Pupil dynamics during very light exercise predict benefits to prefrontal cognition

AUTHOR(S):

Kuwamizu, Ryuta; Yamazaki, Yudai; Aoike, Naoki; Hiraga, Taichi; Hata, Toshiaki; Yassa, Michael A.; Soya, Hideaki

CITATION:

Kuwamizu, Ryuta ...[et al]. Pupil dynamics during very light exercise predict benefits to prefrontal cognition. NeuroImage 2023, 277: 120244.

ISSUE DATE:

2023-08-15

URL:

http://hdl.handle.net/2433/284138

NeuroImage 277 (2023) 120244

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage

Pupil dynamics during very light exercise predict benefits to prefrontal cognition

Ryuta Kuwamizu[a,b], Yudai Yamazaki[a,c], Naoki Aoike[a,c], Taichi Hiraga[a], Toshiaki Hata[a], Michael A. Yassa[c,d], Hideaki Soya[a,c,*]

[a] Laboratory of Exercise Biochemistry and Neuroendocrinology, Faculty of Health and Sport Sciences, University of Tsukuba, Ibaraki 305-8574, Japan
[b] Graduate School of Letters, Kyoto University, Kyoto 606-8501, Japan
[c] Sport Neuroscience Division, Advanced Research Initiative for Human High Performance (ARIHHP), Faculty of Health and Sport Sciences, University of Tsukuba, Ibaraki 305-8574, Japan
[d] Department of Neurobiology and Behavior and Center for the Neurobiology of Learning and Memory, University of California Irvine, Irvine, CA 92679-3800, USA

ARTICLE INFO

Keywords:
Pupillometry
Physical activity
Arousal
Prefrontal cortex
fNIRS

ABSTRACT

Physical exercise, even stress-free very-light-intensity exercise such as yoga and very slow running, can have beneficial effects on executive function, possibly by potentiating prefrontal cortical activity. However, the exact mechanisms underlying this potentiation have not been identified. Evidence from studies using pupillometry demonstrates that pupil changes track the real-time dynamics of activity linked to arousal and attention, including neural circuits from the locus coeruleus to the cortex. This makes it possible to examine whether pupil-linked brain dynamics induced during very-light-intensity exercise mediate benefits to prefrontal executive function in healthy young adults. In this experiment, pupil diameter was measured during 10 min of very-light-intensity exercise (30% $\dot{V}o_{2peak}$). A Stroop task was used to assess executive function before and after exercise. Prefrontal cortical activation during the task was assessed using multichannel functional near-infrared spectroscopy (fNIRS). We observed that very-light-intensity exercise significantly elicited pupil dilation, reduction of Stroop interference, and task-related left dorsolateral prefrontal cortex activation compared with the resting-control condition. The magnitude of change in pupil dilation predicted the magnitude of improvement in Stroop performance. In addition, causal mediation analysis showed that pupil dilation during very-light-intensity exercise robustly determined subsequent enhancement of Stroop performance. This finding supports our hypothesis that the pupil-linked mechanisms, which may be tied to locus coeruleus activation, are a potential mechanism by which very light exercise enhances prefrontal cortex activation and executive function. It also suggests that pupillometry may be a useful tool to interpret the beneficial impact of exercise on boosting cognition.

1. Introduction

An active lifestyle, including proper exercise habits, is expected to promote brain health. Our previous work has revealed that not only acute moderate-intensity exercise, but also short-duration, stress-free, very-light-intensity exercise enhances executive performance (Byun et al., 2014b; Yanagisawa et al., 2010). Very-light-intensity exercise, such as yoga and very slow running, have clinical significance due to a high exercise adherence without an unpleasant mood or hypothalamic-pituitary-adrenal (HPA) axis stress response (Ekkekakis et al., 2008; Soya et al., 2007a; Suwabe et al., 2018). The evidence, including our studies, that exercise enhances neural response during cognitive tasks has been investigated. Our previous study suggested that very-light-intensity exercise elicits dorsolateral prefrontal cortex (DLPFC) activation during cognitive conflict processing (i.e., Stroop interference) (Byun et al., 2014b), however, why this occurs is an important but unresolved question. A conspicuous missing point is the neural substrate during exercise that triggers executive function enhancement. It is clear from animal studies that brain activation is increased during exercise, even stress-free light-intensity (Kurosawa et al., 1993; Nishijima and Soya, 2006; Ohiwa et al., 2006b, 2006a; Pagliari and Peyrin, 1995; Soya et al., 2007b), which has been postulated as the factor for post-exercise cognitive enhancement and neural response change during tasks. However, this is only a hypothesis and has not been proven. Body movement and cardiovascular responses during exercise make it difficult to apply MRI-based neuroimaging technology, so no studies

* Corresponding author at: Laboratory of Exercise Biochemistry and Neuroendocrinology, Sport Neuroscience Division, Advanced Research Initiative for Human High Performance (ARIHHP), Faculty of Health and Sport Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8574, Japan.
E-mail address: soya.hideaki.gt@u.tsukuba.ac.jp (H. Soya).

https://doi.org/10.1016/j.neuroimage.2023.120244.
Received 20 March 2023; Received in revised form 12 June 2023; Accepted 20 June 2023
Available online 21 June 2023.

have been able to identify the trigger neural mechanism during exercise in real time for executive function enhancement.

The upregulation of the ascending arousal system, including the catecholaminergic system originating from the locus coeruleus (LC), is a potential candidate for the neuromodulatory mechanisms underlying the beneficial effect of acute exercise and cortical cognitive function. The catecholaminergic modulation from the LC to the PFC is essential for executive function because they control optimal signal and noise regulation of the DLPFC (Cools and Arnsten, 2022; Grueschow et al., 2020) by interacting with two neuron activity patterns: tonic and phasic (Aston-Jones and Cohen, 2005). Activation of the LC catecholaminergic system increases the network of cortical excitatory and inhibitory neurons resulting in increased cortical activity (Toussay et al., 2013) and this effect may carry over to the post-activation period (Bari et al., 2020; Pagliari and Peyrin, 1995; Totah et al., 2019). Post-exercise executive function enhancement and task-induced prefrontal activity are hypothesized to be triggered by upregulation of the ascending arousal system during exercise.

Our recent work has demonstrated that pupil dilation occurs not only with moderate or higher intensity exercise (assumed to be associated with the HPA-axis-mediated stress-response), but also robustly with very-light-intensity exercise (i.e., < 37% $\dot{V}o_{2peak}$ and stress-free exercise below ventilatory threshold), corresponding to increases in psychological arousal states (Kuwamizu et al., 2022). Accumulating evidence suggests that pupil dynamics, which are part of the autonomic nervous system, can provide a noninvasive readout of brain states related to arousal, attention, and cortical network activity (Joshi and Gold, 2020; Schwalm and Jubal, 2017; Strauch et al., 2022). Mechanistically, there is strong evidence linking pupil dynamics and LC-associated catecholaminergic activity as well as other ascending arousal pathways (e.g., cholinergic activation) (Breton-Provencher and Sur, 2019; Joshi et al., 2016; Lu et al., 2020; Reimer et al., 2016). Pupil dynamics possibly reflect either phasic or tonic LC activity modes (Joshi et al., 2016; Privitera et al., 2020). Recent rodent studies reported that pupil fluctuation correlates with catecholaminergic activity in the cortex (Breton-Provencher and Sur, 2019; Reimer et al., 2016) and cortical physiological signatures (McGinley et al., 2015), synchronizing with locomotion in real time. This evidence from moving rodents suggests that pupil diameter is a powerful tool for elucidating the neural substrate during human exercise. Recently, we confirmed that pupil dilation during very-light-intensity exercise correlates with changes in psychological arousal and LC integrity as measured by neuromelanin imaging (Yamazaki et al., 2023). These results support the theory that pupil change during very-light-intensity exercise can be considered a biomarker of brain arousal state including LC activation.

As described above, pupillometry is a useful brain arousal state marker, but it is both physiologically and anatomically complex and subject to luminosity and cognitive influences (Mathôt, 2018), so a valid methodology is important. Previous human studies have found that cognitive task demands lead to increases in pupil dilation by applying pupillometry during cognitive tasks (van der Wel and van Steenbergen, 2018). In an attempt to build on this, recent studies have challenged the relationship between acute exercise and pupil change during cognitive tasks (Ayala and Heath, 2021; McGowan et al., 2019; Shigeta et al., 2021), but the results of cross-over designs are notable for failing to detect significant pupil change and associations with executive function (McGowan et al., 2019; Shigeta et al., 2021). There might be limitations in extracting components of acute exercise effects from dynamic pupil change that interact with cognitive state change, screen-derived light stimuli, and spontaneous fluctuations. In contrast to these previous studies, the current study focused on pupil dynamics during exercise.

Based on the current evidence, we hypothesized that the upregulation of the pupil-linked neural substrates described above is similarly engaged for very-light-intensity exercise-induced PFC function enhancement. Interestingly, there are large inter-individual differences in exercise-induced pupil dilation compared to heart rate and expiratory parameters (Kuwamizu et al., 2022). Thus, we hypothesized that inter-individual difference in exercise-induced pupil dilation would predict the beneficial effect of exercise on executive function. We used pupillometry to investigate real-time redouts of brain states during exercise and combined it with multichannel functional near-infrared spectroscopy (fNIRS), which enabled us to examine the association between pupil dilation during exercise and exercise-potentiated PFC activation underlying executive performance enhancement on a color-word Stroop task. Our previous work used the combination of fNIRS and the color-word Stroop task as a test of PFC inhibitory control to examine the acute effects of very-light-intensity exercise on executive function mechanisms (Byun et al., 2014b). Thus, we used the same task and method here, with the addition of pupillometry to investigate the associated mechanisms. This experimental design has been established as an experimental model for exercise-neuroimaging that minimizes the effects of non-cortical-derived fNIRS signals generated by exercise (Byun et al., 2014b, 2014a; Yanagisawa et al., 2010). We note that fNIRS allows for an environment with minimal physical restriction compared to other neuroimaging modalities and in which possible environmental effects on cognitive performance and neural activation can be kept to a minimum.

2. Materials and methods

2.1. Participants

Thirty-four healthy young adults (6 females), with no self-reported history of neurological or psychiatric disorders, were recruited for the study. This study was approved by the Institutional Ethics Committee of the University of Tsukuba and was in accordance with the latest version of the Declaration of Helsinki. Written informed consent was obtained from all participants. The following exclusion criteria were used: taking medication that affects the central nervous and/or endocrine systems ($N = 4$), suffering from an abnormal eye condition ($N = 1$), Stroop interference inverse efficiency scores (IES) exceeding 2 SD from the average or a correct response rate ≤ 80% in the pre-session ($N = 3$), and excess fNIRS signals in all regions of interest (ROIs) greater than 2 SD from the average in the pre-session ($N = 1$). In addition, one participant did not follow the instructions and performed the tasks inappropriately. Thus, twenty-four participants (three female) were included in the final analysis. All participants had normal or corrected-to-normal vision and normal color vision. They were all Japanese native speakers. Table 1 depicts the characteristics of the participants. This sample size is acceptable for the following reasons. Based on calculations in Green (1991), at least 31 data sets are required in the case of three predictor variables for multiple regression analysis assuming a large effect size (f^2 >0.35) used in causal mediation analyses. The current study exceeded this number because 24 participants performed 2 conditions (EX and CTL) and a total of 48

Table 1
Participant demographics data.

Measure	Male			Female		
Sample Size [n]	21			3		
Age [year]	22.2	±	1.5	21.7	±	1.2
Height [cm]	171.5	±	5.4	159.3	±	3.1
Weight [kg]	68.8	±	13.4	51.6	±	5.8
BMI [kg/m^2]	23.3	±	3.9	20.3	±	2.0
Graded Exercise Test						
$\dot{V}o_{2peak}$ [ml/min/kg]	48.6	±	7.1	39.2	±	5.0
HR_{peak} [bpm]	180.8	±	10.8	181.0	±	3.6
WR_{peak} [w]	243.0	±	30.1	156.3	±	22.5
RPE_{peak} [score]	19.7	±	0.6	19.0	±	1.0
BDI-II [score]	6.1	±	5.3	11.7	±	4.6

Values are presented as mean ± SD. BMI, body mass index; $\dot{V}o_{2peak}$, peak oxygen uptake; HR, heart rate; WR, work rate; RPE, rating of perceived exertion; BDI, Beck Depression Inventory.

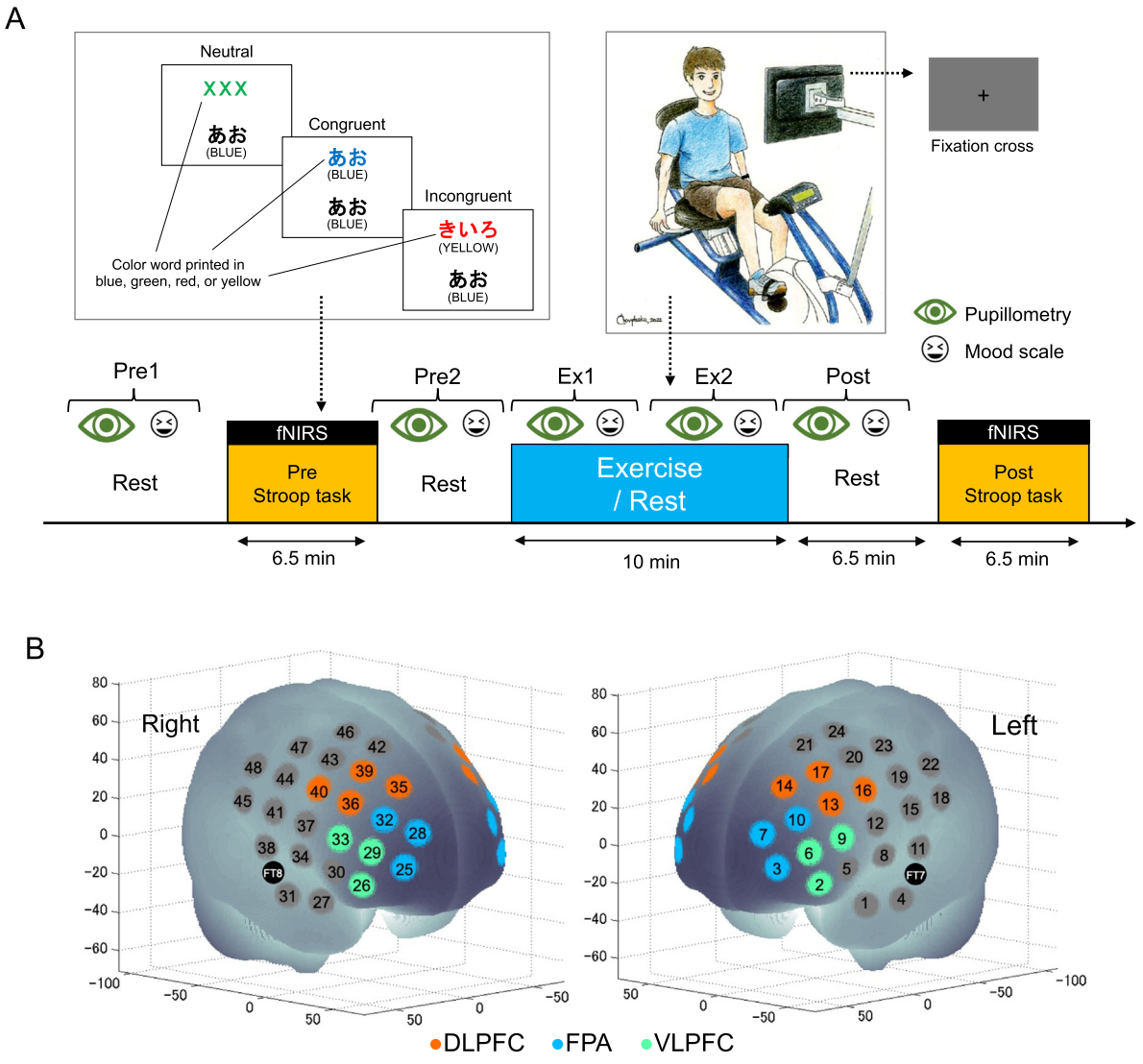

Fig. 1. (A) Example of experimental paradigm and the Japanese version of the color-word-matching Stroop task. Examples of the task stimulus for single trials of the neutral, congruent, and incongruent tasks are presented. (B) The spatial profiles of fNIRS channels and ROI segmentation used in the present study (introduced in previous studies (Okamoto and Dan, 2005; Tsuzuki et al., 2007)).

data sets were analyzed. Additionally, post-hoc sensitivity analysis, computed using G*Power 3.1, performed based on the current sample size (24 participants) with 80% power and an alpha of 0.05 demonstrated sufficient sensitivity for detecting *t*-test differences exceeding $d = 0.60$ (with a two-tailed alpha), which is consistent with our previous studies (Byun et al., 2014b; Damrongthai et al., 2021; Yanagisawa et al., 2010).

2.2. Procedure

The main experiment consisted of two trial conditions: the resting control (CTL) condition and the very-light-intensity exercise (EX) condition. In the EX condition, participants performed very-light-intensity exercise, an individualized load corresponding to 30% of each participant's $\dot{V}o_{2peak}$ using a cycle ergometer (see *Cardiorespiratory fitness assessment* in the Supplemental information for detailed instructions). We set the exercise duration to 10 min because our previous work has suggested that a minimum of 10 min of exercise improves Stroop task performance (Yanagisawa et al., 2009). According to the American College of Sports Medicine (ACSM, 2018), 30% $\dot{V}o_{2peak}$ is classified as "very light". In the CTL condition, participants sat on the ergometer and remained at rest (Fig. 1A). All participants underwent the CTL and EX conditions on separate days in a quasi-randomized order using a crossover design. The two visits were separated by a minimum of 48 h. Participants were asked to refrain from exercise and the consumption of alcohol for at least 24 h prior to the experiment and caffeine on experimental days so as to control for outside factors. In the EX condition, participants performed the Stroop task before and 6.5 min after the exercise. Pupil diameter and mood were measured before, during, and after the exercise. For measurements taken during exercise, the exercise time was divided into two 5-min periods (start to 5 min: 1st half of exercise; 5 min to end: 2nd half of exercise; no break). During each 5-min period, 3 min were spent looking at a fixation cross (pupillometry) and two min were spent answering mood-state questions (see *Psychological mood states* in the Supplemental information for detailed results) which were displayed on the screen (Fig. 1A). Eye blink rate was also measured before and after the exercise (data not shown). The intensity of illumination in the room was maintained at about 1440-1530 lx, measured with an illuminance meter placed on a central desk in the experiment room.

2.3. Acquisition and preprocessing of pupillometry data

Participants' pupil diameters were recorded using a screen-based eye-tracker (Tobii pro nano, Tobii AB, Danderyd, Sweden). Since we focused on long-lasting pupil dilation during exercise compared to the

pupil diameter at resting states, the eye-tracking data were recorded continuously for 3 min during each session. Participants focused on a fixation cross presented on a screen attached to the ergometer and located 70 cm from the participant. The fixation cross was a black cross presented at the center of a blank gray screen (background: RGB: 120, 120, 120, fixation cross: RGB: 0, 0, 0). During this time, participants were asked not to move their heads or look away from the fixation cross for as long as they were able. The data were sampled at 60 Hz. The analysis software (Tobii Pro Lab, Tobii AB, Danderyd, Sweden) automatically removed missing and invalid pupil data in cases where the participant blinked or looked away from the screen. Raw data were averaged every 15 s, and the average pupil diameter over 3 min of looking at the fixation cross was calculated. The right eye was used for analysis, as in previous studies (Hayashi et al., 2010; Kuwamizu et al., 2022). There was a high correlation with the left eye (median within-participant correlation coefficient $r = 0.94$).

2.4. Stroop task

Executive function was assessed using the event-related version of the color-word-matching Stroop task as in our previous fNIRS studies (Byun et al., 2014b; Damrongthai et al., 2021; Fukuie et al., 2022; Kuwamizu et al., 2021; Suwabe et al., 2021; Yanagisawa et al., 2010). Participants were required to respond using either “yes” or “no” buttons with their left or right forefingers. The percentage of answers assigned to yes and no was 50%. Two rows of letters (or words) were displayed on the screen, and participants were asked to respond to whether the color of the letters in the upper row corresponded to the color name shown in the lower row (Fig. 1A). The task consisted of 30 trials, including 10 neutral, 10 congruent, and 10 incongruent trials, presented in random order. For the neutral condition, the upper row contained crosses (XXXX) printed in yellow (RGB: 255, 244, 38), blue (RGB: 4, 0, 159), green (RGB: 8, 162, 0), or red (RGB: 255, 0, 0) and the bottom row contained the words YELLOW, BLUE, GREEN, or RED printed in black. For the congruent condition, the upper row contained the words YELLOW, BLUE, GREEN, or RED printed in a color congruent with the color name printed in black in the lower row. For incongruent conditions, the color word in the upper row was printed in a color incongruent with the color name printed in black in the lower row to elicit cognitive interference between the color word and the color name (i.e., Stroop interference). All words were written in Japanese. Each trial was separated by an interstimulus interval showing a fixation cross for 10–12 s to avoid prediction of the timing of the following trial (Damrongthai et al., 2021). The lower row was presented 0.1 s after the upper row to achieve sequential visual attention of participants (Byun et al., 2014b; Damrongthai et al., 2021; Ochi et al., 2022; Suwabe et al., 2021; Yanagisawa et al., 2010). The stimulus was presented on the screen for 2 s. Mean reaction time (RT) and accuracy rate were recorded for the neutral and incongruent conditions. Each RT was integrated with each accuracy using the IES (inverse efficiency score = RT [ms]/accuracy [%] ×100) to control for the speed-accuracy trade-off as in our previous study (Kuwamizu et al., 2021). Stroop interference was calculated as the [incongruent – neutral] contrast, which is an index of executive function performance. Finally, the Stroop interference IES [incongruent IES – neutral IES] was used as a performance measure of executive function in the current study.

2.4.1. fNIRS measurements

We assessed hemodynamic responses to the Stroop task in the lateral prefrontal cortex using a multichannel fNIRS optical topography system (ETG-7000, Hitachi Medical Corporation, Japan) applying two wavelengths of near-infrared light (785 and 830 nm). With this method, we were able to calculate signals reflecting the oxygenated hemoglobin (oxy-Hb) concentration changes in a unit of millimolar-millimeter (mM•mm) (Maki, 1995). The configuration and placement of the fNIRS probe holder followed the same procedure as in our previous studies (Byun et al., 2014b; Damrongthai et al., 2021; Ochi et al., 2022; Suwabe et al., 2021; Yanagisawa et al., 2010). We adopted a virtual registration method to register fNIRS data to Montreal Neurological Institute (MNI) standard brain space (Brett et al., 2002; Tsuzuki et al., 2007; Tsuzuki and Dan, 2014). With this method, a virtual probe holder is placed on the scalp. By simulating the deformation of the holder, the probes and channels can be registered to the reference brain in the MRI database (Okamoto, 2004; Okamoto and Dan, 2005). Statistical analysis of the MNI coordinate values of the fNIRS channels was performed to estimate the most likely location of a particular channel and its spatial variability for a group of participants (Singh et al., 2005). Finally, the estimated sites were given anatomical labels using a MATLAB function that reads anatomical labeling information coded in a macro anatomical brain atlas (Shattuck et al., 2008).

2.4.2. fNIRS data analysis

Each channel’s timeline data were preprocessed using a band-pass filter (high-pass 0.04 Hz, low-pass 0.3 Hz). Channel-wise and subject-wise contrasts were calculated using the inter-trial mean of differences between the baseline (0–2 s before the onset of the trial) and the peak (6–8 s after the onset of the trial) periods. Oxy-Hb signal change reflecting the Stroop interference effect was calculated as the difference of [incongruent trial signal change – neutral trial signal change] (Byun et al., 2014b; Damrongthai et al., 2021; Suwabe et al., 2021; Yanagisawa et al., 2010). As in previous studies (Byun et al., 2014b; Damrongthai et al., 2021; Suwabe et al., 2021; Yanagisawa et al., 2010), we combined 3 or 4 adjacent channels to form each ROI using LBPA40, which is a widely-used method among anatomical labeling systems (Shattuck et al., 2008). The ROIs included the left dorsolateral PFC (l-DLPFC; channels 13, 14, 16, and 17), the left ventrolateral PFC (l-VLFPC; channels 2, 6, and 9), the left frontopolar area (l-FPA; channels 3, 7, and 10), the right dorsolateral PFC (r-DLPFC; channels 35, 36, 39, and 40), the right ventrolateral PFC (r-VLPFC; channels 26, 29, and 33), and the right frontopolar area (r-FPA; channels 25, 28, and 32) (Fig. 1B). This method is considered valid because the optical properties of adjacent channels are known to be similar (Katagiri et al., 2010). To control for the low proportion of false positives, we limited ROI analyses and used a statistical threshold of $q < 0.05$ false-discovery-rate (FDR) corrected.

2.5. Statistical analyses

We assessed pupil diameter with a repeated measures two-way ANOVA with condition (EX, CTL) and time (Pre1, Pre2, Ex1, Ex2, Post) as within-subject factors, followed by Bonferroni-corrected post hoc tests. The effect of acute very-light-intensity exercise on task performance (i.e., Stroop interference IES) and cortical activation (Oxy-Hb change) was analyzed using a paired *t*-test. For cortical activation, ROI-wise analysis with FDR was conducted to control for false positives. The difference in the degree of task performance and cortical activation between post- and pre-sessions was examined: the contrasts ([incongruent - neutral] of post-session Stroop) - ([incongruent – neutral] of pre-session Stroop) for both the EX and CTL conditions were respectively calculated to compare the EX and CTL results. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 28 (SPSS Inc., Chicago, IL). Statistical significance was set apriori at $P < 0.05$.

2.6. Causal mediation analyses

Causal mediation analyses were performed in R 4.1.2. The R package “mediation” (Shigeta et al., 2021; Tingley et al., 2014) was used to examine whether pupil dilation during exercise serves as an indication of the neural substrate, which is hypothesized to be linked to pupil dilation, that mediates the effects of exercise on Stroop task performance. The contrast (change in the EX and CTL conditions) was calculated for pupil diameter and Stroop interference IES separately. The changes were calculated as follows: ΔPupil diameter: [mean of Ex1 and

Ex2] – Pre2 and ΔStroop interference IES: Post – Pre. The total effect, average direct effect (ADE), and average causal mediation effect (ACME) were calculated. A quasi- Bayesian approximation was used for confidence intervals and the number of simulations was 1000. Additionally, we performed a sensitivity analysis in order to examine the robustness of the results of the ACME against potential violations of sequential ignorability (i.e., whether or not an unmeasured confounder affected the mediation). The mediating effect of pupil diameter was also tested for cortical activity in a method consistent with Stroop interference IES.

3. Results

3.1. Verification of acute very-light-intensity exercise

We evaluated whether participants could perform 10 min of very-light-intensity exercise by monitoring RPE and HR. Average RPE and HR were 8.7 ± 1.7 (points) and 105.3 ± 12.3 (bpm), respectively, which are within the range of very-light-intensity exercise according to the guidelines of the American College of Sports Medicine (ACSM, 2018).

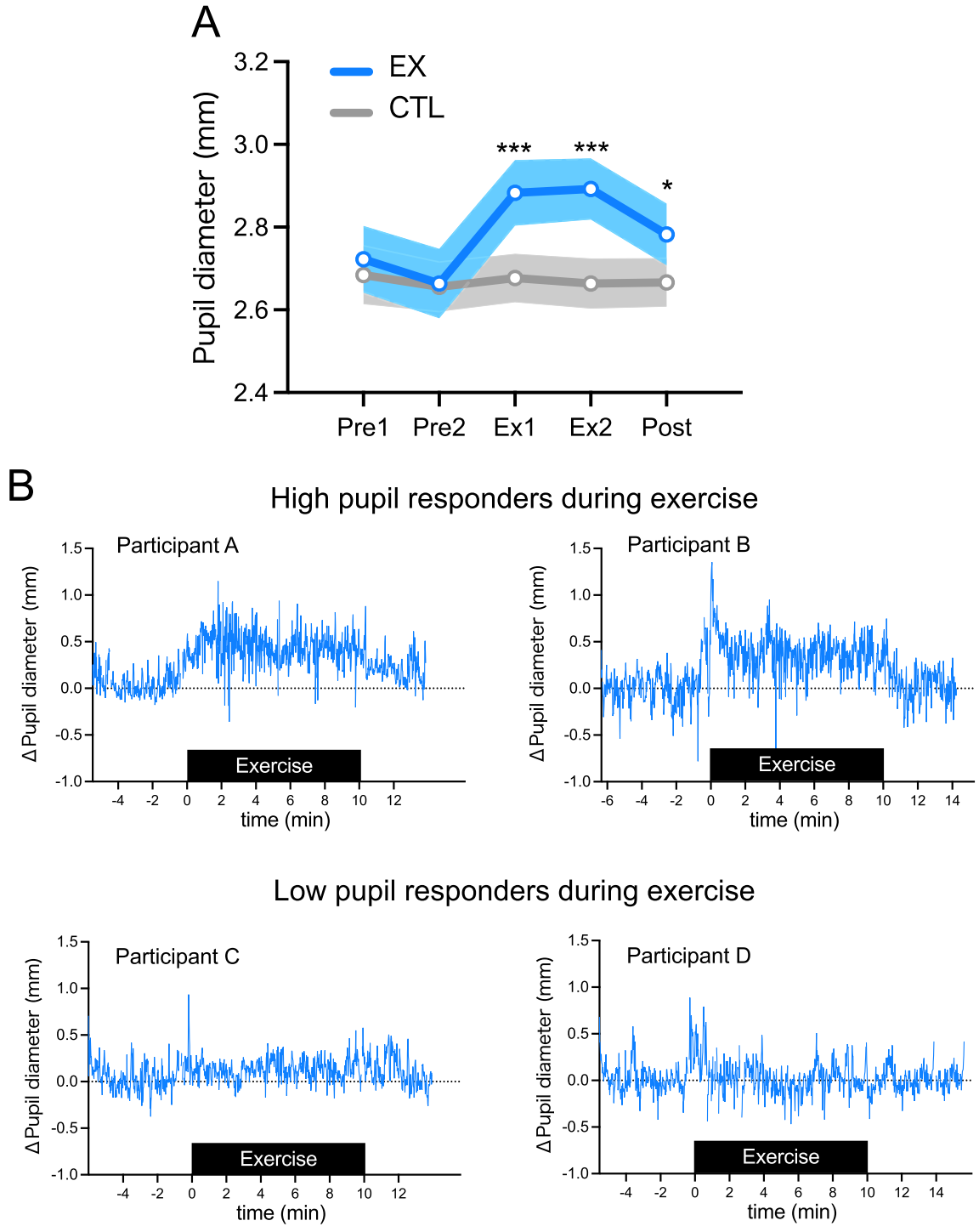

Fig. 2. (A) Pupil diameter in both CTL and EX conditions. Data are mean ± SE. $^{*}P < 0.05$, $^{***}P < 0.001$vs CTL. (B) Typical examples of pupil dilation during exercise (two high pupil responders and two low pupil responders). The difference from baseline (Pre2, just before exercise) is indicated.

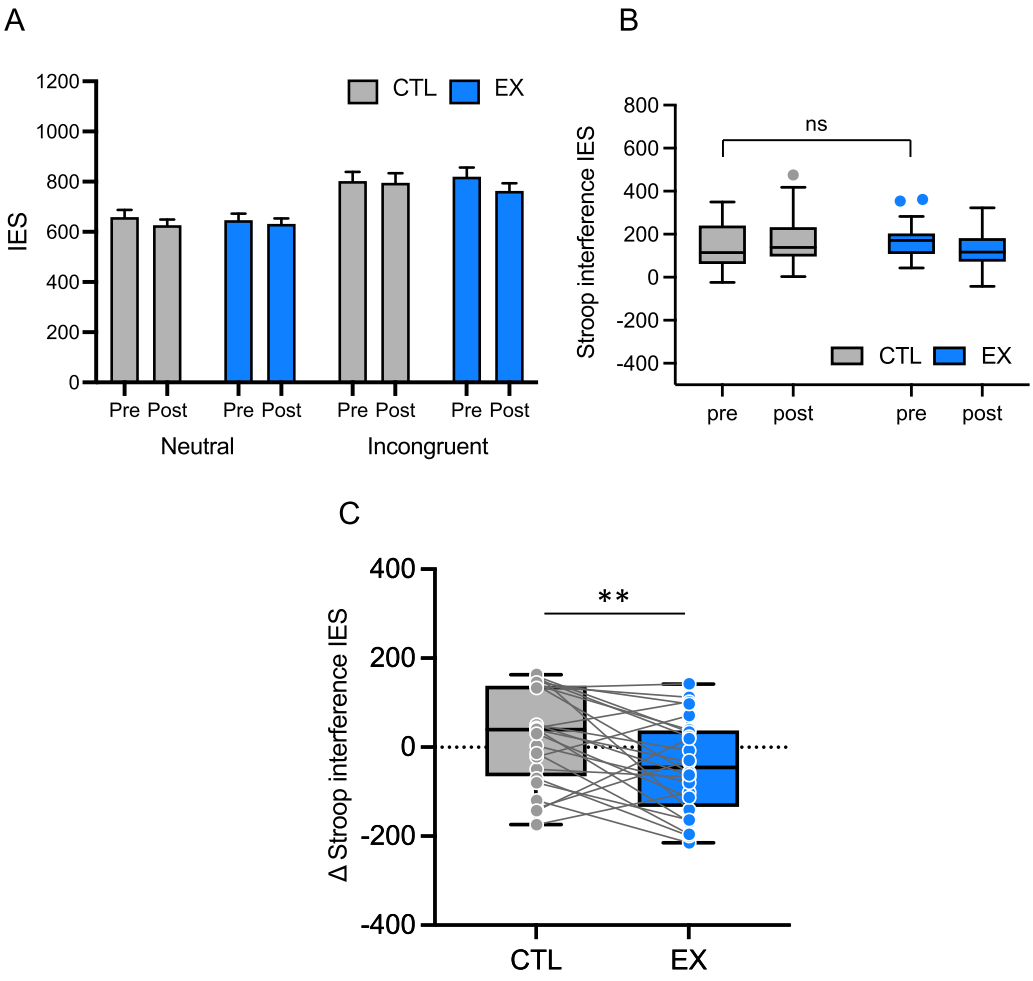

Fig. 3. (A)Results for neutral and incongruent tasks. Mean reaction time (RT) was adjusted for accuracy (inverse efficiency score (IES) = RT [ms] /accuracy [%] ×100). Data are mean ± SE. (B) The Stroop interference IES for each condition. (C) The differences in Stroop interference IES for both CTL and EX conditions are shown. Notes: ns not significant, $**P < 0.01$, The box-and-whisker plot is drawn in the Tukey manner. Line plots represent individual data.

3.2. Pupil diameter

Pupil diameter was measured as participants looked at a fixation cross before, during, and after exercise. A repeated measures two-way ANOVA for pupil diameter revealed a significant interaction between condition and time (F (2.54, 58.30) = 17.47, $P < 0.001$,$\eta_p^2 = 0.43$). Bonferroni-corrected post hoc comparisons revealed that pupil diameter in the EX condition was significantly higher than that in the CTL condition during and after exercise (EX1 (i.e., 1st half of exercise): F (1, 23) = 21.18, $P < 0.001$, $\eta_p^2 = 0.48$; EX2 (i.e., 2nd half of exercise): F (1, 23) = 28.88, $P < 0.001$, $\eta_p^2 = 0.56$; Post: F (1, 23) = 7.14, $P = 0.014$, $\eta_p^2 = 0.24$), but not significantly different before exercise ($Ps > 0.35$, $\eta_p^2 < 0.037$) (Fig. 2A).

3.3. Cognitive performance: Stroop interference

Fig. 3A shows the IES results for neutral and incongruent tasks. To examine the exercise session interaction for Stroop interference IES, we calculated the difference in the degree of Stroop interference IES between post- and pre-sessions. The change of Stroop interference IES was significantly negative in the EX condition compared to that in the CTL condition (t (23) = 2.98, $P = 0.007$, $d = 0.61$, paired t-test) (Fig. 3C). These results demonstrate that very-light-intensity exercise improved Stroop task performance (i.e., shorter reaction time and higher accuracy) compared with resting control. This result is also supported by the repeated measures two-way ANOVA (condition: EX/CTL, time: pre/post) demonstrating a significant interaction between condition and time factors for Stroop interference IES (F (1, 23) = 8.85, $P = 0.007$, $\eta_p^2 = 0.28$). However, Stroop interference IES did not statistically differ between pre-sessions (EX/CTL) (F (1, 23) = 2.89, $P = 0.10$,$\eta_p^2 = 0.11$) (Fig. 3B).

3.3.1. fNIRS results: cortical activation

To examine the exercise session interaction, we calculated the differences in hemodynamic response due to Stroop interference between post- and pre-sessions (Fig. 4). The paired t-test performed on each of the six ROIs revealed significant differences between conditions in the left DLPFC (t (23) = 3.00, $P = 0.006$, $q < 0.05$, $d = 0.61$, FDR corrected). The left FPA had a significant difference between conditions (t (23) = 2.32, $P = 0.030$, $d = 0.47$), but this finding did not survive FDR-correction.

3.4. Correlation analyses

Inter-individual differences in pupil dilation with exercise correlated with task performance change (i.e., reduced Stroop interference IES) (r (24) = -0.63, $P = 0.001$) (Fig. 5A). There is no significant correlation between increased left DLPFC activation and two variables: task performance change (r (24) = 0.12, $P = 0.56$) and pupil diameter change (r (24) = 0.05, $P = 0.82$).

3.5. Causal mediation analyses

To identify an underlying causal mechanism for very-light-intensity exercise effects on Stroop task performance, we conducted causal me-

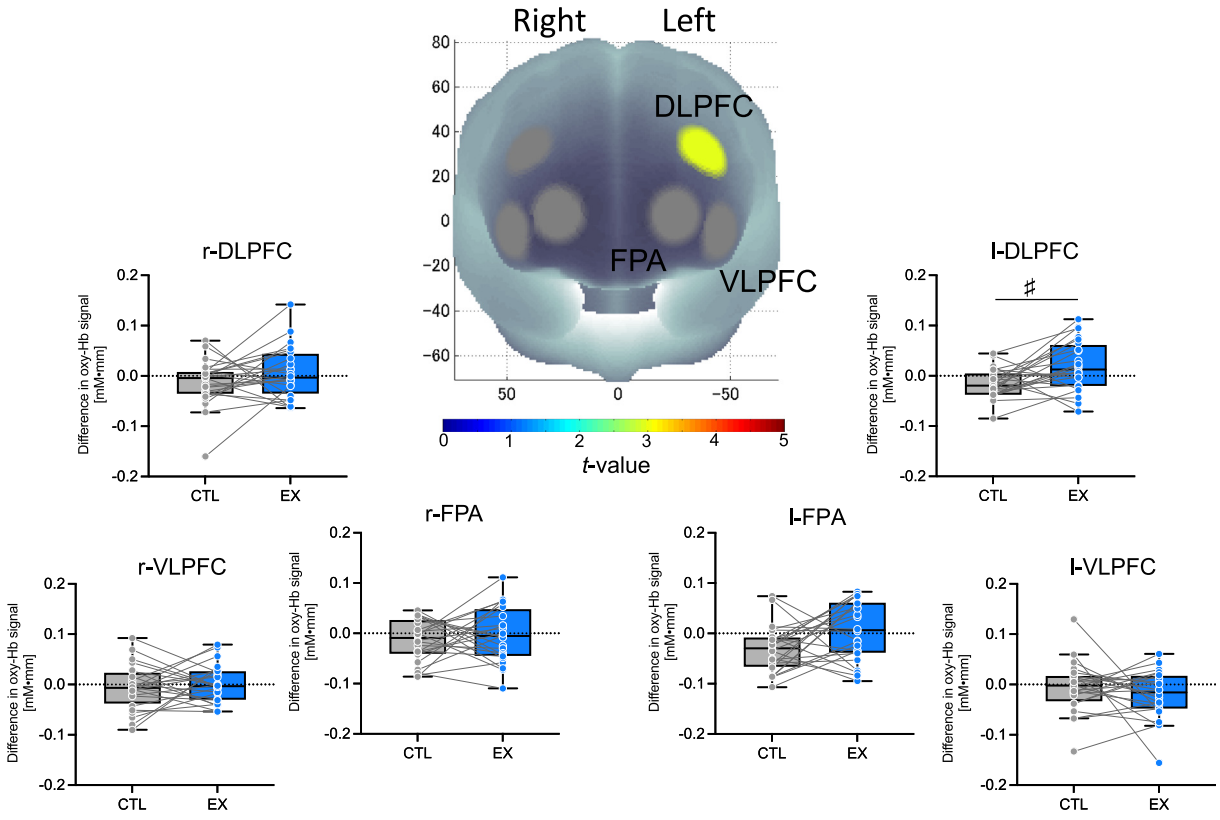

Fig. 4. Cortical loci of exercise-enhanced activation related to Stroop interference. The difference in oxygenated hemoglobin (oxy-Hb) signal changes corresponding to Stroop interference in both CTL and EX conditions are shown. The *t*-map of oxy-Hb signal changes reflects a very-light-intensity exercise effect. Among the six (ROIs, significant difference can be seen in the l-DLPFC (FDR-corrected). Notes: # $q < 0.05$, The box-and-whisker plot is drawn in the Tukey manner. Line plots represent individual data.

diation analyses (Imai et al., 2010) to examine whether pupil dilation during exercise indicates a mediative effect of very-light-intensity exercise on task performance. It should be noted that the hypothesized mediator is a neural substrate which is linked to pupil dilation. Consistent with our hypothesis, pupil dilation (M) was associated in such a way that it appeared as if it were mediating the association between very-light-intensity exercise intervention (X) and Stroop task performance enhancement (Y) (average causal mediation effect (ACME): -102.9, 95% CI [-167.8, -45.5], $P < 0.0001$; average direct effect (ADE): 35.8, 95% CI [-26.0, 102.0], $P = 0.27$; total effect: 67.1, 95% CI [-111.7, -22.5], $P = 0.004$; prop. mediated: 1.54, 95% CI [0.65, 4.5], $P = 0.004$) (Fig. 5B). Fig. 5C shows a model of the mediation analysis. The sensitivity analysis indicated that the assumption of sequential ignorability for the estimated ACME is maintained unless $\rho < -0.6$, which shows that the effect is reasonably robust to the violation of this assumption.

For cortical activation, we conducted causal mediation analyses to examine whether pupil-dilation-linked brain states during exercise mediated the effect of very-light-intensity exercise on Stroop interference-related l-DLPFC activation. There was no significant mediation effect of pupil dilation (M) on the association between very-light-intensity exercise intervention (X) and l-DLPFC activation (Y) (ACME: 0.0045, 95% CI [-0.029, 0.04], $P = 0.80$; average direct effect (ADE): 0.030, 95% CI [-0.011, 0.07], $P = 0.15$; total effect: 0.034, 95% CI [0.011, 0.06], $P = 0.004$; prop. mediated: 0.12, 95% CI [-1.15, 1.45], $P = 0.80$).

4. Discussion

We found that pupil dilation during very-light-intensity exercise was associated as if it were mediating the exercise-induced executive function enhancement with task-related subregion activation in the PFC. This is the first evidence to support the hypothesis that the upregulation of pupil-linked neural substrates, linking to arousal states and cortical network activity driven by neuromodulatory systems, during very-light-intensity exercise causes prefrontal cognitive potentiation.

Through the behavioral measurements showing a higher IES (i.e., integration score of longer RT and lower accuracy rate) in the incongruent task compared with that in the neutral task, we verified that the Stroop effect could be stably observed both before and after exercise. Based on this observation, we tested the effect of an acute bout of very-light-intensity exercise on Stroop interference IES, and confirmed significant enhancement of performance. Hence, the present study confirmed that an acute bout of 10 min of very-light-intensity exercise enhances cognitive functions to cope with Stroop interference. This result is consistent with previous studies, including our own, that have demonstrated that an acute bout of moderate or very-light-intensity exercise enhances inhibitory control functions (Byun et al., 2014b; Chang et al., 2012; McGowan et al., 2019; Shigeta et al., 2021; Yanagisawa et al., 2010).

Significant pupil dilation during exercise was observed, consistent with our previous work (Kuwamizu et al., 2022). These pupil dynamics suggest that pupil-synchronized brain states, related to arousal, attention, and cortical network activity, are upregulated during very-light-intensity exercise (Joshi and Gold, 2020; Schwalm and Jubal, 2017; Strauch et al., 2022). As we predicted, there were inter-individual differences (i.e., high and low responders) (Fig. 2B), which highly correlated with inter-individual differences in Stroop task performance enhancement. In addition, causal mediation analysis showed that pupil dilation while exercising mediated the executive performance enhancement that was observed after exercise. The ADE of exercise intervention on cognitive performance, after excluding the pupil dilation effect, was not significant, which indicates this effect is fully mediated by pupil dilation, strongly suggesting a mechanistic connection with this peripheral

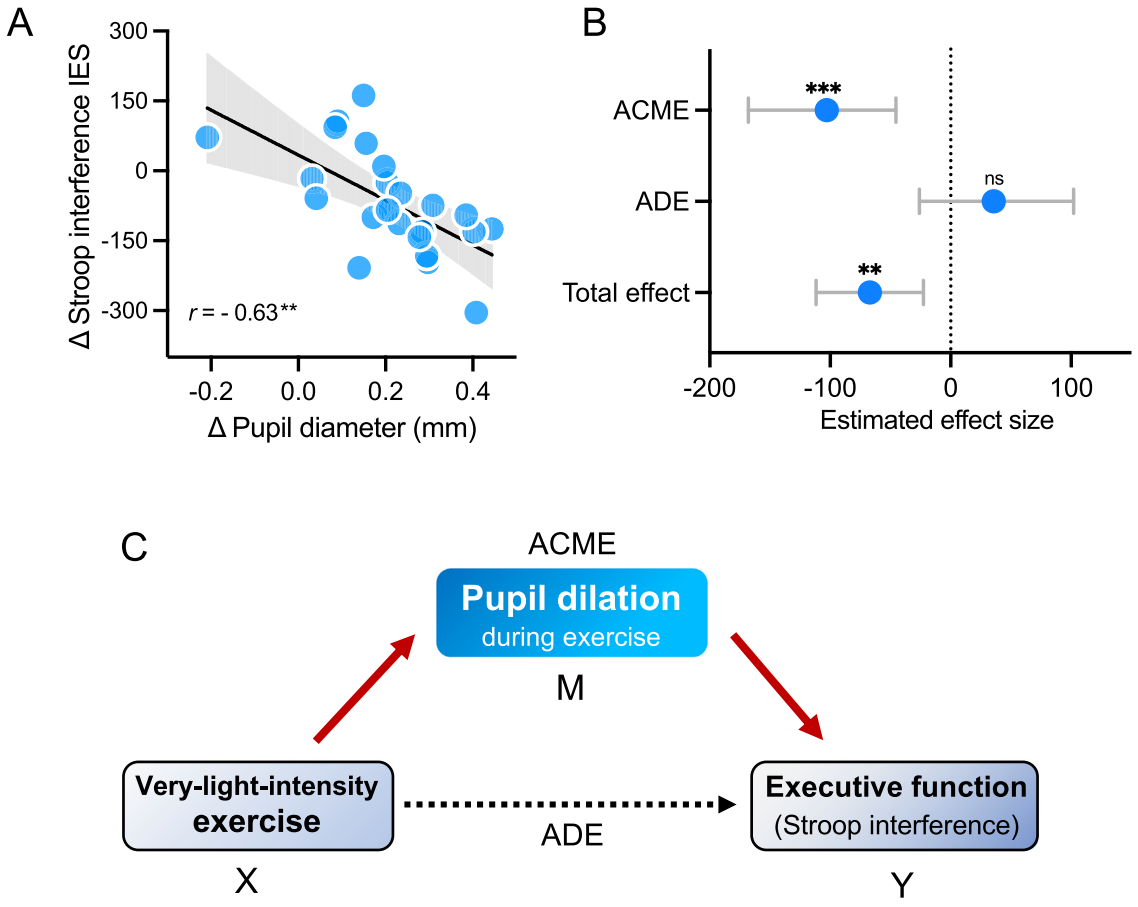

Fig. 5. (A) Association between Δpupil diameter (ΔEX-ΔCTL) and ΔStroop interference (ΔEX-ΔCTL). The black line represents linear regression and the gray band represents 95% confidence bands. (B) Results of causal mediation analysis. Pupil dilation had a significant ACME with regard to improving Stroop task performance with very-light-intensity exercise intervention, whereas the ADE was not significant. Data are estimated effect size ± 95% CI. (C) Mediation model of the relationship between very-light-intensity exercise intervention (X: exposure), pupil dilation (M: mediator), and Stroop task performance (Y: outcome). Notes: $^{**}P < 0.01$, $^{***}P < 0.001$, ns not significant

biomarker. These results indicate that identifying the neurological underpinning of cognitive enhancement after very-light-intensity exercise depends on pupil dilation, which is to say the ascending arousal system represented by LC-catecholaminergic modulation, during exercise. Several previous studies have relied on peripheral circulating biomarkers, such as plasma catecholamine, due to a lack of predictive measures of brain activation during exercise; significant changes in these measures were not observed with a short period of very-light-intensity exercise (McMorris et al., 2016), and there were no significant associations between these biomarkers and the effect of acute exercise on cognitive function (Ando et al., 2022). Two recent studies have examined pupil diameter in post-exercise tasks but did not link it to cognitive function improved by exercise (McGowan et al., 2019; Shigeta et al., 2021).

We observed a significant increase in Stroop interference-related l-DLPFC activation after exercise, corresponding to the enhancement of Stroop interference processing detected via pupil dilation (Fig. 4). This left-lateralized effect of ergometer exercise is consistent with our previous work in young adults, which demonstrated enhanced l-DLPFC activation with improved Stroop interference processing (Byun et al., 2014b; Yanagisawa et al., 2010). The DLPFC is implicated in Stroop interference processing (Frings et al., 2018; MacDonald et al., 2000) and it is also the projection site of pupil-linked neuromodulatory systems such as the catecholaminergic and cholinergic systems (Cools and Arnsten, 2022; Joshi and Gold, 2020; Strauch et al., 2022). The ascending arousal system is essential for executive function because it controls the optimal signal and noise regulation of the DLPFC (Cools and Arnsten, 2022). Activation of LC-catecholaminergic neurons increases cortical activity via the modulation of cortical glutamatergic neurotransmission, which is the main determinant for the coupling between increased cortical activity and hemodynamic alterations (Toussay et al., 2013). It is compelling that very-light-intensity exercise upregulates pupil-linked neural substrates, possibly modulating oxy-Hb signal changes in l-DLPFC-related Stroop interference. Although it was clear that l-DLPFC activation changed in the same direction mean-wise as both pupil dilation and decreased Stroop interference, the correlation analysis, which focused on individual differences, did not reach a significant linear association between l-DLPFC activity, and two variables; Stroop interference and pupil diameter. Therefore, the causal mediation analysis did not support that pupil dilation mediates the effect of very-light-intensity exercise on l-DLPFC activation. One reason for this is that the complexity of the experimental model limits the parametricity of the repeated subtracted components. As pointed out by previous studies (Byun et al., 2014b; Hyodo et al., 2012; Kujach et al., 2018; Yanagisawa et al., 2009), there are oxy-Hb signals that entail substantial individual differences and a subtraction procedure to contrast out Stroop interference (Incongruent-Neutral) further jeopardizes the quantification and narrows the range of parameters. In addition, trial intervention models that use the difference-in-difference model (i.e., EX(post-pre) - CTL(post-pre)) reduce the power to detect significant linear relationships between indicators. To close the further causal inference in the furture, it would be helpful to investigate whether experimental pharmacological inhibition of the catecholaminergic arousal system, with noted ethical considerations allowed, leads to a reduction in pupil dilation during exercise and activation of l-DLPFC and consequent attenuation of improve-

ments in task performance. Another point that should be considered is the brain regions that are involved in Stroop interference processing by interacting with the DLPFC. LC-catecholaminergic neurons project widely throughout the brain. The anterior cingulate and parietal cortical regions form a network with the DLPFC and contribute to efficient executive function processing (MacDonald et al., 2000; Mather et al., 2020). LC activation may trigger the release of striatum dopamine from the ventral tegmental area and the substantia nigra and the striatum dopamine also may contribute to executive function by interacting with the DLPFC (Nagano-Saito et al., 2008; Vernaleken et al., 2007). This would be an excellent subject for further study.

Given the involvement of catecholaminergic modulation from the LC to the cortex indicated by pupil dilation, which is a mechanism implicated in Alzheimer's, ADHD, and Parkinson's disease (Kremen et al., 2019; Poe et al., 2020), it is possible that brief mild exercise, such as yoga and very slow running, could be developed into more promising lifestyle interventions as natural and low-risk strategies to ameliorate cognitive impairment. The evidence presented here demonstrates that mild exercise works as an efficacious method of brain stimulation for enhancing cognitive function and that this effect can be assessed by observing pupil dynamics. In addition, pupil dynamics were also demonstrated to be significantly synchronized with psychological arousal and pleasure moods (see *Psychological mood states* in the Supplemental information for detailed results). These findings indicate that pupillometry could be a new non-invasive tool to clarify the beneficial impact of exercise in boosting brain health, something which cannot be accomplished with neuroimaging technology that is affected by body movement and cardiovascular responses.

There are remaining points with regard to the generalization of these findings. First, although healthy young adults of both sexes were included in this study, the male/female proportion was unbalanced. Further research that addresses differences in gender and populace would provide a generalization of this finding. Second, participants were seated on the ergometer and at rest in the control condition to minimize pupil responses to various environmental stimuli. While mind wandering may remain a potential factor related to arousal and cognition, there were no changes in pupil diameter during resting.

5. Conclusion

The current study reveals that very-light-intensity exercise-induced cognitive enhancement, with task-related PFC subregion activation, depends on pupil dilation during exercise. This suggests that neural mechanisms associated with pupil dilation may be associated with the beneficial impact of exercise on prefrontal cognitive function.

Credit author statement

Ryuta Kuwamizu: Conceptualization, Methodology, Performing the experiments, Data analysis, Visualization, Writing- Original draft preparation, Project administration, Funding acquisition, **Yudai Yamazaki**: Performing the experiments, Data analysis, Writing- Reviewing and Editing, **Naoki Aoike**: Performing the experiments, Data analysis, Writing- Reviewing and Editing, **Taichi Hiraga**: Writing- Reviewing and Editing, **Toshiaki Hata**: Writing- Reviewing and Editing, **Michael A. Yassa**: Writing- Reviewing and Editing, **Hideaki Soya**: Conceptualization, Writing- Reviewing and Editing, Supervision, Funding acquisition.

Source of funding

This work was supported in part by the Japan Society for the Promotion of Science (JSPS) 16H06405 (H.S), 18H04081 (H.S.), 21H04858 (H.S.), 20J20893 (R.K.), and 23KJ1169 (R.K.); the Japan Science and Technology Agency (JST) Grant JPMJMI19D5 (H.S.); and the Meiji Yasuda Life Foundation of Health and Welfare Grant (R.K.). This work was also supported in part by the Inviting Overseas Educational Research Units in University of Tsukuba (2016–2023) (to H.S.).

Declaration of Competing Interest

The authors do not declare any competing interests.

Data availability

All data that support the findings of this study are available upon request from the corresponding author within the limits set by the Institutional Ethics Committee of the University of Tsukuba, which ensures that personal information will not be disclosed.

Acknowledgments

The authors are grateful to H. Imoarai, C. Damrongthai, A. Nagano-Saito, M. Okamoto (University of Tsukuba, Japan); K. Suwabe (Ryutsu Keizai University, University of Tsukuba, Japan); and G. Ochi (Niigata University of Health and Welfare, Japan) for data collection support and discussion. They thank R. Shimoda (University of Tsukuba, Japan) and R. Hosokawa (Tokyo Institute of Technology, Japan) for analysis support. They express their gratitude to M. Noguchi (ELCS English Language Consultation Services, Japan) for helping with the manuscript. The illustration (cycling man in Fig. 1A) was drawn by C. Damrongthai.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2023.120244.

References

American College of Sports Medicine, 2018. ACSM's Guidelines for Exercise Testing and Prescription, 10th Ed Wolters Kluwer Health.

Ando, S., Komiyama, T., Tanoue, Y., Sudo, M., Costello, J.T., Uehara, Y., Higaki, Y., 2022. Cognitive improvement after aerobic and resistance exercise is not associated with peripheral biomarkers. Front. Behav. Neurosci. 16, 1–10. doi:10.3389/fnbeh.2022.853150.

Aston-Jones, G., Cohen, J.D., 2005. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu. Rev. Neurosci. 28, 403–450. doi:10.1146/annurev.neuro.28.061604.135709.

Ayala, N., Heath, M., 2021. Pupillometry reveals the role of arousal in a postexercise benefit to executive function. Brain Sci. 11, 1048. doi:10.3390/brainsci11081048.

Bari, A., Xu, S., Pignatelli, M., Takeuchi, D., Feng, J., Li, Y., Tonegawa, S., 2020. Differential attentional control mechanisms by two distinct noradrenergic coeruleo-frontal cortical pathways. Proc. Natl. Acad. Sci. U. S. A. 117, 29080–29089. doi:10.1073/pnas.2015635117.

Breton-Provencher, V., Sur, M., 2019. Active control of arousal by a locus coeruleus GABAergic circuit. Nat. Neurosci. 22, 218–228. doi:10.1038/s41593-018-0305-z.

Brett, M., Johnsrude, I.S., M.Owen, A., 2002. The problem of functional localization in the human brain. Nat. Rev. Neurosci. 3, 243–249. doi:10.1038/NRN756.

Byun, K., Hyodo, K., Suwabe, K., Kujach, S., Kato, M., Soya, H., Ochi, G., Sakairi, Y., Kato, M., Dan, I., Soya, H., 2014a. Possible influences of exercise-intensity-dependent increases in non-cortical hemodynamic variables on NIRS-based neuroimaging analysis during cognitive tasks: Technical note. J. Exerc. Nutr. Biochem. 18, 327–332. doi:10.1016/j.neuroimage.2014.04.067.

Byun, K., Hyodo, K., Suwabe, K., Ochi, G., Sakairi, Y., Kato, M., Dan, I., Soya, H., 2014b. Positive effect of acute mild exercise on executive function via arousal-related prefrontal activations: an fNIRS study. Neuroimage 98, 336–345. doi:10.1016/j.neuroimage.2014.04.067.

Chang, Y.K., Labban, J.D., Gapin, J.I., Etnier, J.L., 2012. The effects of acute exercise on cognitive performance: a meta-analysis. Brain Res. 1453, 87–101. doi:10.1016/j.brainres.2012.02.068.

Cools, R., Arnsten, A.F.T., 2022. Neuromodulation of prefrontal cortex cognitive function in primates: the powerful roles of monoamines and acetylcholine. Neuropsychopharmacology 47, 309–328. doi:10.1038/s41386-021-01100-8.

Damrongthai, C., Kuwamizu, R., Suwabe, K., Ochi, G., Yamazaki, Y., Fukuie, T., Adachi, K., Yassa, M.A., Churdchomjan, W., Soya, H., 2021. Benefit of human moderate running boosting mood and executive function coinciding with bilateral prefrontal activation. Sci. Rep. 11, 1–12. doi:10.1038/s41598-021-01654-z.

Ekkekakis, P., Hall, E.E., Petruzzello, S.J., 2008. The relationship between exercise intensity and affective responses demystified: To crack the 40-year-old nut, replace the 40-year-old nutcracker! Ann. Behav. Med. 35, 136–149. doi:10.1007/s12160-008-9025-z.

Frings, C., Brinkmann, T., Friehs, M.A., van Lipzig, T., 2018. Single session tDCS over the left DLPFC disrupts interference processing. Brain Cogn. 120, 1–7. doi:10.1016/j.bandc.2017.11.005.

Fukuie, T., Suwabe, K., Kawase, S., Shimizu, T., Ochi, G., Kuwamizu, R., Sakairi, Y., Soya, H., 2022. Groove rhythm stimulates prefrontal cortex function in groove enjoyers. Sci. Rep. 12, 1–14. doi:10.1038/s41598-022-11324-3.

Green, S.B., 1991. How many subjects does it take to do a regression analysis? Multivar. Behav. Res. 26, 499–510. doi:10.1207/s15327906mbr2603_7.

Grueschow, M., Kleim, B., Ruff, C.C., 2020. Role of the locus coeruleus arousal system in cognitive control. J. Neuroendocrinol. 32, 1–11. doi:10.1111/jne.12890.

Hayashi, N., Someya, N., Fukuba, Y., 2010. Effect of intensity of dynamic exercise on pupil diameter in humans. J. Physiol. Anthropol. 29, 119–122. doi:10.2114/jpa2.29.119.

Hyodo, K., Dan, I., Suwabe, K., Kyutoku, Y., Yamada, Y., Akahori, M., Byun, K., Kato, M., Soya, H., 2012. Acute moderate exercise enhances compensatory brain activation in older adults. Neurobiol. Aging 33, 2621–2632. doi:10.1016/j.neurobiolaging.2011.12.022.

Imai, K., Keele, L., Tingley, D., 2010. A general approach to causal mediation analysis. Psychol. Methods 15, 309–334. doi:10.1037/a0020761.

Joshi, S., Gold, J.I., 2020. Pupil size as a window on neural substrates of cognition. Trends Cogn. Sci. 24, 466–480. doi:10.1016/j.tics.2020.03.005.

Joshi, S., Li, Y., Kalwani, R.M., Gold, J.I., 2016. Relationships between pupil diameter and neuronal activity in the locus coeruleus, colliculi, and cingulate cortex. Neuron 89, 221–234. doi:10.1016/j.neuron.2015.11.028.

Katagiri, A., Dan, I., Tuzuki, D., Okamoto, M., Yokose, N., Igarashi, K., Hoshino, T., Fujiwara, T., Katayama, Y., Yamaguchi, Y., Sakatani, K., 2010. Mapping of optical pathlength of human adult head at multi-wavelengths in near infrared spectroscopy. Adv. Exp. Med. Biol. 662, 205–212. doi:10.1007/978-1-4419-1241-1_29.

Kremen, W.S., Panizzon, M.S., Elman, J.A., Granholm, E.L., Andreassen, O.A., Dale, A.M., Gillespie, N.A., Gustavson, D.E., Logue, M.W., Lyons, M.J., Neale, M.C., Reynolds, C.A., Whitsel, N., Franz, C.E., 2019. Pupillary dilation responses as a midlife indicator of risk for Alzheimer's disease: association with Alzheimer's disease polygenic risk. Neurobiol. Aging 83, 114–121. doi:10.1016/J.NEUROBIOLAGING.2019.09.001.

Kujach, S., Byun, K., Hyodo, K., Suwabe, K., Fukuie, T., Laskowski, R., Dan, I., Soya, H., 2018. A transferable high-intensity intermittent exercise improves executive performance in association with dorsolateral prefrontal activation in young adults. Neuroimage 169, 117–125. doi:10.1016/j.neuroimage.2017.12.003.

Kurosawa, M., Sato, A., Uchida, S., 1993. Extracellular release of acetylcholine, noradrenaline and serotonin increases in the cerebral cortex during walking in conscious. rats 161, 73–76. doi:10.1016/0304-3940(93)90143-9 Paperpile.

Kuwamizu, R., Suwabe, K., Damrongthai, C., Fukuie, T., Ochi, G., Hyodo, K., Hiraga, T., Nagano-Saito, A., Soya, H., 2021. Spontaneous eye blink rate connects missing link between aerobic fitness and cognition. Med. Sci. Sport. Exerc. 53, 1425–1433. doi:10.1249/mss.0000000000002590.

Kuwamizu, R., Yamazaki, Y., Aoike, N., Ochi, G., Suwabe, K., Soya, H., 2022. Pupil-linked arousal with very light exercise: pattern of pupil dilation during graded exercise. J. Physiol. Sci. 72, 1–9. doi:10.1186/s12576-022-00849-x.

Lu, L., Ren, Y., Yu, T., Liu, Z., Wang, S., Tan, L., Zeng, J., Feng, Q., Lin, R., Liu, Y., Guo, Q., Luo, M., 2020. Control of locomotor speed, arousal, and hippocampal theta rhythms by the nucleus incertus. Nat. Commun. 11, 1–16. doi:10.1038/s41467-019-14116-y.

MacDonald, A.W., Cohen, J.D., Stenger, V.A., Carter, C.S., 2000. Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. Science 288, 1835–1838. doi:10.1126/science.288.5472.1835.

Maki, A., 1995. Spatial and temporal analysis of human motor activity using noninvasive NIR topography. Med. Phys. 22, 1997–2005. doi:10.1118/1.597496.

Mather, M., Huang, R., Clewett, D., Nielsen, S.E., Velasco, R., Tu, K., Han, S., Kennedy, B.L., 2020. Isometric exercise facilitates attention to salient events in women via the noradrenergic system. Neuroimage 210, 116560. doi:10.1016/j.neuroimage.2020.116560.

Mathôt, S., 2018. Pupillometry: psychology, physiology, and function. J. Cogn. 1, 1–23. doi:10.5334/joc.18.

McGinley, M.J., David, S.V., McCormick, D.A., 2015. Cortical membrane potential signature of optimal states for sensory signal detection. Neuron 87, 179–192. doi:10.1016/j.neuron.2015.05.038.

McGowan, A.L., Chandler, M.C., Brascamp, J.W., Pontifex, M.B., 2019. Pupillometric indices of locus-coeruleus activation are not modulated following single bouts of exercise. Int. J. Psychophysiol. 140, 41–52. doi:10.1016/j.ijpsycho.2019.04.004.

McMorris, T., Turner, A., Hale, B.J., Sproule, J., 2016. Beyond the catecholamines hypothesis for an acute exercise–cognition interaction: a neurochemical perspective. In: McMorris, T. (Ed.), Exercise-Cognition Interaction: Neuroscience Perspectives. Elsevier Academic Press, pp. 65–103. doi:10.1016/j.optmat.2011.11.002.

Nagano-Saito, A., Leyton, M., Monchi, O., Goldberg, Y.K., He, Y., Dagher, A., 2008. Dopamine depletion impairs frontostriatal functional connectivity during a set-shifting task. J. Neurosci. 28, 3697–3706. doi:10.1523/JNEUROSCI.3921-07.2008.

Nishijima, T., Soya, H., 2006. Evidence of functional hyperemia in the rat hippocampus during mild treadmill running. Neurosci. Res. 54, 186–191. doi:10.1016/j.neures.2005.11.005.

Ochi, G., Kuwamizu, R., Suwabe, K., Fukuie, T., Hyodo, K., Soya, H., 2022. Cognitive fatigue due to exercise under normobaric hypoxia is related to hypoxemia during exercise. Sci. Rep. 12, 9835. doi:10.1038/s41598-022-14146-5.

Ohiwa, N., Saito, T., Chang, H., Nakamura, T., Soya, H., 2006a. Differential responsiveness of c-Fos expression in the rat medulla oblongata to different treadmill running speeds. Neurosci. Res. 54, 124–132. doi:10.1016/j.neures.2005.10.014.

Ohiwa, N., Saito, T., Chang, H., Omori, T., 2006b. Activation of A1 and A2 noradrenergic neurons in response to running in the rat. Neurosci. Lett. 395, 46–50. doi:10.1016/j.neulet.2005.10.053.

Okamoto, M., 2004. Three-dimensional probabilistic anatomical cranio-cerebral correlation via the international 10–20 system oriented for transcranial functional brain mapping. Neuroimage 21, 99–111. doi:10.1016/j.neuroimage.2003.08.026.

Okamoto, M., Dan, I., 2005. Automated cortical projection of head-surface locations for transcranial functional brain mapping. Neuroimage 26, 18–28. doi:10.1016/j.neuroimage.2005.01.018.

Pagliari, R., Peyrin, L., 1995. Norepinephrine release in the rat frontal cortex under treadmill exercise: a study with microdialysis. J. J. Appl. Physiol. 78, 2121–2130. doi:10.1152/japplphysiol.90378.2008.

Poe, G.R., Foote, S., Eschenko, O., Johansen, J.P., Bouret, S., Aston-Jones, G., Harley, C.W., Manahan-Vaughan, D., Weinshenker, D., Valentino, R., Berridge, C., Chandler, D.J., Waterhouse, B., Sara, S.J., 2020. Locus coeruleus: a new look at the blue spot. Nat. Rev. Neurosci. 21, 644–659. doi:10.1038/s41583-020-0360-9.

Privitera, M., Ferrari, K.D., von Ziegler, L.M., Sturman, O., Duss, S.N., Floriou-Servou, A., Germain, P.L., Vermeiren, Y., Wyss, M.T., De Deyn, P.P., Weber, B., Bohacek, J., 2020. A complete pupillometry toolbox for real-time monitoring of locus coeruleus activity in rodents. Nat. Protoc. 15, 2301–2320. doi:10.1038/s41596-020-0324-6.

Reimer, J., McGinley, M.J., Liu, Y., Rodenkirch, C., Wang, Q., McCormick, D.A., Tolias, A.S., 2016. Pupil fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. Nat. Commun. 7, 1–7. doi:10.1038/ncomms13289.

Schwalm, M., Jubal, E.R., 2017. Back to pupillometry: how cortical network state fluctuations tracked by pupil dynamics could explain neural signal variability in human cognitive neuroscience. eNeuro 4. doi:10.1523/ENEURO.0293-16.2017.

Shattuck, D.D.W., Mirza, M., Adisetiyo, V., Hojatkashani, C., Salamon, G., Narr, K.L., Poldrack, R.A., Bilder, R.M., Toga, A.W., 2008. Construction of a 3D probabilistic atlas of human cortical structures. Neuroimage 39, 1064–1080. doi:10.1016/j.neuroimage.2007.09.031.

Shigeta, T.T., Morris, T.P., Henry, D.H., Kucyi, A., Bex, P., Kramer, A.F., Hillman, C.H., 2021. Acute exercise effects on inhibitory control and the pupillary response in young adults. Int. J. Psychophysiol. 170, 218–228. doi:10.1016/j.ijpsycho.2021.08.006.

Singh, A.K., Okamoto, M., Dan, H., Jurcak, V., Dan, I., 2005. Spatial registration of multichannel multi-subject fNIRS data to MNI space without MRI. Neuroimage 27, 842–851. doi:10.1016/j.neuroimage.2005.05.019.

Soya, H., Mukai, A., Deocaris, C.C., Ohiwa, N., Chang, H., Nishijima, T., Fujikawa, T., Togashi, K., Saito, T., 2007a. Threshold-like pattern of neuronal activation in the hypothalamus during treadmill running: establishment of a minimum running stress (MRS) rat model. Neurosci. Res. 58, 341–348. doi:10.1016/j.neures.2007.04.004.

Soya, H., Nakamura, T., Deocaris, C.C., Kimpara, A., Iimura, M., Fujikawa, T., Chang, H., McEwen, B.S., Nishijima, T., 2007b. BDNF induction with mild exercise in the rat hippocampus. Biochem. Biophys. Res. Commun. 358, 961–967. doi:10.1016/j.bbrc.2007.04.173.

Strauch, C., Wang, C.-A., Einhäuser, W., Van der Stigchel, S., Naber, M., 2022. Pupillometry as an integrated readout of distinct attentional networks. Trends Neurosci. 1–13. doi:10.1016/j.tins.2022.05.003.

Suwabe, K., Byun, K., Hyodo, K., Reagh, Z.M., Roberts, J.M., Matsushita, A., Saotome, K., Ochi, G., Fukuie, T., Suzuki, K., Sankai, Y., Yassa, M.A., Soya, H., Soyaa, H., Fukuie, T., 2018. Rapid stimulation of human dentate gyrus function with acute mild exercise. Proc. Natl. Acad. Sci. U. S. A. 115, 10487–10492. doi:10.1073/pnas.1805668115.

Suwabe, K., Hyodo, K., Fukuie, T., Ochi, G., Inagaki, K., Sakairi, Y., Soya, H., 2021. Positive mood while exercising influences beneficial effects of exercise with music on prefrontal executive function: a functional NIRS study. Neuroscience 454, 61–71. doi:10.1016/j.neuroscience.2020.06.007.

Tingley, D., Yamamoto, T., Hirose, K., Keele, L., Imai, K., 2014. mediation: R package for causal mediation analysis. J. Stat. Softw. 59, 1–38. doi:10.18637/jss.v059.i05.

Totah, N.K.B., Logothetis, N.K., Eschenko, O., 2019. Noradrenergic ensemble-based modulation of cognition over multiple timescales. Brain Res. 1709, 50–66. doi:10.1016/j.brainres.2018.12.031.

Toussay, X., Basu, K., Lacoste, B., Hamel, E., Cbf, T., 2013. Locus coeruleus stimulation recruits a broad cortical neuronal network and increases cortical perfusion. J. Neurosci. 33, 3390–3401. doi:10.1523/JNEUROSCI.3346-12.2013.

Tsuzuki, D., Dan, I., 2014. Spatial registration for functional near-infrared spectroscopy: from channel position on the scalp to cortical location in individual and group analyses. Neuroimage 85, 92–103. doi:10.1016/j.neuroimage.2013.07.025.

Tsuzuki, D., Jurcak, V., Singh, A.K., Okamoto, M., Watanabe, E., Dan, I., 2007. Virtual spatial registration of stand-alone fNIRS data to MNI space. Neuroimage 34, 1506–1518. doi:10.1016/j.neuroimage.2006.10.043.

van der Wel, P., van Steenbergen, H., 2018. Pupil dilation as an index of effort in cognitive control tasks: a review. Psychon. Bull. Rev. 25, 2005–2015. doi:10.3758/s13423-018-1432-y.

Vernaleken, I., Buchholz, H.-G., Kumakura, Y., Siessmeier, T., Stoeter, P., Bartenstein, P., Cumming, P., Gründer, G., 2007. 'Prefrontal' cognitive performance of healthy subjects positively correlates with cerebral FDOPA influx: an exploratory [18F]-fluoro-L-DOPA-PET investigation. Hum. Brain Mapp. 28, 931–939. doi:10.1002/hbm.20325 .

Yamazaki, Y., Suwabe, K., Nagano-saito, A., Saotome, K., Kuwamizu, R., Hiraga, T., Torma, F., Suzuki, K., Sankai, Y., Yassa, M.A., Soya, H., 2023. A Possible contribution of the locus coeruleus to arousal enhancement with mild exercise: evidence from pupillometry and neuromelanin imaging. Cereb. Cortex Commun. 4, tgad010. doi:10.1093/texcom/tgad010.

Yanagisawa, H., Dan, I., Tsuzuki, D., Kato, M., Okamoto, M., Kyutoku, Y., Soya, H., 2010. Acute moderate exercise elicits increased dorsolateral prefrontal activation and improves cognitive performance with Stroop test. Neuroimage 50, 1702–1710. doi:10.1016/j.neuroimage.2009.12.023.

Yanagisawa, H., Soya, H., Dan, I., 2009. Effect of duration of acute moderate exercise on exercise-elicited cortical activation and cognitive performance on stroop task: a preliminary examination. Int. J. Hum. Mov. Sci. 3, 111–132.