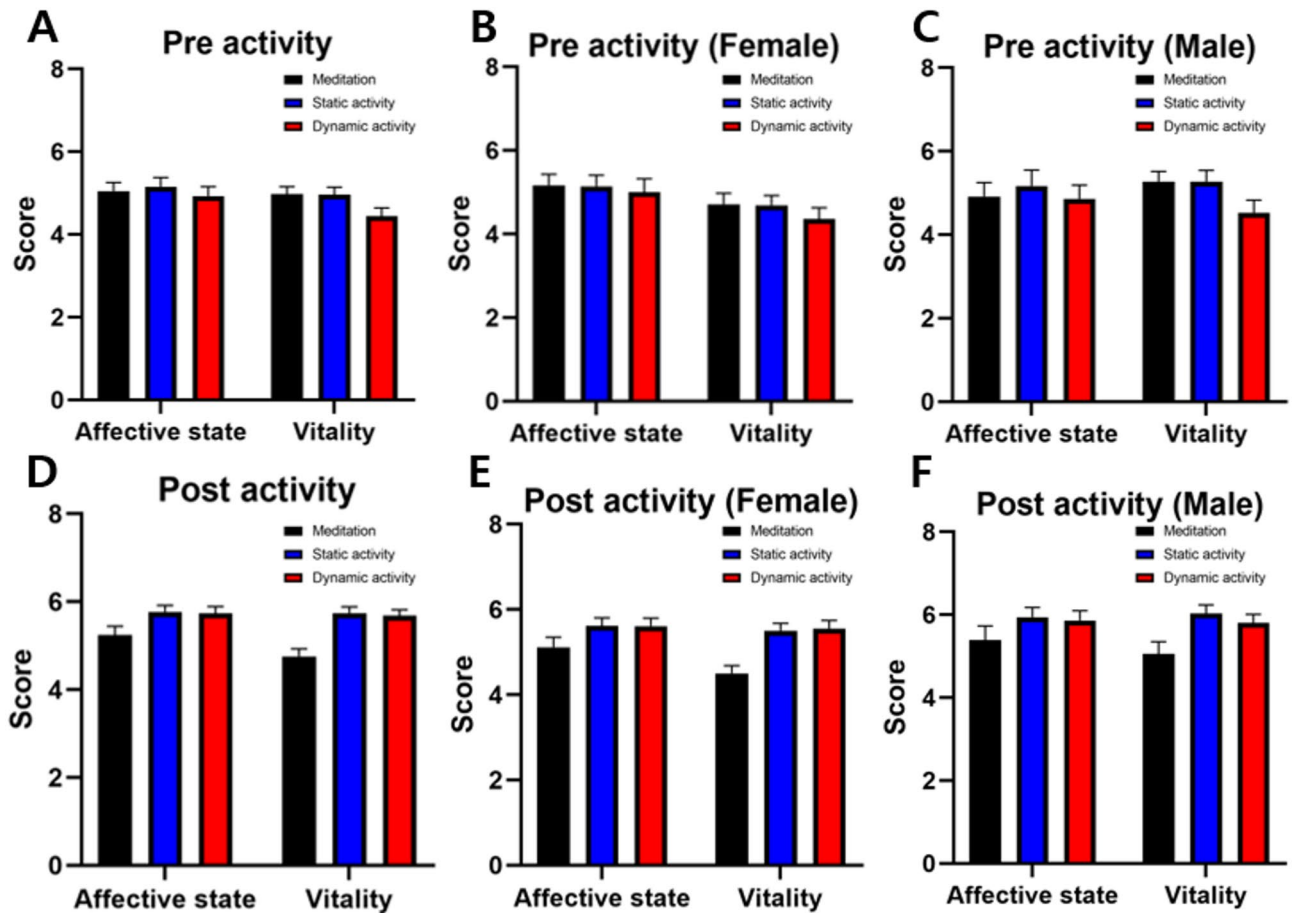


Fig. 3. Pre- and post-activity scores in semantic differential method (SDM) factor scores for all participants, females, and males. SDM items were categorized via PFA into two factors: Affective state (feel at ease, stable, calm) and Vitality (cheerful, energetic, optimistic). Bars represent mean \pm SEM for meditation (black), static activity (blue), and dynamic activity (red). (A–C) Pre-activity results for all participants, females, and males, respectively. (D–F) Post-activity results for all participants, females, and males, respectively. No significance markers are shown; statistical inference is reported in the results based on linear mixed-effects models (SAS PROC MIXED; REML; Kenward–Roger degrees of freedom) and estimated marginal mean contrasts with multiplicity-adjusted p values and 95% confidence intervals.

and activity ($F(2, 21.2) = 4.80, p = .0190, \eta^2 = 0.312$) were most consistent with, and the activity \times time interaction showed limited evidence of differences ($F(2, 32) = 9.27, p = .0007, \eta^2 = 0.367$). However, Sidak-adjusted across-activity post hoc contrasts at each time point did not identify any statistically robust between-activity differences (all adjusted $p > .05$), indicating that the significant omnibus interaction reflects an overall difference in pre–post change patterns across activities rather than multiplicity-adjusted between-condition effects at a specific time point.

For affective state, planned within-activity pre–post contrasts (MD = post–pre) indicated MD = 0.339 (adjusted 95% CI [–0.532, 1.211], adjusted $p = .9778$) for meditation, MD = 0.714 (adjusted 95% CI [–0.158, 1.585], adjusted $p = .1920$) for static activity, and MD = 1.034 (adjusted 95% CI [0.104, 1.963], adjusted $p = .0202$) for dynamic activity (Fig. 4). Sex-stratified within-activity pre–post contrasts did not provide clear evidence of change after adjustment in females (meditation: MD = 0.097, adjusted 95% CI [–1.297, 1.491], adjusted $p = 1.000$; static: MD = 0.594, adjusted 95% CI [–0.800, 1.988], adjusted $p = .9998$; dynamic: MD = 1.014, adjusted 95% CI [–0.576, 2.603], adjusted $p = .7900$) or in males (meditation: MD = 0.582, adjusted 95% CI [–0.924, 2.087], adjusted $p = 1.000$; static: MD = 0.833, adjusted 95% CI [–0.672, 2.339], adjusted $p = .9574$; dynamic: MD = 1.053, adjusted 95% CI [–0.452, 2.559], adjusted $p = .5961$). Sex differences at matched activity \times time conditions were not detected (meditation pre: female–male MD = 0.093, adjusted 95% CI [–2.387, 2.573], adjusted $p = 1.000$; meditation post: MD = –0.392, adjusted 95% CI [–2.872, 2.088], adjusted $p = 1.000$; static pre: MD = –0.098, adjusted 95% CI [–2.578, 2.382], adjusted $p = 1.000$; static post: MD = –0.337, adjusted 95% CI [–2.817, 2.143], adjusted $p = 1.000$; dynamic pre: MD = –0.072, adjusted 95% CI [–2.605, 2.461], adjusted $p = 1.000$; dynamic post: MD = –0.111, adjusted 95% CI [–2.644, 2.422], adjusted $p = 1.000$) (Fig. 4). Overall, affective state increased from pre to post at the model level, but between-activity differences and sex-related differences were not supported; the only statistically reliable pre–post increase under multiplicity control was observed for dynamic activity in the total sample.

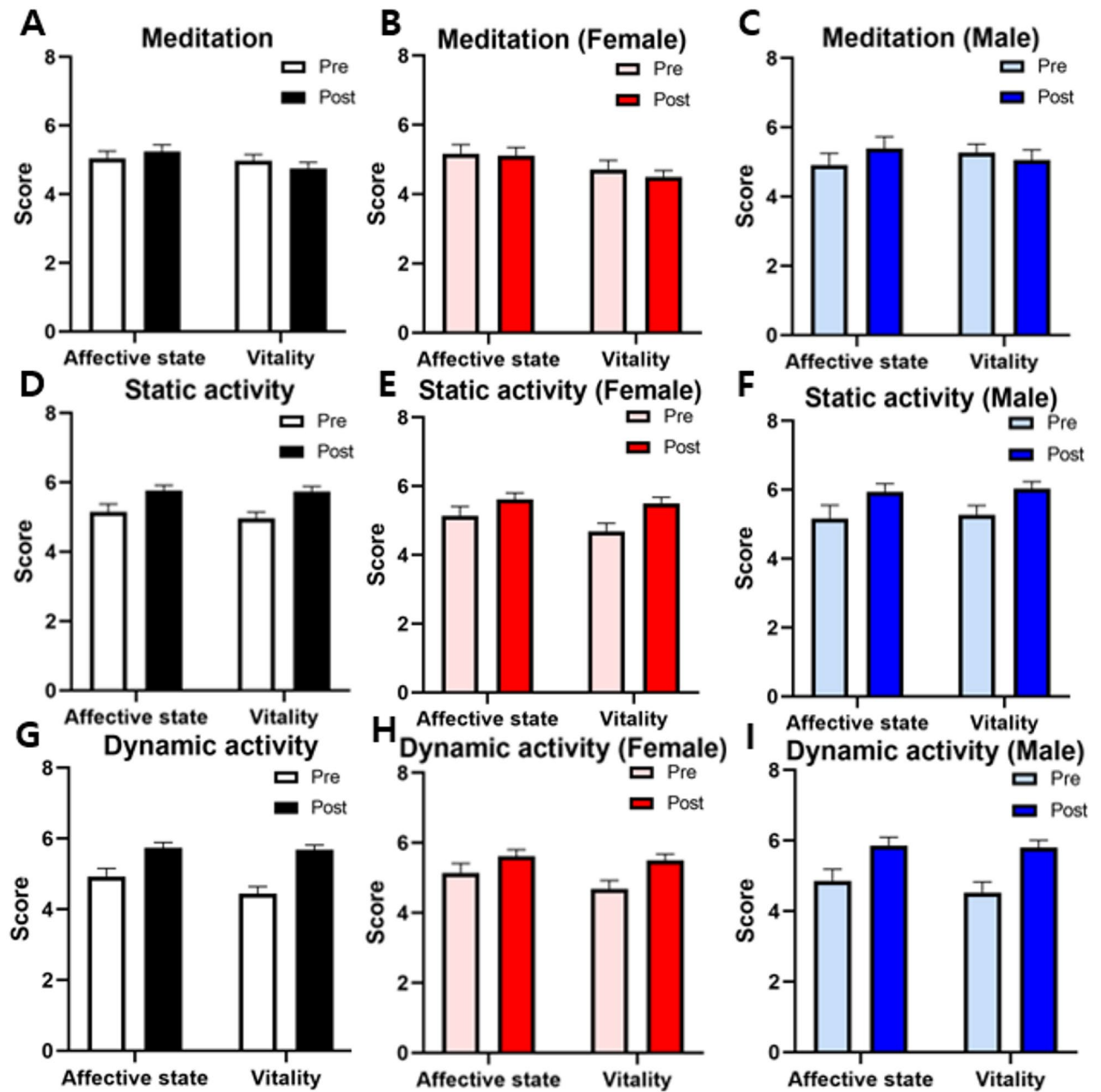


Fig. 4. Pre- and post-changes in SDM factor scores for each activity. The SDM items were categorized via PFA into two factors: Affective state (feel at ease, stable, calm), and Vitality (cheerful, energetic, optimistic). Within each panel, bars show pre-scores (lighter) and post-scores (darker). Values are presented as mean \pm SEM. (A–C) Results for meditation in all participants, females, and males, respectively. (D–F) Results for static activity in all participants, females, and males, respectively. (G–I) Results for dynamic activity in all participants, females, and males, respectively. No significance markers are shown; statistical inference is reported in the results based on PROC MIXED and estimated marginal mean contrasts with multiplicity-adjusted p values and 95% confidence intervals.

For vitality, planned within-activity pre–post contrasts (MD = post–pre) showed MD = -0.219 (adjusted 95% CI [$-0.981, 0.544$], adjusted $p = .9990$) for meditation, MD = 0.780 (adjusted 95% CI [$0.018, 1.543$], adjusted $p = .0415$) for static activity, and MD = 1.236 (adjusted 95% CI [$0.444, 2.027$], adjusted $p = .0004$) for dynamic activity. Sex-stratified within-activity pre–post contrasts did not provide clear evidence of change after adjustment in females (meditation: MD = -0.216 , adjusted 95% CI [$-1.432, 1.001$], adjusted $p = 1.000$; static: MD = 0.809 , adjusted 95% CI [$-0.408, 2.025$], adjusted $p = .7201$; dynamic: MD = 1.193 , adjusted 95% CI [$-0.121, 2.507$], adjusted $p = .1216$) or in males (meditation: MD = -0.222 , adjusted 95% CI [$-1.536, 1.092$], adjusted $p = 1.000$; static: MD = 0.752 , adjusted 95% CI [$-0.562, 2.066$], adjusted $p = .9389$; dynamic: MD = 1.278 , adjusted 95% CI [$-0.036, 2.592$], adjusted $p = .0654$) (Fig. 4). Sex differences at matched activity \times time conditions were not

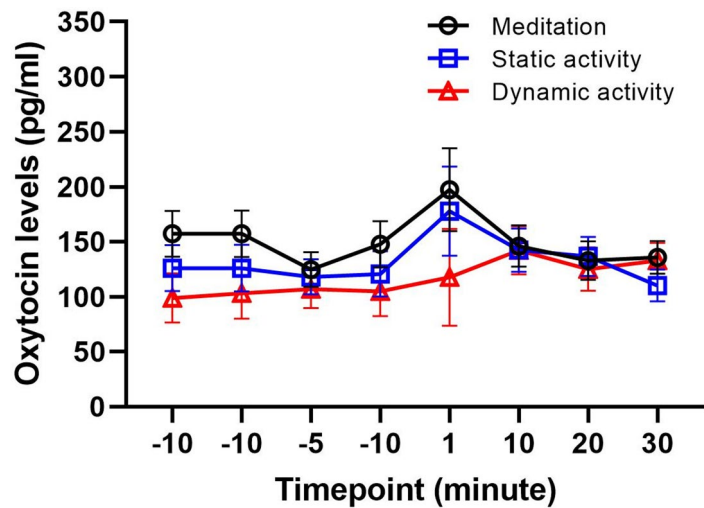


Fig. 5. Time-series changes in salivary oxytocin levels across different activity types. This figure shows time-series changes in salivary oxytocin levels across multiple time points for three activity types in all participants: meditation (black circles), static activity (blue squares), and dynamic activity (red triangles).

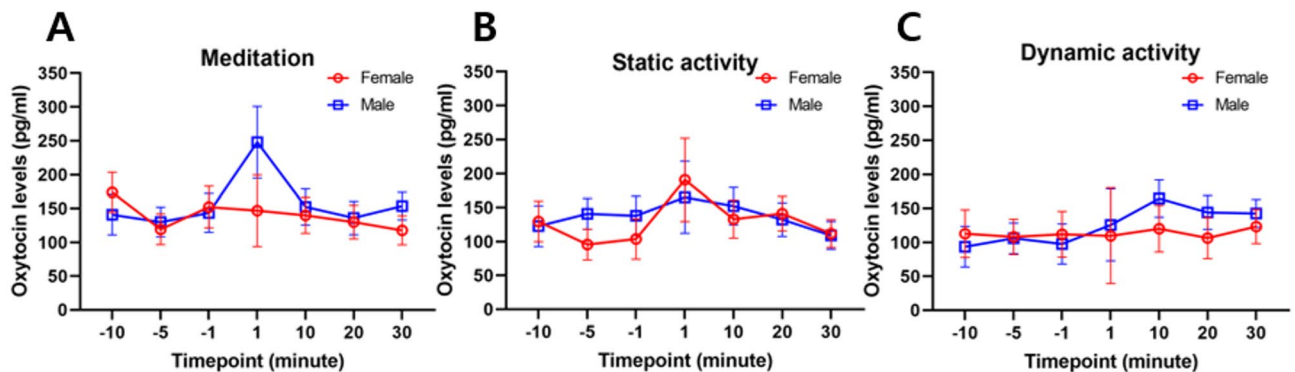


Fig. 6. Sex-specific time-series oxytocin levels pre- and post-each activity. Oxytocin levels were measured at different time points pre- and post-each activity. Red circles and blue squares denote females and males, respectively. (A–C) Meditation, static activity, and dynamic activity, respectively.

detected (female–male: meditation pre MD = -0.564 , adjusted 95% CI [$-2.377, 1.248$], adjusted $p = 1.000$; meditation post MD = -0.558 , adjusted 95% CI [$-2.370, 1.254$], adjusted $p = 1.000$; static pre MD = -0.585 , adjusted 95% CI [$-2.398, 1.227$], adjusted $p = 1.000$; static post MD = -0.528 , adjusted 95% CI [$-2.341, 1.284$], adjusted $p = 1.000$; dynamic pre MD = -0.156 , adjusted 95% CI [$-2.006, 1.695$], adjusted $p = 1.000$; dynamic post MD = -0.241 , adjusted 95% CI [$-2.091, 1.610$], adjusted $p = 1.000$) (Fig. 4). Overall, vitality increased from pre- to post-session, and the pattern of contrasts indicated that the post-session advantage was specific to static and dynamic activities in the total sample, with no evidence of sex-related differences under multiplicity control.

Salivary Oxytocin concentration following different activity types

For all participants, time-series profiles of salivary oxytocin across the three activity types are shown in Fig. 5. For oxytocin, PROC MIXED showed no main effect of time ($F(6, 15.4) = 1.71, p = .1860, \eta^2 = 0.3996$), activity ($F(2, 11.6) = 1.45, p = .2729, \eta^2 = 0.1999$), or sex ($F(1, 8.94) = 0.19, p = .6743, \eta^2 = 0.0207$), and no evidence for interaction effects (sex \times time: $F(6, 15.4) = 1.40, p = .2779, \eta^2 = 0.3525$; activity \times time: $F(12, 21.9) = 0.93, p = .5384, \eta^2 = 0.3365$; sex \times activity: $F(2, 11.6) = 0.05, p = .9539, \eta^2 = 0.0081$; sex \times activity \times time: $F(12, 21.9) = 0.87, p = .5828, \eta^2 = 0.3235$). Planned post hoc contrasts did not survive Sidak correction; no sex differences at matched activity \times time conditions and no within-sex time-point changes provided robust evidence under multiplicity control (all adjusted $p > .05$; Supplementary Table S4).

Sex-stratified time-series oxytocin levels for each activity type are presented in Fig. 6.

Time-series changes in salivary oxytocin levels for each activity type are shown in Fig. 7.

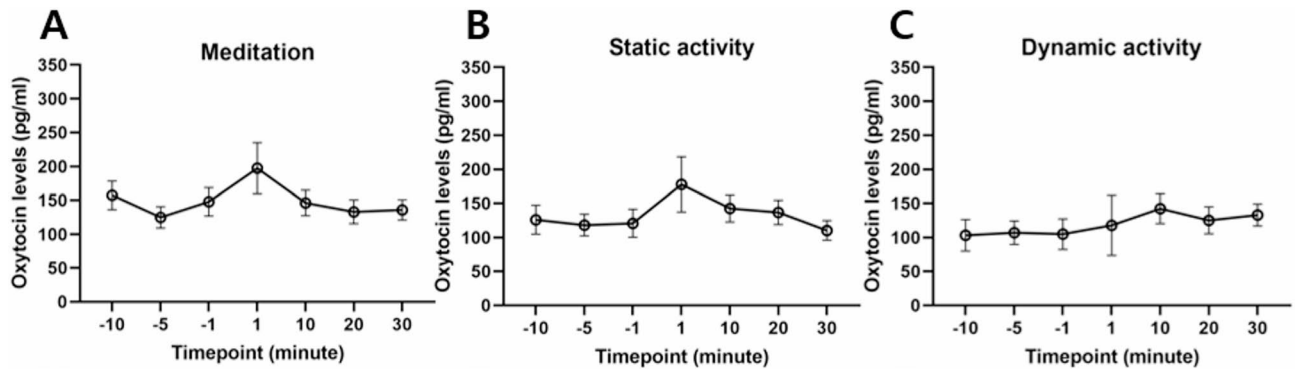


Fig. 7. Time-series changes in salivary oxytocin levels pre- and post-each activity in all participants. Oxytocin levels were measured at different time points pre- and post-each activity. Black circles denote all participants. (A–C) Meditation, static activity, and dynamic activity, respectively.

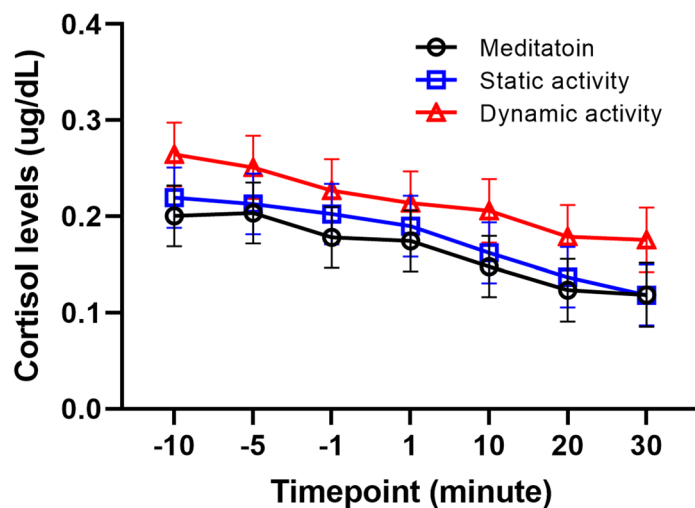


Fig. 8. Time-series changes in salivary cortisol levels across different activity types. This figure shows time-series changes in salivary cortisol levels measured at multiple time points for three activity types in all participants: meditation (black circles), static activity (blue squares), and dynamic activity (red triangles).

Salivary cortisol concentration following different activity types

For cortisol, PROC MIXED indicated evidence of a time-related pattern ($F(6, 21.4) = 3.75, p = .0105, \eta^2 = 0.513$), consistent with an overall temporal decrease in cortisol from baseline to later post-activity sampling points (Fig. 8). In contrast, the activity term did not provide evidence of differences ($F(2, 27.2) = 0.85, p = .4392, \eta^2 = 0.0586$), and activity-related interactions were not supported (activity \times time: $F(12, 30.9) = 0.64, p = .7945, \eta^2 = 0.198$; sex \times activity: $F(2, 27.2) = 1.49, p = .2435, \eta^2 = 0.0985$; sex \times activity \times time: $F(12, 30.9) = 0.66, p = .7752, \eta^2 = 0.2039$). Together, these results suggest that cortisol showed a broadly similar time course across activity types under this model (Supplementary Table S5).

Sex-stratified time-series cortisol levels for each activity type are presented in Fig. 9. Planned within-activity contrasts occasionally yielded nominal p values below .05 prior to adjustment; however, after Sidak correction, the contrasts did not provide clear evidence of within-activity changes (all adjusted $p > .05$).

Time-series changes in salivary cortisol levels for each activity type are shown in Fig. 10. Full planned-contrasts outputs are provided in Supplementary Table S5-5.

EEG following different activity types

For relative alpha power across electrodes, Type III tests indicated evidence consistent with activity-related differences across several electrodes (Fp1, Fp2, T3, T4, P3, P4; all $p \leq .0325$), whereas activity \times time interactions did not show clear evidence of effects at these sites ($p > .05$). Sidak-adjusted post hoc comparisons at the post indicated higher alpha power in dynamic activity than meditation at F3 (estimate = -0.111 , 95% adjusted CI [$-0.209, -0.013$], adjusted $p = .0166$), T3 (estimate = -0.104 , 95% adjusted CI [$-0.197, -0.012$], adjusted $p = .0171$), T4 (estimate = -0.109 , 95% adjusted CI [$-0.210, -0.0069$], adjusted $p = .0286$), P3 (estimate = -0.211 , 95% adjusted CI [$-0.371, -0.050$], adjusted $p = .0034$), and P4 (estimate = -0.170 , 95% adjusted CI [$-0.322, -0.0188$],

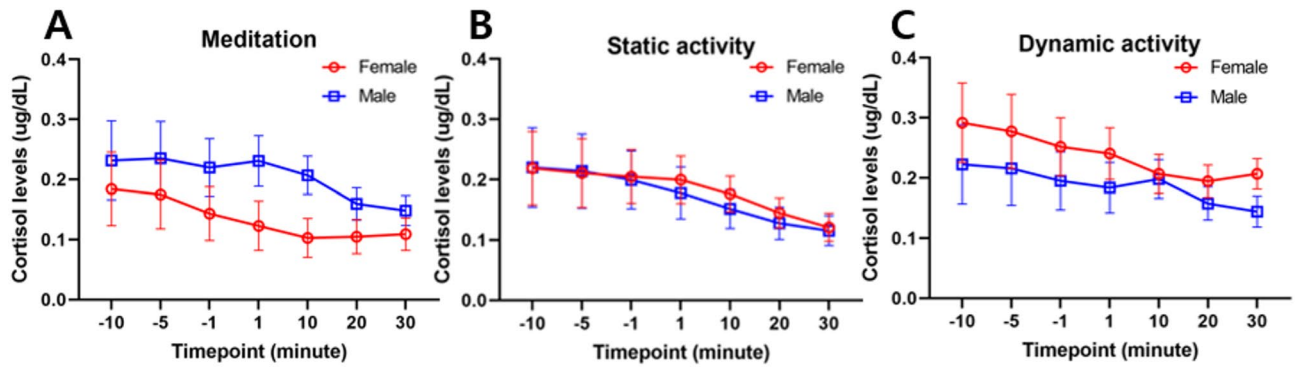


Fig. 9. Sex-specific time-series cortisol levels pre- and post-each activity. Cortisol levels were measured at different time points pre- and post-each activity. Red circles and blue squares denote females and males, respectively. (A–C) Meditation, static activity, and dynamic activity, respectively.

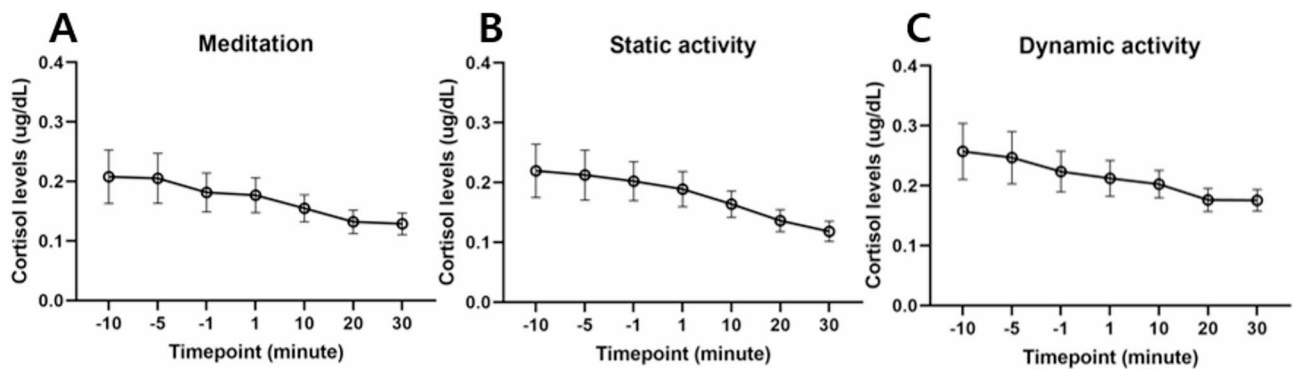


Fig. 10. Time-series changes in salivary cortisol levels pre- and post-each activity in all participants. Cortisol levels were measured at different time points pre- and post-each activity. Black circles denote all participants. (A–C) Meditation, static activity, and dynamic activity, respectively.

adjusted $p = .0179$). In addition, at P3, static activity exceeded meditation post-intervention (estimate = -0.178 , 95% adjusted CI [$-0.335, -0.0217$], adjusted $p = .0158$). At pre, no Sidak-adjusted between-activity contrasts were supported in the provided LSMeans outputs (all adjusted $p > .05$), as shown in Fig. 11.

For sex-related effects, omnibus tests revealed evidence consistent with sex differences at Fp1 ($F(1,11.1) = 5.98$, $p = .0324$, $\eta^2 = 0.351$), Fp2 ($F(1,11) = 7.20$, $p = .0212$, $\eta^2 = 0.395$), F4 ($F(1,11.1) = 8.35$, $p = .0145$, $\eta^2 = 0.428$), and T4 ($F(1,11.2) = 6.87$, $p = .0235$, $\eta^2 = 0.381$). Additionally, sex \times activity interactions showed evidence of effects at Fp1 ($F(2,21.1) = 4.72$, $p = .0202$, $\eta^2 = 0.309$) and Fp2 ($F(2,21.1) = 5.18$, $p = .0148$, $\eta^2 = 0.329$), whereas at other electrodes these interaction terms did not show clear evidence of effects ($p > .05$). However, cell-wise female–male comparisons at matched activity \times time levels did not provide clear evidence after Sidak correction in the available LSMeans difference tables (all adjusted $p > .05$ and adjusted 95% CIs included 0), indicating that these omnibus sex-related terms did not translate into Sidak-adjusted within-cell differences (Fig. 12). Likewise, Sidak-adjusted within-activity pre–post contrasts (overall and sex-stratified) did not provide clear evidence of change (all adjusted $p > .05$).

For relative low-beta power across electrodes, Type III tests provided evidence consistent with activity-related differences at T3 and T4 (T3: $F(2, 21.1) = 4.73$, $p = .0201$, $\eta^2 = 0.309$; T4: $F(2, 21.3) = 4.12$, $p = .0308$, $\eta^2 = 0.279$), whereas other electrodes and activity \times time interactions did not provide evidence of effects ($p > .05$). Sidak-adjusted post hoc pairwise comparisons among activity \times time LSMeans did not provide clear evidence of differences at any electrode (all adjusted $p > .05$) (Fig. 11).

No evidence of sex-related main effects or interactions was observed in relative low-beta power, and Sidak-adjusted sex differences within each activity \times time cell were not supported (all adjusted $p > .05$). Within-activity pre–post contrasts (overall and sex-stratified) likewise did not provide clear evidence of change after Sidak adjustment (all adjusted $p > .05$), as shown in Fig. 13.

For relative high-beta power across electrodes, activity \times time interaction did not show clear evidence of effects, and most within-time activity pairwise comparisons did not provide clear evidence of differences after Sidak correction (all adjusted $p > .05$). However, F3 showed evidence consistent with activity-related differences ($F(2,21.3) = 4.82$, $p = .0188$, $\eta^2 = 0.3119$), with a post difference indicating lower values in meditation than static activity: estimate = -0.02566 , $df = 39.2$, adjusted $p = .0127$, 95% adjusted CI [$-0.04772, -0.00361$]. No other electrode showed clear Sidak-adjusted evidence of activity differences within the same time point (Fig. 11).

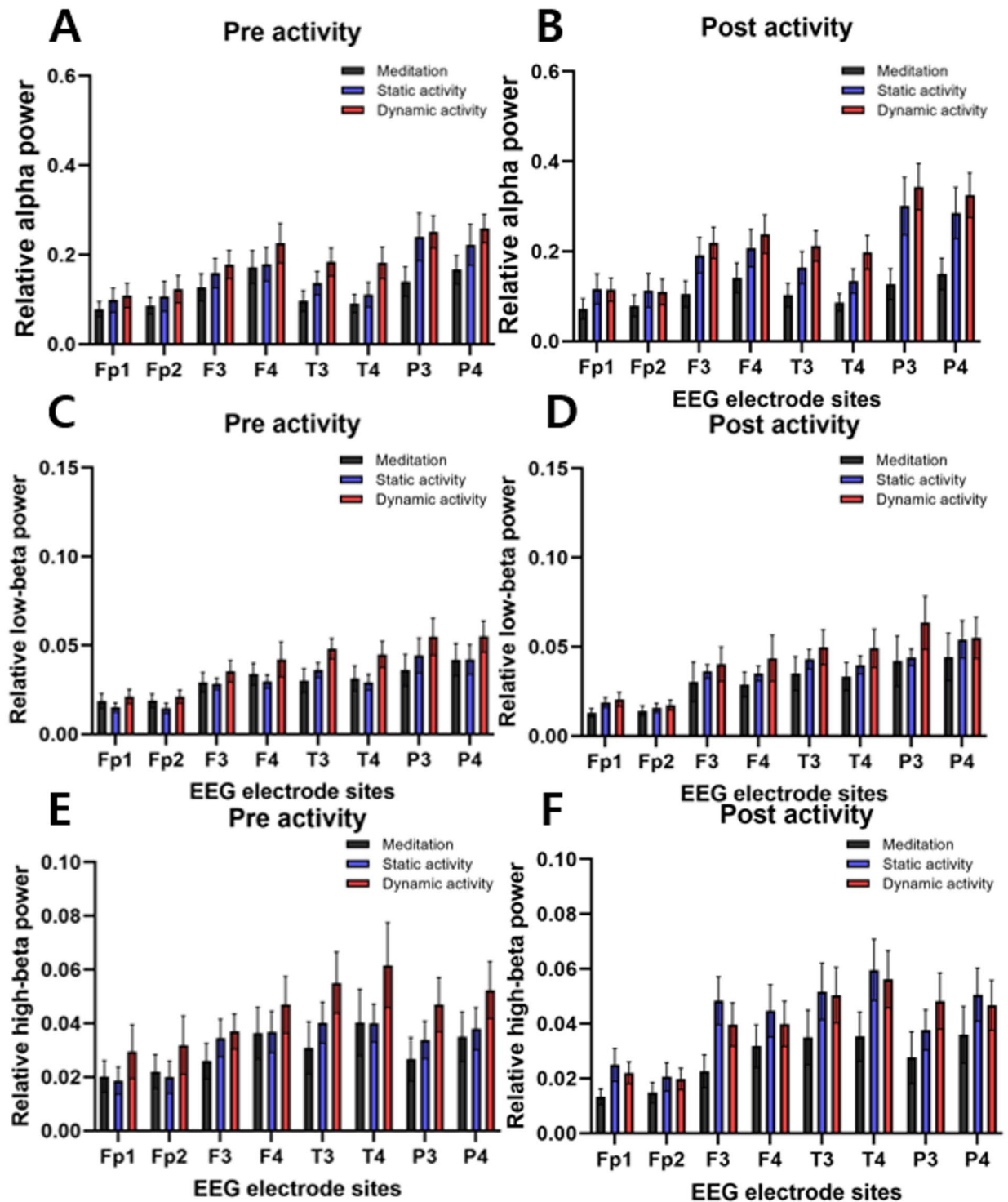


Fig. 11. Relative alpha, low-beta, and high-beta power across activities. Bars denote meditation (black), static activity (blue), and dynamic activity (red). Values present mean \pm SEM. Electrode sites follow the International 10–20 system (Fp1, Fp2, F3, F4, T3, T4, P3, P4). (A, B) Relative alpha power before and after the activities, respectively. (C, D) Relative low-beta power before and after the activities, respectively. (E, F) Relative high-beta power before and after the activities, respectively.

For relative high-beta power across electrodes, no clear evidence of a main effect of sex was observed. Likewise, sex \times activity, sex \times time, and sex \times activity \times time did not show clear evidence of effects across electrodes (all $p > .05$). Accordingly, no clear evidence of Sidak-corrected female–male differences was observed within any activity \times time cell (all adjusted $p > .05$) (Fig. 14). Across electrodes, Sidak-adjusted sex contrasts and within-activity pre–post contrasts did not provide robust evidence (all adjusted $p > .05$; Fig. 14).

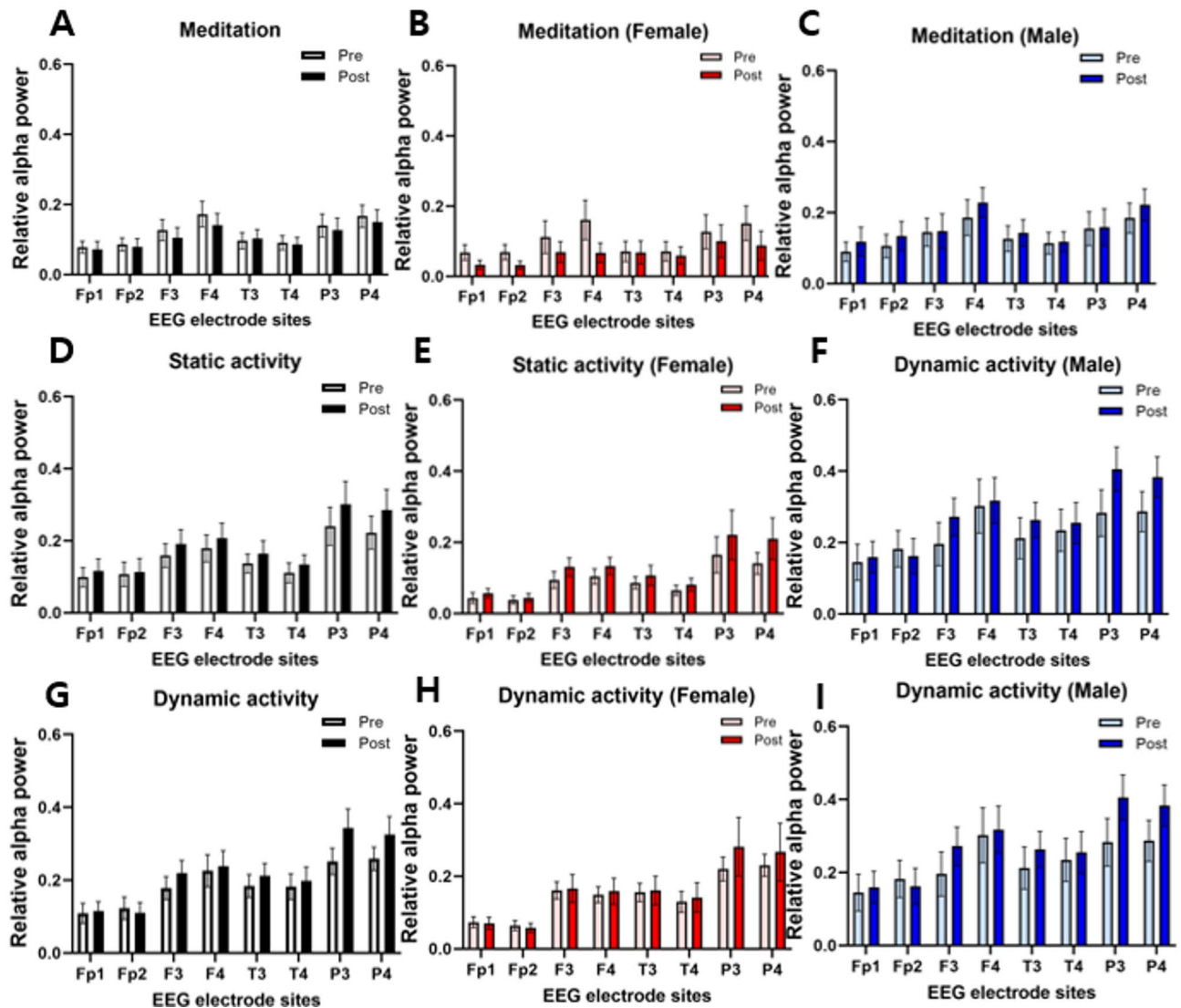


Fig. 12. Changes in relative alpha power pre- and post-three activities. Relative alpha power across electrode sites following the International 10–20 system (Fp1, Fp2, F3, F4, T3, T4, P3, P4) is shown. Within each panel, bars show pre-values (lighter) and post-values (darker). Values present mean \pm SEM. (A–C) Relative alpha power pre- and post-meditation: (A) all participants, (B) females, (C) males. (D–F) Relative alpha power pre- and post-static activity: (D) all participants, (E) females, (F) males. (G–I) Relative alpha power pre- and post-dynamic activity: (G) all participants, (H) females, (I) males.

HRV

Across PNS index, SDNN, RMSSD, and LF/HF ratio, the fixed-effect terms and interactions generally showed limited evidence of effects (all key effects $p > .05$). For PNS index, sex \times time term showed weak, exploratory evidence of an interaction ($F(1,30.3) = 3.58, p = .0680, \eta^2 = 0.10572$), but Sidak-adjusted pairwise comparisons did not provide clear evidence of differences (all adjusted $p > .05$). Similarly, SDNN showed limited evidence of fixed effects or interactions ($p > .05$). For RMSSD, the sex \times time term also showed weak, exploratory evidence ($F(1,29.4) = 3.66, p = .0655, \eta^2 = 0.11065$), but Sidak-adjusted pairwise comparisons did not provide clear evidence (all adjusted $p > .05$). The LF/HF ratio showed weak, exploratory evidence of a time-related pattern ($F(1,32) = 3.16, p = .0850, \eta^2 = 0.08978$); however, Sidak-adjusted post hoc comparisons and slice tests did not provide clear evidence of effects (all adjusted $p > .05$), as shown in Fig. 15.

For the SNS index, Type III tests of fixed effects showed evidence consistent with an activity \times time interaction ($F(2,30.3) = 7.25, p = .0027, \eta^2 = 0.32388$). In Sidak-adjusted post hoc comparisons, meditation at post was lower than dynamic activity at post (estimate = $-1.0703, df = 36.4, adjusted p = .0065, 95\%$ adjusted CI [$-1.9314, -0.2092$]), and dynamic activity showed a pre-post decrease (estimate = $-0.7276, df = 30.3, adjusted p = .0304, 95\%$ adjusted CI [$-1.4131, -0.04204$]). Consistently, slice tests indicated an activity effect at post ($F(2,37.2) = 7.85, p = .0014$) and a time effect within dynamic activity ($F(1,30.3) = 11.37, p = .0021$). However, sex \times activity \times time pairwise contrasts did not provide clear evidence of differences after Sidak correction (all adjusted $p > .05$), as shown in Fig. 16.

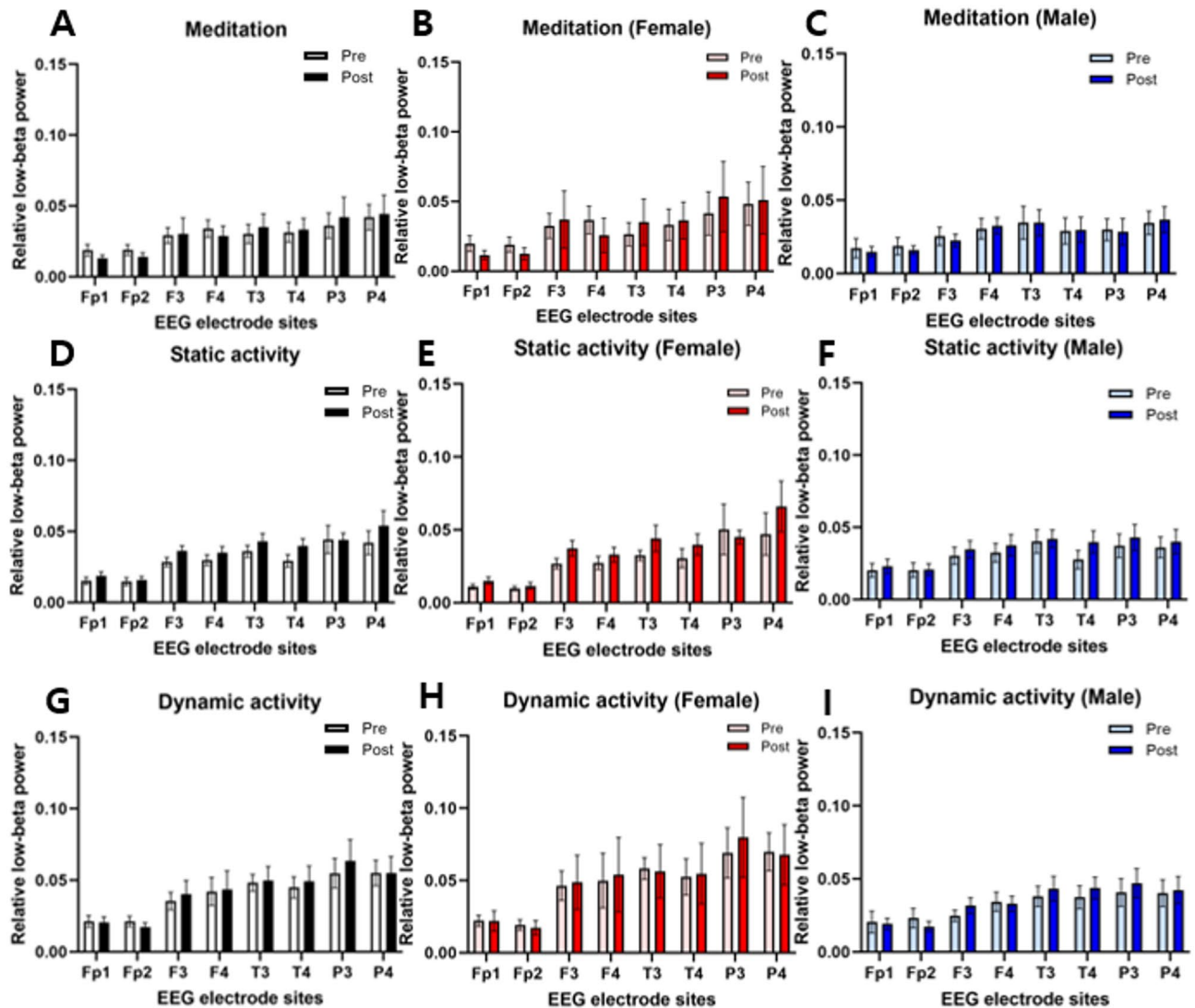


Fig. 13. Changes in relative low-beta power pre- and post-three activities. Relative alpha power across electrode sites following the International 10–20 system (Fp1, Fp2, F3, F4, T3, T4, P3, P4) is shown. Within each panel, bars show pre-values (lighter) and post-values (darker). Values present mean \pm SEM. (A–C) Relative low-beta power pre- and post-meditation: (A) all participants, (B) females, (C) males. (D–F) Relative low-beta power pre- and post-static activity: (D) all participants, (E) females, (F) males. (G–I) Relative low-beta power pre- and post-dynamic activity: (G) all participants, (H) females, (I) males.

For the stress index, Type III tests of fixed effects indicated evidence consistent with an activity \times time interaction ($F(2,31.9) = 3.34, p = .0482, \eta^2 = 0.17307$). In Sidak-adjusted post hoc testing, meditation at post was lower than dynamic activity at post (estimate = $-2.2759, df = 47.9, adjusted p = .0411, 95\% adjusted CI [-4.4980, -0.05386]$). Furthermore, slice tests showed an activity effect at post ($F(2,48.1) = 5.25, p = .0087$), whereas the activity effect at pre did not show clear evidence of differences ($F(2,48.3) = 0.23, p = .7936$). Although the time effect within dynamic activity had a nominal p value below 0.05 ($p = .0237$), the corresponding within-activity pre–post contrast did not provide clear evidence of change after Sidak adjustment (adjusted $p = .3025$), as shown in Fig. 16.

Discussion

This study investigated the physiological and psychological effects of AAI, focusing on differences in sex and activity intensity. To address the limitations of self-reported measures, physiological markers, including salivary hormones, EEG, and HRV, were quantified. By comparing the effects of static and dynamic activities, this study provides a comprehensive understanding of the effects of AAI on physiological and psychological responses, and the influence of sex on these responses.

One of the key findings of this study is that AAI positively affects the emotional states of participants. Across all participants, affective states did not show robust between-activity differences. However, a pre–post increase was observed within the dynamic activity. Vitality showed evidence consistent with time and activity effects

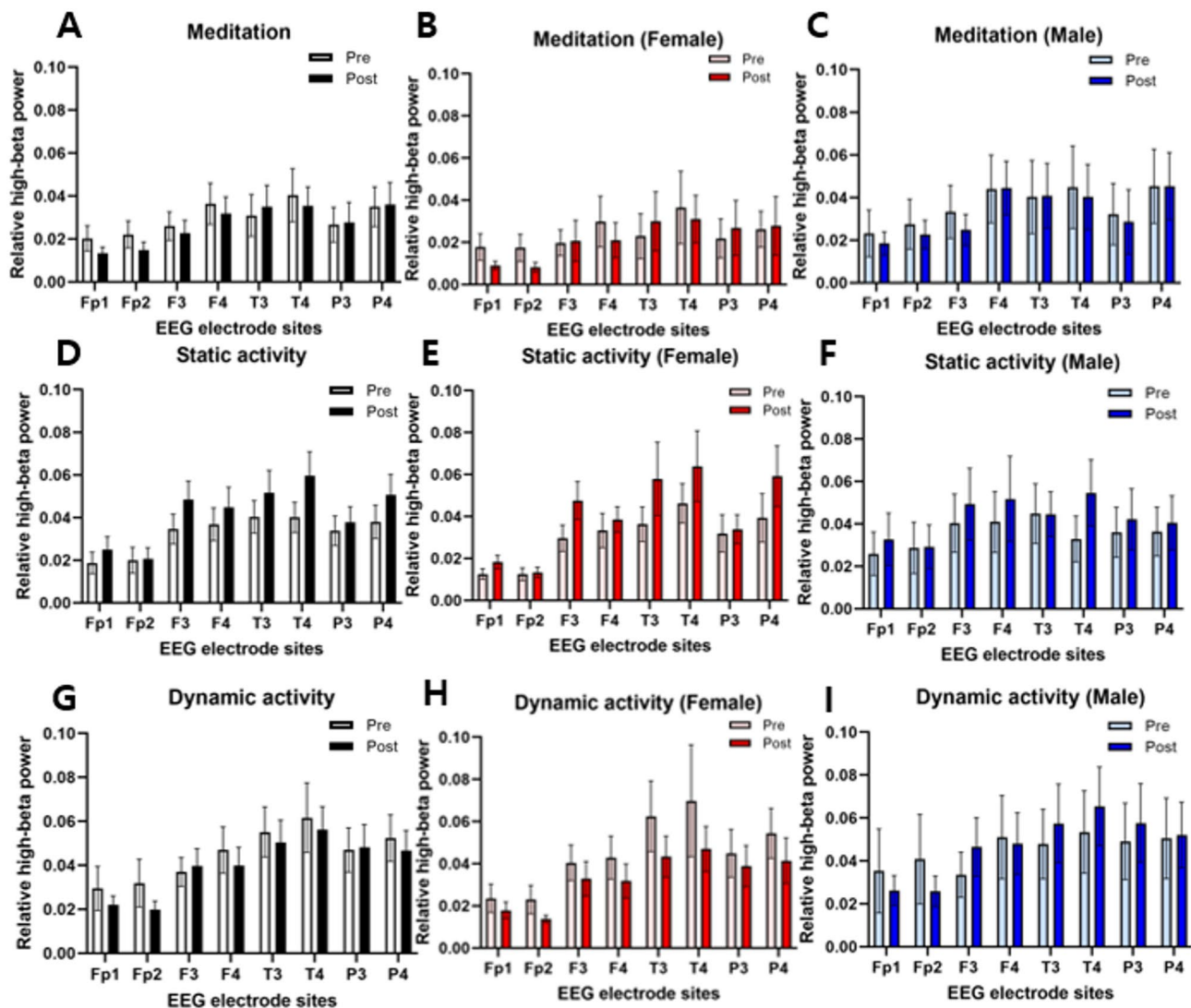


Fig. 14. Changes in relative high-beta power pre- and post-three activities. Relative alpha power across electrode sites following the International 10–20 system (Fp1, Fp2, F3, F4, T3, T4, P3, P4) is shown. Within each panel, bars show pre-values (lighter) and post-values (darker). Values present mean \pm SEM. (A–C) Relative high-beta power pre- and post-meditation: (A) all participants, (B) females, (C) males. (D–F) Relative high-beta power pre- and post-static activity: (D) all participants, (E) females, (F) males. (G–I) Relative high-beta power pre- and post-dynamic activity: (G) all participants, (H) females, (I) males.

with an activity-by-time interaction, with pre–post increases following both static and dynamic activities. These findings indicate that both static and dynamic activities contributed to increased vigor and positive emotional states. Additionally, SDM results showed that participants' subjective emotional states improved after static and dynamic activities compared to before. Participants reported improved emotional states and vigor following these interventions. These findings suggest that emotional states improved after participating in both static and dynamic activities. Similar to this study, a previous study evaluated emotional responses using the Profile of Mood States and the SDM⁶³. The results showed that AAI improved emotional states. Another study found that AAI in an acute care setting successfully enhanced positive emotions in children⁴¹. Another study reported that AAI improves memory, attention, and emotional regulation while alleviating symptoms of depression⁴². Overall, the findings of this and previous studies indicate that AAI can lead to positive emotional changes.

This improvement in subjective emotional state is also consistent with the EEG results. The results showed that relative alpha power in the frontal and parietal lobes increased after dynamic activity compared to meditation, and that relative alpha power in the parietal lobes was higher after static activity than after meditation. An increase in relative alpha power is typically associated with a state of relaxed alertness, focused attention, or reduced cognitive load^{64,65}. The evaluation of relative alpha power following AAI appears to be linked to activities that induce a comfortable yet focused state in participants. Positive interactions with nature are known to reduce stress and enhance cognitive functioning^{66,67}. An increase in alpha wave activity has been proposed as one of the neurophysiological mechanisms underlying these beneficial effects. Therefore, the higher alpha

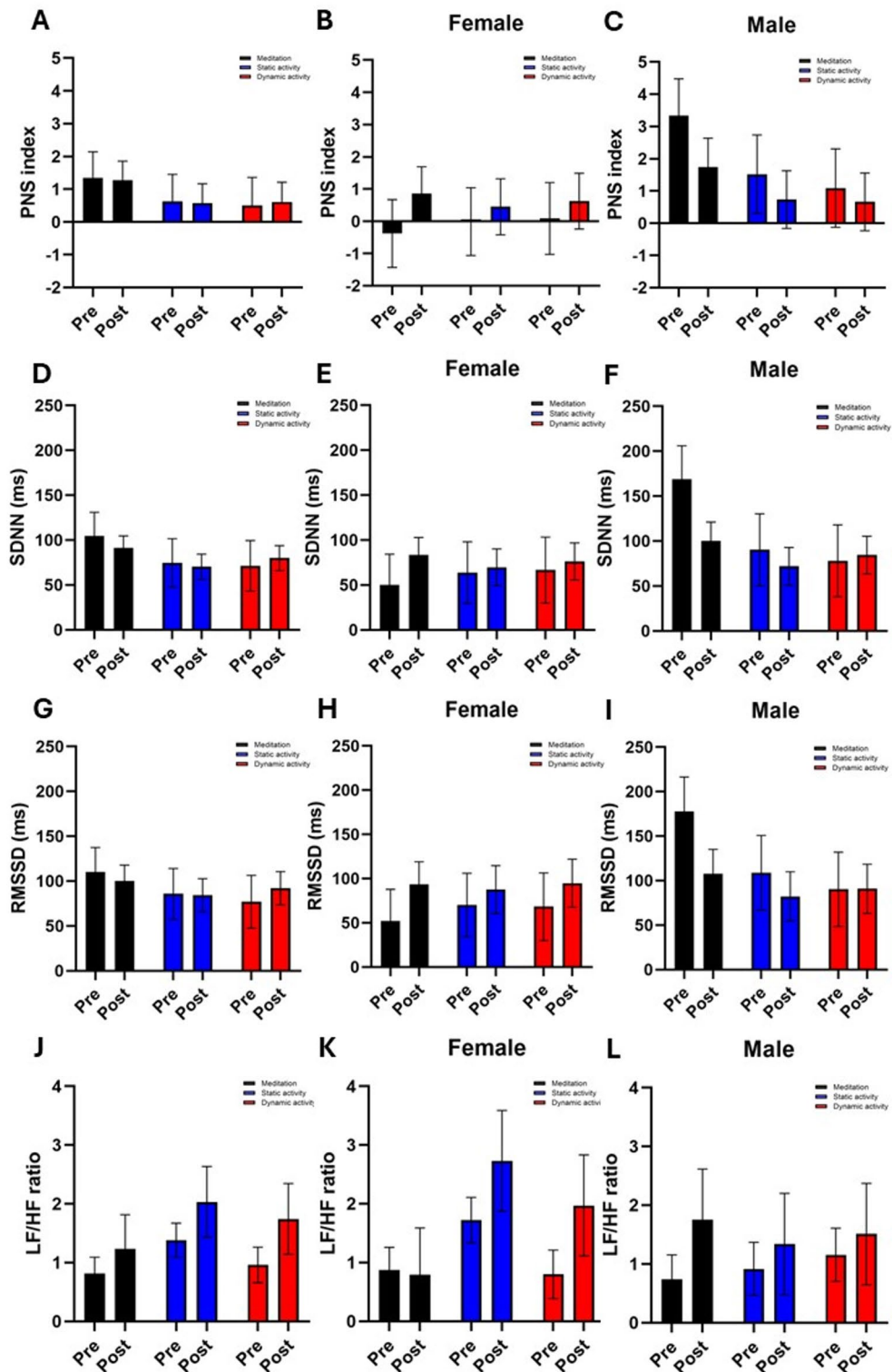


Fig. 15. PNS index, SDNN, RMSSD, and LF/HF ratio pre- and post-each activity. (A–C) PNS index pre- and post-each activity type: meditation (black bars), static activity (blue bars), and dynamic activity (red bars). (A) PNS index of all participants, (B) PNS index of female participants, and (C) PNS index of male participants. (D–F) SDNN pre- and post- each activity type: meditation (black bars), static activity (blue bars), and dynamic activity (red bars). (D) SDNN of all participants, (E) SDNN of female participants, and (F) SDNN of male participants. (G–I) RMSSD pre- and post- each activity type: meditation (black bars), static activity (blue bars), and dynamic activity (red bars). (G) RMSSD of all participants, (H) RMSSD of female participants, and (I) RMSSD of male participants. (J–L) LF/HF ratio pre- and post- each activity type: meditation (black bars), static activity (blue bars), and dynamic activity (red bars). (J) LF/HF ratio of all participants, (K) LF/HF ratio of female participants, and (L) LF/HF ratio of male participants. All bars represent LSM means and standard error.

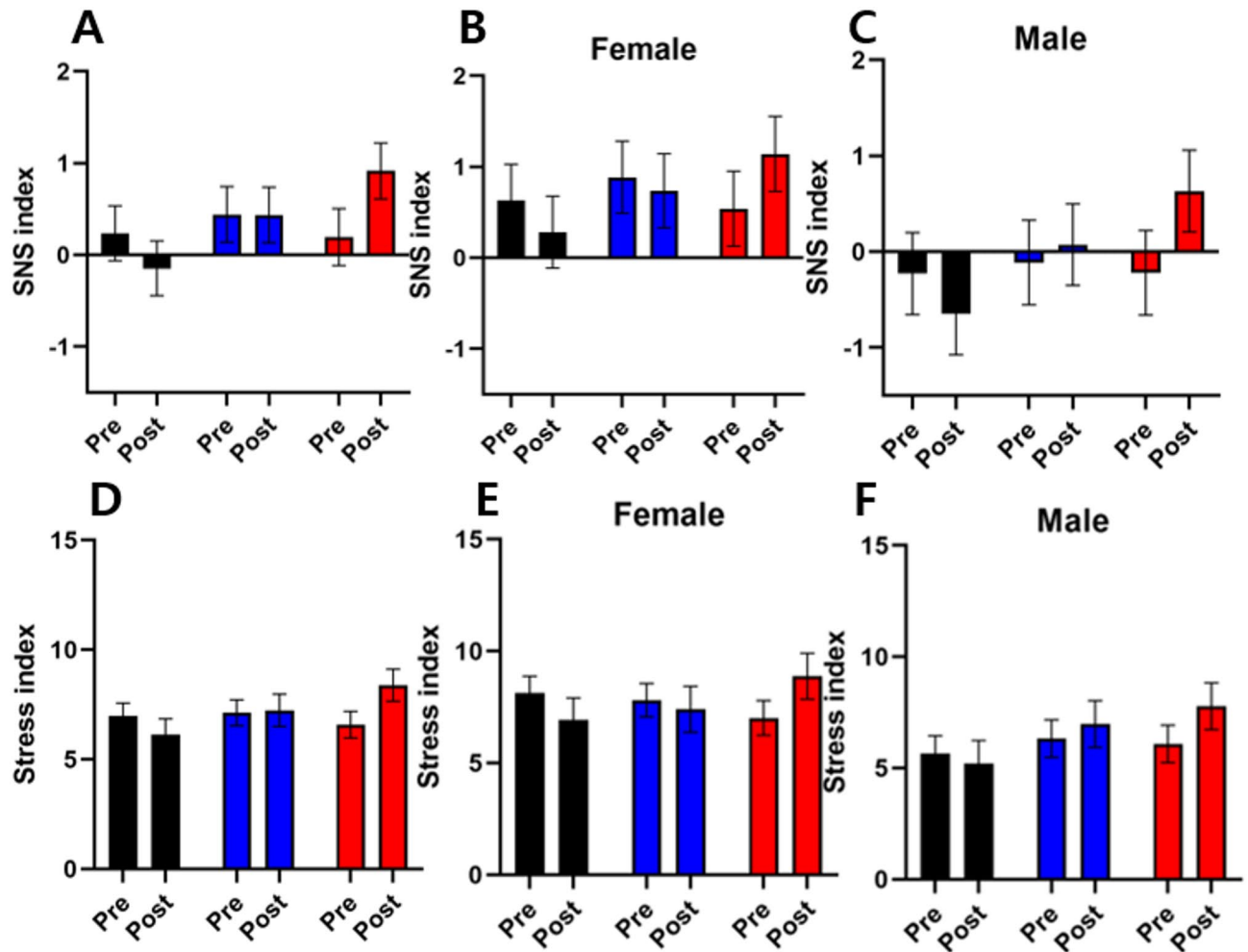


Fig. 16. SNS index and stress index pre- and post-each activity. (A–C) SNS index pre- and post-each activity type: meditation (black bars), static activity (blue bars), and dynamic activity (red bars). (A) PNS index of all participants, (B) PNS index of female participants, and (C) PNS index of male participants. (D–F) Stress index pre- and post- each activity type: meditation (black bars), static activity (blue bars), and dynamic activity (red bars). (D) Stress index of all participants, (E) Stress index of female participants, and (F) Stress index of male participants. All bars represent LSM means and standard error.

power observed after dynamic and static activities compared to meditation can be attributed to the positive and engaged relaxation state promoted by animal interactions. AAI increased the relative alpha power associated with a sense of stability and also elicited brain activation.

Among HRV-derived indices, PNS index, SDNN, RMSSD, and the LF/HF ratio did not show robust effects. In contrast, the SNS index and stress index demonstrated activity-by-time interactions, with higher post-activity values after dynamic activity compared with meditation. Furthermore, within-condition contrasts indicated a pre-post increase in the SNS index following dynamic activity, whereas the corresponding stress index pre-post contrast did not provide clear evidence of change after multiplicity adjustment. Because the Kubios-derived SNS and stress indices are composite HRV metrics, they were interpreted as reflecting autonomic modulation/arousal rather than direct sympathetic nerve activity. Accordingly, these findings are consistent with higher post-activity autonomic arousal following dynamic activity than meditation, as reflected by higher Kubios-derived SNS and stress indices⁶⁸. This sympathetic activation is likely due to the novel and engaging nature of interacting with animals, which can naturally elicit an alertness response. Previous studies have shown that exposure to new or unpredictable stimuli can activate the SNS, increasing attention and responsiveness^{69,70}. AAI may therefore induce an initial arousal response, indexed by SNS activation, owing to the novelty and engagement involved in interacting with animals^{71,72}. Therefore, the heightened SNS and stress indices observed after dynamic activity, compared to meditation, can be attributed to participants' physiological arousal and attentional responses to the novelty of animal interaction.

With respect to salivary oxytocin concentrations, oxytocin did not show clear evidence of main effects of sex, activity, or time, nor their interactions, and planned contrasts did not provide clear evidence of differences after Sidak correction. Therefore, any apparent activity-specific fluctuations should be interpreted as exploratory. Meditation is known to effectively enhance empathy, and one of the key mechanisms underlying this effect

involves the oxytocin system⁷³. A previous study examining the effects of short meditation sessions on positive and negative emotions, state anxiety, and salivary oxytocin levels in psychology students⁷³ found that participants in the meditation group showed a significant reduction in negative emotions and state anxiety, along with a significant increase in salivary oxytocin concentrations compared to the control group. These findings suggest that meditation can facilitate emotional and physiological states that promote empathy. Additionally, physical activity, such as high-intensity interval exercise (HIIE), has also been shown to increase oxytocin levels⁷⁴. In healthy males, both arterial and venous oxytocin concentrations increased after two identical HIIE sessions, suggesting that physical activity can elevate circulating oxytocin levels. This indicates that oxytocin may act as one of the exerkines released during exercise. Similarly, another study reported that salivary oxytocin levels increased after physical activities such as running, rising within 10 min, and peaking around 30 min post-exercise⁷⁵. These findings imply that oxytocin levels are also likely to increase during dynamic physical activities involving dogs, such as disc exercises or agility tasks.

For salivary cortisol concentrations, cortisol showed evidence consistent with a time-related pattern, whereas activity-related effects showed limited evidence of differences. Planned within-activity contrasts failed Sidak correction, indicating that the changes observed may be due to time-related factors instead of condition-specific effects. Meditation is known to provide various health benefits and is particularly effective in reducing stress and anxiety⁷⁶. This effect is attributed to its influence on the HPA axis, which helps regulate cortisol secretion. Several studies have reported a potential association between meditation and decreased cortisol levels⁷⁷. Static activity can also alleviate stress by promoting emotional closeness and providing psychological stability⁷⁸. Such interactions are generally known to decrease cortisol levels through the regulation of HPA axis activity. Particularly, human–dog interactions induce positive emotions, which may further contribute to lowering cortisol concentrations⁷⁹. These activities involve direct physical contact and smooth communication, which effectively relieve participants' stress levels. Although increases in cortisol levels are typically observed during most physical activities, in the present study, cortisol showed evidence consistent with an overall time-related pattern, and planned within-activity contrasts did not provide clear evidence after Sidak correction⁸⁰. Accordingly, cortisol changes should not be attributed to a specific activity condition.

In females, SDM scores were higher after both static and dynamic activities. EEG results showed changes in relative alpha power in multiple regions, while beta-band findings were inconsistent and limited across electrodes. For salivary oxytocin, however, the mixed model tests and Sidak-adjusted planned contrasts did not provide clear evidence of robust sex- or activity-specific changes. The descriptive trajectories/nominal contrasts suggested that oxytocin values could be higher at selected post-static sampling points in females. Nevertheless, these comparisons were not retained after multiplicity adjustment; therefore, this pattern should be regarded as exploratory. Salivary cortisol concentrations showed an overall time-related decrease across sampling points, without evidence that time courses differed by activity under the model. Taken together, the female data do not support a definitive conclusion that static, contact-focused interactions reliably increase oxytocin, and any mechanistic interpretation should be presented as a hypothesis for future confirmation. Nonetheless, females showed improvements in subjective outcomes after dog-assisted conditions, and the contact-focused static format may be a practical candidate for sex-tailored AAI program design, pending confirmation in adequately powered studies.

Consistent with the results, oxytocin- and cortisol-related patterns in females should be interpreted cautiously as exploratory because these effects were not retained after Sidak correction. Accordingly, any mechanistic interpretation is hypothesis-generating and requires confirmation in adequately powered studies. Although contact-focused AAI could plausibly engage oxytocin-related pathways, the present salivary oxytocin data do not provide robust evidence of an activity-specific increase under multiplicity control. Cortisol, a major HPA-axis stress hormone, showed an overall time-related decline without evidence that trajectories differed by activity type; therefore, cortisol changes should not be attributed to a specific activity.

In males, SDM scores were higher after dynamic activity. EEG results showed increases in both relative alpha and high-beta power following dynamic activity. These findings may reflect activity-related psychophysiological changes. However, the endocrine pattern was not specific to dynamic activity. Salivary oxytocin concentrations did not provide clear evidence of sex- or activity-specific changes after Sidak correction; hence, any apparent sex-stratified patterns should be regarded as exploratory. Salivary cortisol concentrations showed an overall time-related decline across sampling points, without evidence that trajectories differed by activity type under the model. Generally, active interaction appears to be more effective in promoting oxytocin release and strengthening social connections in males^{81,82}. Previous studies have reported that cortisol levels typically increase during exercise in males^{83,84}. This has been interpreted as a physiological response wherein physical activity, particularly high-intensity exercise, temporarily stresses the body. However, in the present dataset, cortisol showed an overall time-related decline without evidence of activity-specific trajectories under the model; thus, any condition-specific interpretation should be treated as exploratory. The dynamic activity in this study was not merely intense physical exercise but also included a unique component, interaction with animals. Several studies have shown that positive interactions with animals can reduce stress hormone levels and promote relaxation^{23,85}. Therefore, a potential modulatory role of positive animal interaction on perceived stress cannot be excluded, but this interpretation remains speculative given the lack of Sidak-adjusted, activity-specific cortisol effects. Collectively, for males, dynamic activity was associated with stronger engagement-related signals, whereas endocrine measures did not provide robust, activity-specific evidence after Sidak adjustment. Although several oxytocin contrasts were nominally significant before multiplicity adjustment, these effects were not retained after Sidak correction; thus, any apparent fluctuations should be interpreted cautiously as exploratory.

Overall, the present findings suggest that AAI can elicit positive subjective changes across activity formats but that physiological responses vary by modality and metric. From a program-design perspective, a contact-focused static format may align with female participants' subjective improvements, whereas a more active

dynamic format may align with males' stronger engagement-related signals. However, because the patterns of endocrine hormones—particularly oxytocin—did not provide robust condition-specific evidence after multiplicity correction, sex-tailored interpretations of hormonal mechanisms should be treated as hypothesis-generating and tested in larger, adequately powered studies.

Despite its contributions, this study has several limitations. First, the sample size was small, highlighting the need for future research with a larger sample size to improve statistical power and ensure generalizability. Second, this study focused exclusively on short-term effects and did not investigate the long-term implications of AAI. This highlights the importance of longitudinal studies to evaluate enduring hormonal and psychological changes. Third, although sex differences were observed, future studies should involve more rigorous analyses of sex-specific hormonal responses and employ a broader array of validated psychological assessment tools to detect subtle affective changes following AAI. Fourth, this study did not account for the menstrual cycle phase in female participants, despite the well-known effects of estrogen and progesterone on hormone levels and neurophysiological responses to stress. An additional limitation included possible confounding related to order and intensity. Meditation was conducted in the first week due to scheduling constraints, whereas the static and dynamic activities were randomized in the following 2 weeks. Therefore, order/time effects cannot be fully excluded. Moreover, the three conditions differed in physical intensity (MET values), and post-activity EEG/HRV measures may be susceptible to acute exertion and respiratory influences; notably, respiration was not paced or monitored during HRV acquisition. Accordingly, some physiological differences—particularly after higher-intensity dynamic activity—may reflect exercise-related effects rather than animal presence per se. Future studies should counterbalance the condition order, incorporate dog-present vs. dog-absent control conditions matched for physical activity intensity, and standardize recovery and respiratory monitoring to isolate AAI-specific effects more effectively. Future studies should explicitly account for cycle phase or measure relevant sex hormones to more effectively characterize sex-specific hormonal responses to AAI and improve the precision of physiological interpretations, given potential interactions with androgens discussed in this paper. Finally, the PAS was used to assess participants' attitudes toward animals for grouping purposes. However, most participants in this study demonstrated high PAS scores, resulting in limited grouping of the participants. Consequently, this limited the ability to examine correlations between attitudes toward animals and hormonal responses. A previous study reported a greater improvement in mood states in individuals with negative attitudes toward animals than in those with positive attitudes following horse-stroking interventions⁸⁶. Future research should include participants with a broader range of attitudes toward animals to better evaluate the relationship between PAS scores and hormone levels.

This study provides insight into the psychological and physiological mechanisms of AAI, particularly the influence of sex differences and activity intensity on physiological responses. The findings offer a theoretical basis for designing sex-tailored AAI programs that account for sex- and sex-related physiological and psychological characteristics. These contributions may advance both the practical implementation and theoretical development of AAI.

Data availability

The datasets generated and/or analyzed during the current study are included in this published article and its supplementary information files.

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Author contributions

Conceptualization: Y.S., Y.J., M.Y.; Methodology: Y.S., Y.J., M.Y.; Investigation (including participant recruitment and scheduling): Y.S., Y.J., K.W.Y., Y.H.; Formal analysis: Y.S.; Writing—original draft: Y.S., Y.J.; Writing—review & editing: Y.J., M.Y.; Resources: K.Y.Y.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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