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Delving into the relationship between regular physical exercise and cardiac interoception in two cross-sectional studies.

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Abstract

Cardiac interoception, the ability to sense and process cardiac afferent signals, has been shown to improve after a single session of acute physical exercise. However, it remains unclear whether repetitive engagement in physical exercise over time leads to long-term changes in cardiac interoceptive accuracy. It is also unknown whether those changes affect the neural activity associated with the processing of afferent cardiac signals, assessed by the heart-evoked potential (HEP). In this study, we aimed to investigate this hypothesis through two cross-sectional studies, categorizing participants as active or inactive based on physical fitness (Study I; N = 45) or self-reported physical activity levels (Study II; N = 60). Interoception was assessed at rest using the HEP (Studies I and II), the Heartbeat Counting task (Study II), and the Rubber Hand Illusion (Study II). Study I showed strong evidence of better cardiovascular fitness in the active group (N=24 males, 22 years old) than in the inactive group (N=21 males, 23 years old), as well as robust between-group differences in electrocardiogram (ECG) recordings. Study 2 (N=15 males and 15 females, 24 years old, in the active; N=9 males and 21 females, 24 years old, in the inactive group) replicated the clear differences in ECG as a function of regular physical activity. Those results were expected due to clear differences in physical activity habits. In contrast, there were no reliable and clear between-group differences in any of the measures of interoception. Consequently, our results do not provide convincing evidence to support the notion that regular physical exercise leads to an increase in cardiac interoception.

Keywords: Regular physical activity, interoceptive accuracy, physical fitness, heartbeat-evoked potential, rubber hand illusion, cardiac signal, cardiac interoception.

1. Introduction

Interoception, the process of sensing and interpreting the internal state of the body (Khalsa et al., 2018), can be influenced by attentional —top-down— processes (Suksasilp & Garfinkel, 2022). Previous research has demonstrated that directing attention to interoceptive stimuli, such as heartbeats, increases interoceptive accuracy (IAcc), the ability to detect them accurately (Garfinkel et al., 2015). Similarly, auditory feedback has been found to improve IAcc when individuals tapped to their own heartbeat (Canales-Johnson et al., 2015). Moreover, Canales-Johnson et al. showed modulation of the magnitude of the heartbeat-evoked potential (HEP), which is considered a basic neural index of interoception (Park & Blanke, 2019), whereby the brain's cortical activity (measured by means of electroencephalography; EEG) is time-locked to the R- or T-wave of the electrocardiogram (ECG) by averaging consecutive cardiac events.

Evidence however suggests that interoception is also susceptible to bottom-up modulations. Particularly, physical exercise is one of these bottom-up processes that might reliably affect interoception. During physical exercise, the transition from a resting to an aroused state intensifies the cardiac signal, as reflected in parameters such as heart rate (HR), stroke volume, and blood pressure. If the cardiac afferent signal is altered, one would then expect a change in the processing of that signal at the level of the central nervous system. Indeed, a single session of physical exercise has been shown to increase the perception of heartbeats (Antony et al., 1995; Jones & Hollandsworth, 1981; Montgomery et al., 1984; Wallman-Jones et al., 2022)

When performed regularly over a relatively long time, physical exercise induces physiological adaptations at, for example, metabolic, muscular, and cardiovascular levels (Garber et al., 2011; Hellsten & Nyberg, 2015). For instance, Brown et al. (2020) found heart adaptations in cyclists that included increased left ventricular (LV) chamber volume and wall thickness. This ventricular remodeling in elite road cyclists is typically associated with higher cardiac outputs necessary for sustained high-intensity exercise, which can impact heart rate (HR), stroke volume, and consequently the electrocardiogram (ECG) signal.

Again, if afferent signals from these body systems to the brain are consistently and robustly altered by the regular practice of physical exercise, the logic follows that interoception would be affected likewise. This hypothesis has been put forward recently by Wallman-Jones et al. (Wallman-Jones et al., 2021), who thoroughly reviewed the scarce evidence to date. Yet, even if there are articles reporting a positive association of chronic physical exercise with behavioral measures of interoception (e.g., (Georgiou et al., 2015)) to the best of our knowledge, no study to date has investigated its potential link with the amplitude of the HEP, currently one of the most commonly used indexes of interoception at the neural level.

In expanding upon Wallman-Jones et al. (2021), the present study investigates the potential correlation between regular physical exercise and cardiac interoception. Building on the well-established role of HEP as a neural index of interoception, our research explores its link with physical activity, a connection not previously assessed in the literature. The HEP, intricately linked with brain structures such as the insula, anterior cingulate cortex, amygdala, and somatosensory cortex, serves as a robust measure of cardiac interoception (Kern et al., 2013; Park & Blanke, 2019). While the literature often highlights the broader aspects of bodily awareness and processing associated with HEP, our study concentrates on its specific relevance to cardiac interoception which has been particularly emphasized in current research. Nevertheless, we acknowledge that the HEP is a complex construct associated with multiple mechanisms beyond cardiac interoception, such as emotion processing and body awareness. Given its early stage of development as a measure, we approach its utilization with rigor and caution to avoid potential misinterpretations. As such, in light of the absence of consensus on specific regions of interest and latency ranges for reported HEP differences (Coll et al., 2021), we decided to employ a data-driven cluster-based analysis without a-priori decisions on regions or time windows.

In Study I, physically active and inactive participants were also characterized by a cardiovascular fitness test, to further ensure group differences in terms of cardiac adaptations to physical exercise. The study involved novel analyses conducted on a dataset obtained from a previous investigation by Luque-Casado et al. (Luque-Casado et al., 2016) focusing on the relationship between aerobic fitness and sustained attention capacity. In contrast, Study I explored a different research question involving a different construct, cardiac interoception, and employing a novel measure, the HEP. Cardiac interoception was assessed by means of the HEP in Studies I and II, and behaviorally using the heartbeat counting task (HBC) in Study II. Additionally, in Study II we also test group differences in the Rubber Hand Illusion (RHI) (Botvinick & Cohen, 1998).

The RHI involves manipulating bodily cues through multisensory integration, where a rubber hand is touched while the participant's hand is occluded. If the illusion is elicited, the participant feels that the rubber hand is their actual hand, biasing reports of the perceived position of their hand, an effect known as proprioceptive drift. The RHI is thought to inform about the malleability of one’s body representation and therefore should be associated with one’s ability to process body afferent signals. Previous research has tested this hypothesis, showing indeed that better IAcc was associated with a reduced strength of the RHI (Filippetti & Tsakiris, 2017; Tsakiris et al., 2011).

Building upon Wallman-Jones et al. (2021) and guided by existing evidence in exercise physiology and interoception, our study aims to analyze variations in HEP resulting from regular physical activity adaptations, with a complementary focus on changes in ECG. Additionally, we delve into behavioral measures such as the classic IAcc and extend our exploration to proprioceptive drift through the RHI. Specifically, Study I focuses on the impact of chronic physical exercise, anticipating: a) heightened cardiovascular fitness, b) distinct group differences in the cardiac signal measured through electrocardiography, and c) variations in HEP amplitude. In Study II, with a more pronounced emphasis on HEP, we also anticipate improved interoception in the active group, measured by a) discernible group differences in HEP amplitude, b) enhanced Interoceptive Accuracy (IAcc), and/or c) reduced strength of the Rubber Hand Illusion (RHI).

2. Study I

2.1 Materials and Methods

2.1.1 Participants

The analysis was performed on data from an experiment by Luque-Casado et al., (Luque-Casado et al., 2016). Fifty male young adults, without clinical history of cardiovascular or neuropsychological disorders took part in the study. They were recruited from a larger sample of undergraduate students from the University of Granada and athletes from local triathlon clubs. Participants were then divided into two groups (i.e., 25 subjects per group) based on the number of hours of weekly physical training. The active group consisted of participants who reported at least 8h per week of road cycling. The inactive group consisted of participants reporting less than 2h per week of endurance exercise. Five participants were excluded from the analyses for technical issues (see the data reduction section in the original article). Descriptive data from the remaining 45 participants are reported in Table 1. Importantly, participants' cardiorespiratory fitness level was assessed by individuals' performance in an incremental cycle ergometer submaximal effort test based on ventilatory anaerobic threshold (VAT) determination following the protocol established by Luque-Casado et al. (2016) [see Suppl. Material (Study I) for more details of this protocol]. Note that participation in the study was limited to males as the groups themselves consisted exclusively of male members. The study was conducted in accordance with ethical requirements (University of Granada; Code: 201402400001836) and the Helsinki Declaration.

2.1.2 Procedure

In the present study, we only used Luque-Casado et al.’s (2016) data from the baseline electrophysiological recording that consisted of two 5-min blocks of synchronized EEG and ECG recording. Block 1 and Block 2 represented the open and closed eyes conditions, respectively—a standard procedure in baseline recording. Participants began the recording with their eyes open looking at a black monitor and were warned to close their eyes after 5 min with a message on the screen. Then the recording continued with the participants' eyes closed for another 5 min.

2.1.3 Electrophysiological recording and preprocessing

Continuous EEG was recorded at 1024 Hz using a 64-channel BioSemi Active Two amplifier system (Biosemi, Amsterdam, Netherlands). ECG signals were simultaneously recorded using two active electrodes (Ag/AgCl; Biosemi, Amsterdam, Netherlands) arranged at a modified lead I configuration (i.e., right and left wrists). The EEG data were down sampled to 256 Hz and offline bandpass filtered from 0.3 to 30 Hz, following established methodologies in recent studies (Petzschner et al., 2019). The R-peaks of the QRS-ECG complex were automatically detected using the HEPlab Matlab toolbox (Perakakis, 2021), followed by visual inspection for manual artifact correction. EEG preprocessing was performed using the EEGLAB Matlab toolbox (Delorme & Makeig, 2004).

To identify and remove artifacts such as eye blinks and muscle movements, and the cardiac field artifact (CFA) in particular, the IClevel toolbox (Pion-Tonachini et al., 2019; Pion-Tonachini et al., 2017) was used. The identification and classification process in the IClevel requires the researchers to set a minimum accuracy threshold (0 to 100) for each independent component or artifact. A default threshold of >90% was set for eye blinks and muscle movement artifacts, while a low threshold of >10%

was applied for CFA. HEP statistics were analyzed using both the EEGLAB Study structure and Fieldtrip Matlab toolboxes, with CFA processing performed before epoching and entirely on raw data.

2.1.4. CFA detection and removal procedures

Recognizing discrepancies in the literature regarding cardiac artifact cleaning procedures, we decided to tailor our analyses to the characteristics of our data and experimental design. In this regard, we addressed potential biases stemming from significant ECG differences in the active group by implementing a strict CFA removal. Another justification for employing CFA correction aligns with our bottom-up hypothesis. We focused on resting-state recordings, influenced primarily by raw signal characteristics, diverging from active paradigms such as the HBC task (Petzschner et al., 2019; Yoris et al., 2018), where HEP is supposedly influenced by a top-down attentional mechanism. In summary, we reckon that the most rigorous approach was to eliminate biases from the experimental design (i.e., comparing two groups with differences in ECG) and the characteristics of our data (i.e., resting states).

In the IClevel toolbox (Pion-Tonachini et al., 2019; Pion-Tonachini et al., 2017), we set a robust (>10%) threshold for 'heart' detection, contrasting it with >90% threshold for 'eyes' and 'muscles'. This adjustment followed the default parameters yielding zero cardiac components, prompted by the recognition of a tendency among researchers to overlook 'heart' ICs (Pion-Tonachini et al., 2019). To address the risk of false positives, we combined the automatic labeling of IClevel with visual inspection. Using a customized Matlab script (accessible online with all raw data — see our Open Science commitment at the end of the article), we generated a list of IC labels for confirmation or rejection through the IC plotting option in EEGLAB. This decision was grounded in methodological rigor, integrating automatic labeling with cross-verification through visual inspection. Heart ICs were carefully assessed based on our prior HEP analysis experience and the latest labeling practices from the IClevel website.

2.1.5 HEP statistical analysis

The EEG signal was segmented into epochs ranging from -200 ms to 800 ms, time-locked to each individual R-peak. To correct for baseline fluctuations, epochs were baseline-corrected from -200 ms to 0 ms, based on the established literature summarized by (Coll et al., 2021). In this analysis, only cardiac events excluded due to visual inspection for noise were omitted. Statistical analysis of the HEP data was conducted using both the EEGLAB Study (Delorme & Makeig, 2004) and Fieldtrip (Oostenveld et al., 2011) Matlab toolboxes.

To investigate the HEP, separate analyses were performed on the resting state periods, namely eyes closed and eyes open conditions. Due to the absence of consensus on the specific region and time window of interest for studying the HEP (Coll et al., 2021; Park & Blanke, 2019), we adopted a data-driven approach based on cluster-based non-parametric permutation tests (Maris & Oostenveld, 2007). These tests circumvent the need for a priori definition of spatial or temporal regions of interest and account for multiple comparisons in both space and time. Additionally, considering the anticipated ECG group differences (Garber et al., 2011; Hellsten & Nyberg, 2015), we compared ECG amplitude between active and inactive participants using a non-parametric Monte-Carlo test ($p < 0.05$; FDR correction).

Given the marked discrepancies in the methodology for HEP analysis in recent literature, as highlighted by Coll et al. (2021) and the group's internal reviews, we opted for this non-parametric and cluster-based approach to avoid any speculation of confirmation bias.

2.2 Results

Demographic and anthropometric group comparisons (Table 1) found no significant differences in age, weight, height, and Body Mass Index ($p > 0.05$). Importantly, significant differences were found between the groups in terms of cardiorespiratory fitness (Figure 1), with the active group outperforming the inactive group in VO2 consumption at VAT ($p < 0.001$).

Table 1: Mean and standard deviation (SD) of each group's demographic, anthropometric and fitness variables.

	Active	**Inactive**	
Demographic and anthropometric characteristics			**Statistics**
Sample (n)	24 Male	21 Male	
Age (years)	22.52 (± 3.74)	23.23 (± 2.46)	$p = 0.47$
Weight (kg)	69.92 (± 6.51)	77.70 (± 20.14)	$p = 0.08$
Height (cm	177 (± 5.1)	178 (± 7.0)	$p = 0.54$
BMI (kg/m^2)	22.32 (± 1.78)	24.28 (± 4.89)	$p = 0.07$
Cardiorespiratory fitness			
VO_2 at VAT (mL•min-1•kg-1)	43.30 (± 8.50)	18.81 (± 5.11)	$p < .001$*

* Indicates statistically significant differences; VAT, ventilatory anaerobic threshold.

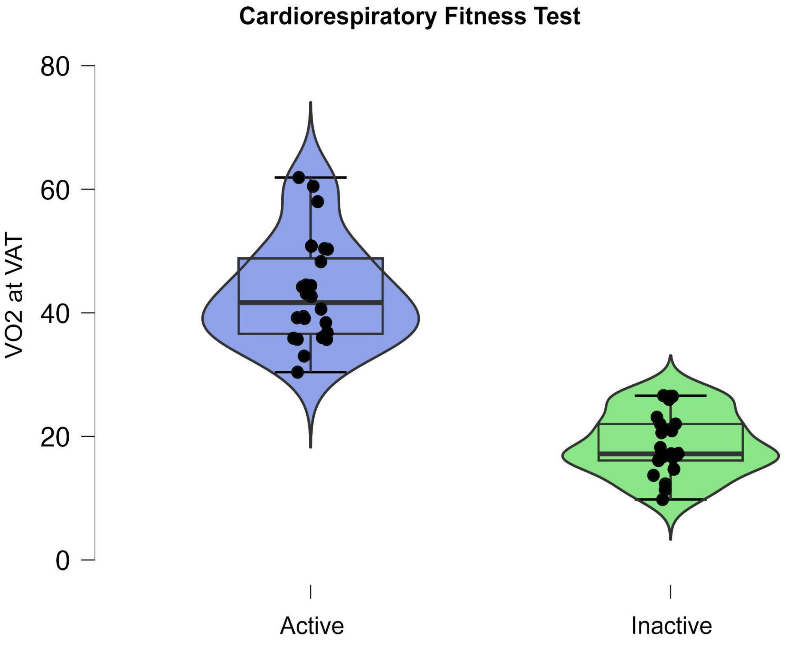

Figure 1: Group performance in the cardiorespiratory fitness test. An independent t-test revealed significant group differences ($p < 0.001$). A complementary Bayesian t-test for independent samples produced a BF_{10} of 3.740×10+11, indicating extreme evidence in favor of the alternative hypothesis (H1).

Participants' performance was measured as VO2 at VAT (mL·min-1·kg-1). Violins depict the distribution of participants within each group. The central horizontal line represents the median, indicating the typical value. The vertical lines represent a 95% confidence interval (CI).

HEP results

Cluster-based permutation tests revealed the presence of 9 positive and 8 negative clusters for the eyes-closed condition. However, in none of these clusters did the analysis reveal statistically significant differences between groups (all *ps* > 0.05). A similar pattern was observed for the open eyes condition, with 8 positive and 2 negative clusters, with no statistically significant differences between groups (Figure 2 A, top and middle).

ECG waveform analysis revealed significant differences between the groups in two-time segments, namely 251-389 ms and 626-800 ms after the R-peak, as determined by the Monte-Carlo permutations test ($p < 0.05$; FDR correction) (Figure 2, A, bottom).

After obtaining inconclusive results in the frequentist analysis, we conducted a Bayesian analysis to assess the strength of evidence for both the null and alternative hypotheses. We first identified the cluster with the lowest *p*-value across all conditions. Then, within this cluster, we computed the average signal from the specified electrodes and the time window for each participant and condition. Finally, these average scores were subjected to a Bayesian t-test for independent samples using JASP (*JASP Team*, 2023).

The selected cluster corresponded to the closed eyes condition (cluster 1; electrodes: F1, FC3, FC1, C1, C3, CP3, CP1, CPz, F2, FC4, FC2, FCz, C2, CP2; time window: 750 - 800 ms; $p = 0.051$). The Bayesian t-test (with a Cauchy prior of .707, two-tailed, and zero-centered) for independent samples resulted in a BF_{10} of 15.708, indicating strong evidence in favor of the alternative hypothesis (H1). Similarly, for the open eyes condition, the analysis yielded a BF_{10} of 2.921, suggesting anecdotal evidence in favor of H1.

For the ECG results, we calculated the average amplitude for each of the two reported time windows and subjected them to Bayes analysis. The results for independent samples in the time window 251-389 ms yielded a BF_{10} of 2.161×10^6, indicating strong evidence in favor of H1. Likewise, a BF_{10} of 9245.291 was obtained for the 626 - 800 ms time window, also supporting H1 strongly.

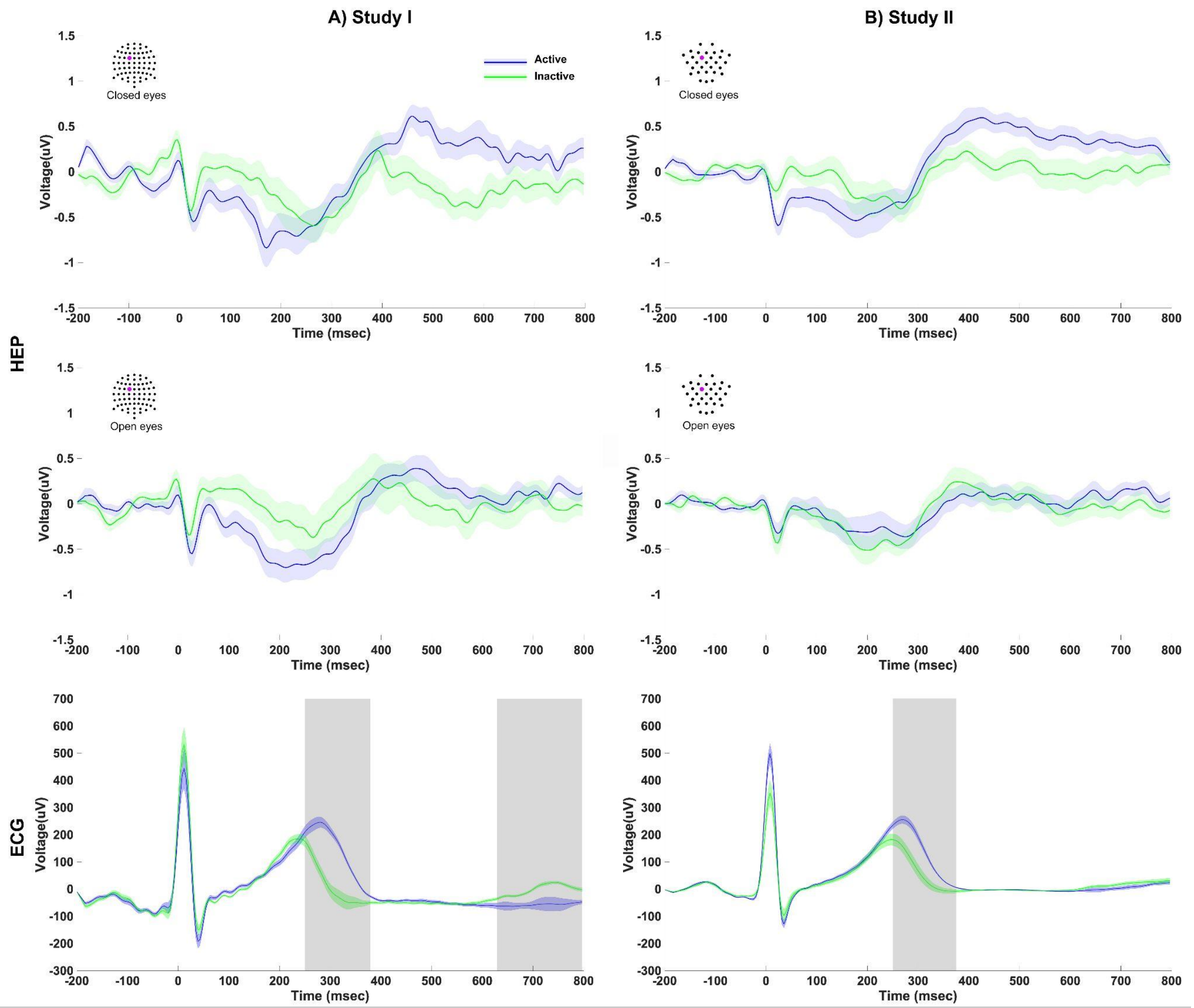

Figure 2: HEP and ECG results in Study I and Study II. In the top panel (A) and (B), for illustrative purposes, we compare the HEP amplitude at electrode FC1 in the closed eyes condition for the active (blue) and inactive (green) groups. Similarly, in the middle

panel (A) and (B), we present the HEP amplitude at electrode FC1 in the open eyes condition. The bottom panel (A) and (B), displays group ECG comparisons. Results of the remaining electrodes both in the closed and open eyes conditions are presented in sections I and II of the Supplementary Material. While no statistically significant differences were found in the HEP, significant group differences were observed in the ECG analysis. Group differences are indicated by the gray-shaded areas indicating the time segments of interest.

3. Study II

3.1 Materials and methods

3.1.1 Participants

Sixty young adults, without clinical history of cardiovascular or neuropsychological disorders participated in the study (Table 2). Participants were recruited from a larger sample of undergraduate students from the University of Granada. Participants were then divided into two groups based on the self-reported number of hours of weekly physical training. Thirty participants were assigned to the active group, which reported exercising at least 8h per week. Another thirty undergraduate students reporting less than 2h per week of physical exercise were assigned to the inactive group. Volunteer participants received 10 euros as monetary compensation. The study was conducted in accordance with ethical requirements (University of Granada; 716/CEIH/2018) and the Helsinki Declaration.

3.1.2 Procedure

Interoception was assessed based on two 5-min synchronized EEG and ECG blocks (open/closed eyes) for HEP analyses. Once completed, the EEG cap was removed, leaving only the ECG electrodes attached to the participant. Consequently, a 10-min HBC task was performed to obtain a measure of IAcc. Proprioceptive drift (see below) was assessed by a 30-min RHI test, following the procedure described by Tsakiris et al. (2011).

3.1.3 Electrophysiological recording, preprocessing and HEP statistical analysis

EEG data were recorded using a 32-channel BrainVision amplifier system (Brain Products, Gilching, Germany) at a sampling rate of 1000 Hz. ECG signals were recorded using two active electrodes (Ag/AgCl) placed on the right and left wrists, configured in a modified lead I setup. The EEG data were resampled at 256 Hz and offline bandpass filtered from 0.3 to 30 Hz. EEG and ECG recording and preprocessing were performed mirroring Study I.

3.1.4 The Heartbeat Counting (HBC) task.

The experimental design is an adaptation of the original experiment by Schandry (Schandry, 1981). Participants were instructed to silently count their heartbeats following their own HR in six conditions of different durations delimited by audiovisual cues (20s, 42s, 53s, 68s, 72s and 86s) randomly ordered across participants, during which ECG was simultaneously measured. IAcc score was calculated by comparing the number of reported heartbeats to the actual number of heartbeats, following the formula:

$$IAcc = 1/6 * \Sigma (1 - (|\text{recorded heartbeats} - \text{counted heartbeats}|) / \text{recorded heartbeats}).$$

IAcc scores ranged from 0 (lowest accuracy) to 1 (highest accuracy), reflecting participants' ability to accurately perceive and estimate their heartbeats in different time intervals.

3.1.5 The Rubber Hand Illusion (RHI) paradigm

The participants sat in front of a desk and placed their left hand inside a bespoke constructed box measuring 36.5 cm in width, 19 cm in height and 29 cm in depth (as in Tsakiris et al., 2011). The distance between their index finger and the index finger of the rubber hand was fixed at 15 cm. The participants were able to see a life-sized prosthetic left hand through the hole on top of the box while their own hand was introduced through a front side hole, concealing their hand from view while allowing the rubber hand to remain visible. The experimenter used two identical paintbrushes to stroke both hands through the backside. A cover (59.5 cm by 29 cm) connected by hinges to the box was used to hide the top of the box. When the induction phase started, the cover was opened to allow the participant to see the rubber hand. At the same time, the opened cover hid the experimenter from the view of the participant.

During the RHI, participants were asked to introduce their left hand in the hole of the box, while the cover remained on top of the box hiding participants' sight of their own left and rubber hand. Then, they were asked to indicate where they felt their left index finger, pointing the position with the index finger of their right hand, while having their eyes closed, by projecting a parasagittal line from their fingertip to the ruler laying on the top of the box. The starting point of their right hand's finger varied randomly along the ruler. Then, they were asked to open their eyes, and with the cover raised, two blocks were completed in a counterbalanced order and lasting 60s each: a synchronous block and an asynchronous block.

The experimenter brushed the index fingers of the rubber hand and the participant's hand, with a frequency of 1 brush per second. The synchronous block consisted of the stimulation of the index fingers of the participant's left hand and the rubber hand, at the same time. During the asynchronous block, they were brushed with the same frequency, also in both index fingers. Critically, while in the synchronous condition, the hands were brushed at the same time, in the asynchronous condition they were brushed 180º out of phase. After these two blocks, the cover was back to its original position, hiding the rubber hand, and the participants were asked again to indicate the position of their left hand's index finger, with their eyes closed, as before the two blocks.

The proprioceptive drift was calculated by comparing the proprioceptive judgments made before and after the induction. Positive values represented a displacement towards the rubber hand, indicating a misperception of localization. Group differences were determined by subtracting effects in the synchronous and asynchronous conditions.

3.1.6 Statistical analyses

HEP and ECG statistical analyses were performed mirroring Study I. Group comparison analyses for demographic variables and IAcc were conducted using independent t-tests. Additionally, to explore the RHI's proprioceptive drift, a paired t-test contrasting participants' performance in the synchronous and asynchronous conditions for each group was performed. Finally, for exploratory purposes, we investigated the relationship between IAcc and RHI with Pearson correlations (see section II. 6. of the Supplementary Material). The analyses were conducted using JASP (*JASP Team*, 2023).

3.2 Results

Demographic and anthropometric group comparisons (see Table 2) found no significant differences in age, weight, height, and Body Mass Index ($p > 0.05$).

Table 2: Mean and standard deviation of demographic variables, IAcc and RHI in each group.

	Active	**Inactive**
Sample (n)	15 (male) 15 (female)	9 (male) 21 (female)
Age (years)	23.8 (± 3.47)	24.47 (± 4.79)
Weight (kg)	64.21 (± 10.68)	62.08 (± 11.16)
Height (cm)	169.2 (± 9.19)	168.92 (± 8.73)
BMI (kg/m^2)	22.31 (± 2.43)	21.64 (± 2.55)
Profile of exercise practice (hours)	8.37 (± 2.36)	0.38 (± 0.67)
IAcc	0.646 (± 0.14)	0.586 (± 0.17)
RHI (sync)	-2.107 (± 4.13)	-3.231 (± 4.65)

RHI (async)	-1.350 (± 3.47)	-1.583 (± 3.73)
RHI (sync - async) *	0.757 (± 2.95)	1.648 (± 3.78)

* Corresponds to proprioceptive drift index

HEP results:

The cluster-based permutation tests did not identify statistically significant between-group differences in any of the positive or negative clusters in either the open-eyes or closed-eyes condition (all *ps* > 0.05) (Figure 2, B, top and middle). Importantly, the analysis of the ECG waveform showed group differences (*p* < 0.05; FDR correction) in the 245-358 ms segment after the R-peak (Figure 1, B, bottom).

For the HEP Bayes factor analysis, it is worth noting that Study I and Study II utilized different scalp configurations, with 64 and 32 channels, respectively. To ensure comparability, we selected electrodes from Study II that best matched Study I's cluster 1 configuration and calculated the average signal for each participant within the same time window. The electrodes considered comparable included F3, FC1, C3, C4, CP1, and CP2.

In the subsequent Bayesian t-tests (utilizing a Cauchy prior of .707, two-tailed, and zero-centered) for independent samples, the results showed a BF_{10} of 0.765, indicating anecdotal evidence in favor of the null hypothesis (H0) in the closed eyes condition. Similarly, for the open eyes condition, the analysis yielded a BF_{10} of 0.357, also suggesting anecdotal evidence in favor of H0.

Regarding the ECG signal, we calculated the averaged amplitude within the reported time window and subjected it to Bayesian analysis. The results in the time window of 245-358 ms produced a BF_{10} of 2.059, indicating anecdotal evidence in favor of the alternative hypothesis (H1).

IAcc results

Independent t-tests showed no-significant difference (*t* (56) = 1.442, *p* = .155) in IAcc between active and inactive individuals (Figure 3, A). The Bayesian t-test for independent samples produced a BF_{10} of 0.631, indicating anecdotal evidence in favor of the null hypothesis (H0).

RHI results

The independent *t*-test showed no significant differences (*t* (57) = -1.011, *p* = 0.316) in the magnitude of the RHI between groups (Figure 3, B). Additionally, as an exploratory analysis, we assessed the magnitude of the RHI effect per group (Figure 3, C). Initially, a paired samples *t*-test comparing the drift in the synchronous and asynchronous conditions indicated that the RHI effect was not statistically significant in the active group (*t* (29) = -1.40, *p* = 0.17), while the inactive group demonstrated a significant effect (*t* (28) = -2.34, p = 0.02). To ensure the robustness of our findings, we conducted an outlier analysis, identifying a participant in the inactive group with an extremely large effect. After excluding this participant, the significant RHI effect in the inactive group persisted (t (27) = -2.46, p = 0.02) (see Figure 3, B and C). The Bayesian t-test for independent samples produced a BF_{10} of 0.318, indicating anecdotal evidence in favor of the null hypothesis (H0).

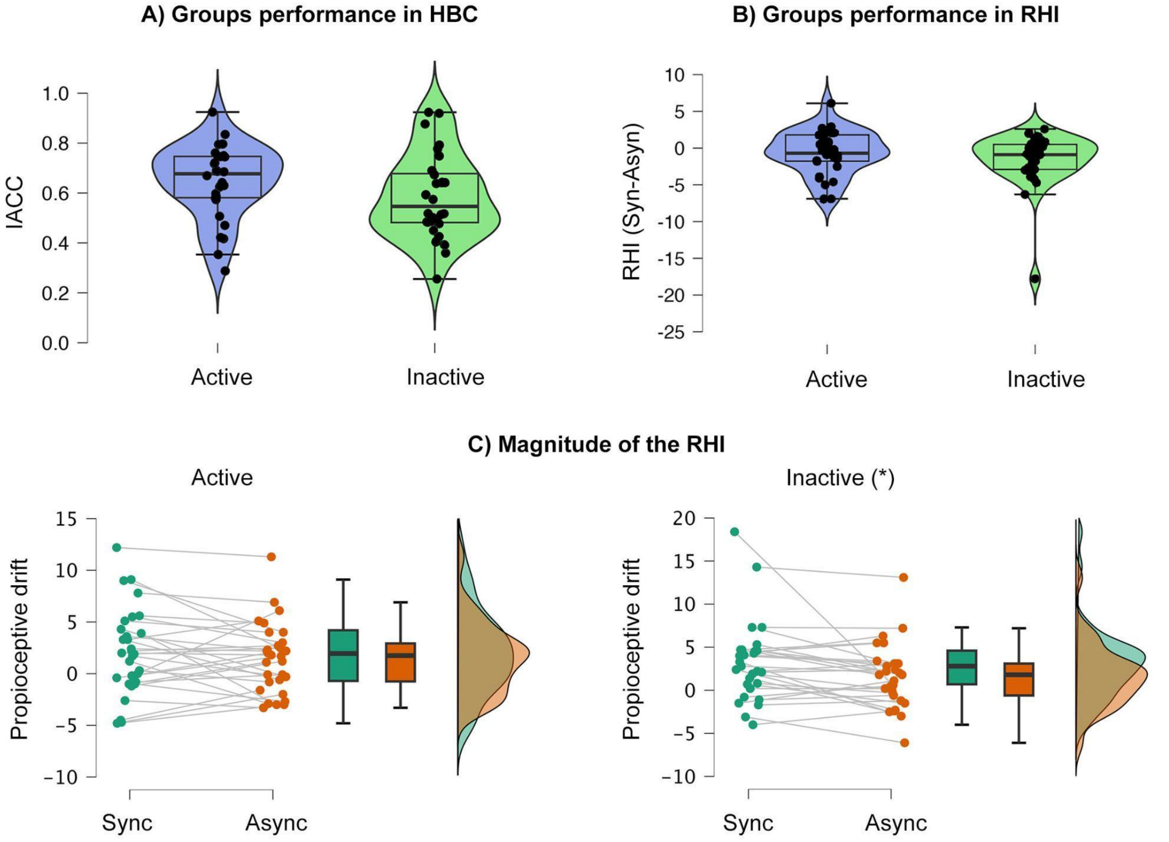

Figure 3. Results in HBC and RHI. A) Participants' Performance in HBC as a measure of IAcc. B) Participants' Performance in RHI was obtained by subtracting Syn-Asyn conditions. C) RHI Magnitude Across Groups is presented by comparing intragroup conditions. Violin plots depict the distribution of participants within each group and the central horizontal line represents the median, indicating the typical value. In A, B and C, the vertical line corresponds to a 95% confidence interval (CI). Proprioceptive drift is expressed in centimeters.

Discussion

This study aimed to investigate the possible relationship between regular physical exercise and cardiac interoception. Specifically, it compared physically active and inactive individuals across multiple measures, including the HEP (Study I and II), IAcc, and propensity to the RHI (Study II). The results showed clear and robust group differences in cardiorespiratory fitness testing (Study I) and ECG waveforms (Study I and II) as a function of the regular practice of physical exercise. However, contrary to our initial expectations, our findings do not support a strong and clear positive association between regular exercise and interoception, either behaviorally or neurally.

Comparison of HEP amplitude between active and inactive groups in both Study I and Study II using a data-driven cluster-based approach, revealed no statistically significant between-group differences. Following a reviewer's advice, who acknowledged the risk of type II errors inherent to the cluster-based methodology, we conducted complementary Bayesian analyses to further assess the strength of evidence for our hypotheses. In Study I, the Bayesian t-test on the cluster with the lowest p-value corresponding to the between-group HEP differences (cluster 1, p = 0.051) during the 'eyes closed' condition showed 'strong' evidence supporting H1, with 'anecdotal' evidence in the 'open eyes' condition. The Bayesian t-test on the HEP data in Study 2 did not show evidence of between-group differences.

HBC and RHI results, commonly used as proxies for IAcc (Schulz et al., 2021) and proprioceptive drift (Tsakiris & Haggard, 2005) respectively, revealed again no clear differences between the active and inactive groups. Note that despite a statistically significant RHI effect solely in the inactive group of Study II, suggesting an enhanced body representation in the active group, the lack of differences in IAcc and HEP and the absence of a significant correlation with RHI magnitude prevent firm conclusions on these exploratory findings.

In contrast, clear differences in cardiac activity emerged in both studies, as evidenced by a cardiorespiratory fitness test and analysis of the ECG waveform. Specifically, the results of the fitness test, assessed via an independent t-test on participants' performance (VO2 at VAT), demonstrated that the active outperformed the inactive group. Similarly, employing a similar approach to the HEP analysis, we compared the ECGs of active and inactive groups using the data-driven Montecarlo Permutations approach. Two temporal windows revealed differences when comparing the groups: one early window coinciding with the T-wave of the ECG (Study I) and another late window related to the peaks P and Q of the consecutive or next cardiac beat (Study I and II). This finding could be linked to the previously reported cardiovascular alterations in elite cyclists (Andersen et al., 2011; Brown et al., 2020; Le Douairon

Lahaye et al., 2022) and, in our view, might impact HEP modulation if attempts are not made to eliminate CFA or if early baseline correction is not performed.

Taken together, our results do not seem to support the existence of a robust relationship between the regular practice of physical exercise and cardiac interoception. However, several considerations should be taken into account in order to interpret our findings. First, the relatively small sample sizes in Studies 1 and 2 represent a potential limitation, as they may have hindered statistical power. Testing larger samples might indeed help capturing small true effects in the proxies of cardiac interoception used here. Second, the likelihood of Type II errors could also have been increased by the use of the data-driven EEG cluster-based analysis. Note, though, that we emphasize the need for this data-driven analysis given the lack of consensus on temporal windows and regions of interest in the HEP literature (see Coll et al., 2021). Another crucial methodological aspect that varies among researchers regards CFA correction. For instance, in a recent discussion by Petzschner et al. (2019), researchers refrain from correction for CFA to avoid speculative assumptions. However, the ECG between-group differences reported in Studies I and II justify adopting a more stringent analysis approach. In fact, the sole evidence in favor of the alternative hypothesis in the exploratory Bayesian t-test in Study 1 was found in a temporal window (750-800 ms), where clear between-group differences in ECG were shown, rendering any neural interpretation of this HEP finding problematic.

Third, it could be argued that the measures utilized in our study might have not fully captured between-group differences in cardiac interoception. However, we mainly followed previous accounts that did argue in favor of the sensitivity and reliability of these indexes to measure interoception. On one hand, the HEP has long been considered a neural marker of cardiac interoception (Park & Blanke, 2019). On the other hand, the selection of the HBC as a behavioral measure of cardiac interoception relied on two main reasons: temporality and prevalence in previous studies. Firstly, the data collection for Study II co-occurred with the publication of criticisms regarding HBC validity, namely test-retest reliability and the influence of prior knowledge about one's heart rate (Desmedt et al., 2018; Murphy et al., 2018; Ring & Brener, 2018). Secondly, the HBC has been widely employed in prior studies on interoception in athletes (Georgiou et al., 2015; Herbert et al., 2007; Koteles, Elias, et al., 2020;

Koteles, Teufel, et al., 2020), making our results potentially comparable with previous evidence. However, aware of those methodological considerations, we acknowledge the limitations of this instrument and the scope of our results. In different circumstances, our choice would have been a selection of more sophisticated analyses reported in the recent literature, such as the measurement of changes in the rate of heartbeats through regression analysis (Larsson et al., 2021). Note that, in line with this, we included the RHI, an index of sensory integration and body representation, to address aspects possibly not fully discernible with conventional interoceptive assessments. Moreover, the inclusion of the RHI was supported by previous reports (Filippetti & Tsakiris, 2017; Tsakiris et al., 2011), which showed that better IAcc was associated with a reduced strength of the body ownership illusion.

Fourth, our target population primarily consisted of endurance cyclists and triathletes in Study I, while the exercise type in Study II was not specifically assessed. This limits the generalizability of our results to broader populations engaging in different forms of physical activity. The unique characteristics of endurance athletes may contribute to distinct interoceptive adaptations, and therefore, caution is warranted when extrapolating these findings to diverse exercise modalities. In other words, specific types of exercise likely have a different impact on interoceptive processing. Our focus was on the potential modulating effect of regular exercise through alterations in afferent cardiovascular signals, aligning with the hypothesis proposed by Wallman-Jones et al. (2021). However, physical activity may induce interoceptive changes through alternative mechanisms unrelated to cardiovascular fitness, warranting further exploration of specific exercise types' effects on interoceptive processing.

Finally, our focus on resting-state recordings for interoceptive assessments may introduce specific biases associated with the absence of directed attention tasks. While aligning with our bottom-up hypothesis, which emphasizes the role of raw signal characteristics, this approach may not fully capture the nuances of interoception during active paradigms. Therefore, the generalizability of our conclusions to scenarios involving focused attention tasks, as in the heartbeat counting task, remains a subject for further investigation.

In summary, while our study contributes with new insights, the issues raised above underscore the complexity of interpreting our results within the broader context of interoceptive research and draw attention to the need for further exploration into the intricacies of interoceptive processing across varied populations and experimental paradigms. In any case, our findings do not seem to support a strong version of the notion of regular physical exertion as a reliable bottom-up factor influencing interoception. While not negating the possibility of interaction, they indicate a relationship that may be less robust than previously hypothesized.

Authors' contribution

DS, PP, AT-J and LC were responsible for conceptualizing the research. AEY was responsible for curating the data and conducting formal analysis. LC, ALC, and CS participated in data acquisition. DS, PP, and AEY took the lead in writing the original draft, with the remaining co-authors contributing to the revision and editing process. Overall, PP and DS provided supervision and guidance throughout the entire project.

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Data from both Study I and II included in this article are available at Open Science Framework (OSF) (*https://osf.io/xrsgn/*) and the Zenodo repository (DOI 10.5281/zenodo.8130405).

Supplementary Material

Integrated Supplementary Material of Studies I and II

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, no LLMs or other AI technology was used for the preparation of this manuscript.

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