

# No evidence of a relationship between regular physical exercise and cardiac interoception

Adrian Yoris<sup>1</sup>, Luis Ciria<sup>2</sup>, Antonio Luque-Casado<sup>3</sup>, Caterina Salvotti<sup>4</sup>, Ana Tajadura-Jiménez<sup>5</sup>, Chiara Avancini<sup>6</sup>, Daniel Sanabria<sup>6</sup>, and Pandelis Perakakis<sup>7</sup>

<sup>1</sup>Instituto de Neurociencia Cognitiva y Traslacional (INCYT)

<sup>2</sup>University of Granada

<sup>3</sup>Universidad Rey Juan Carlos

<sup>4</sup>Affiliation not available

<sup>5</sup>Universidad Carlos III de Madrid

<sup>6</sup>Universidad de Granada

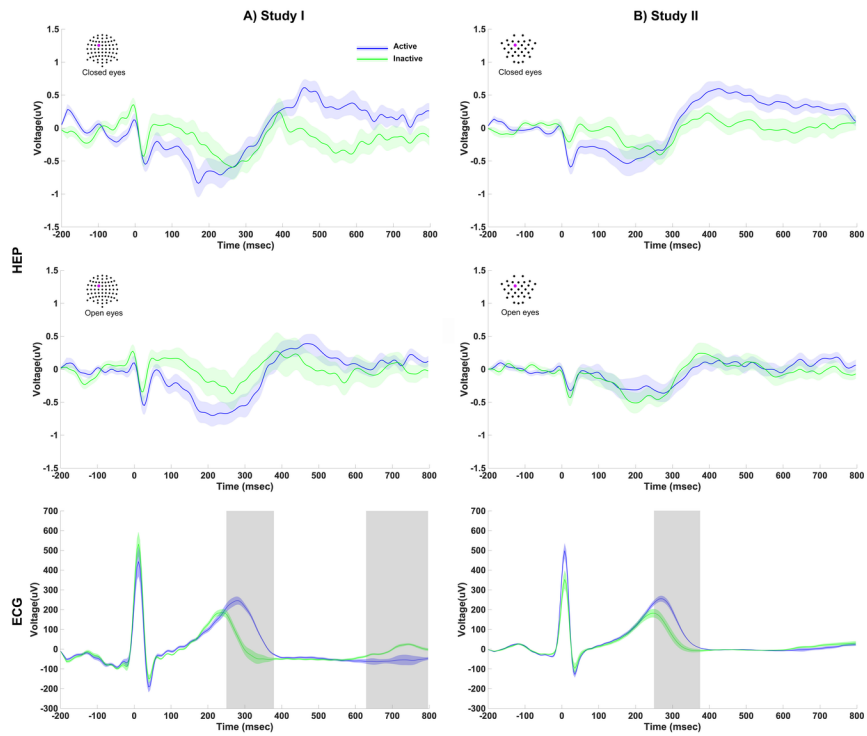
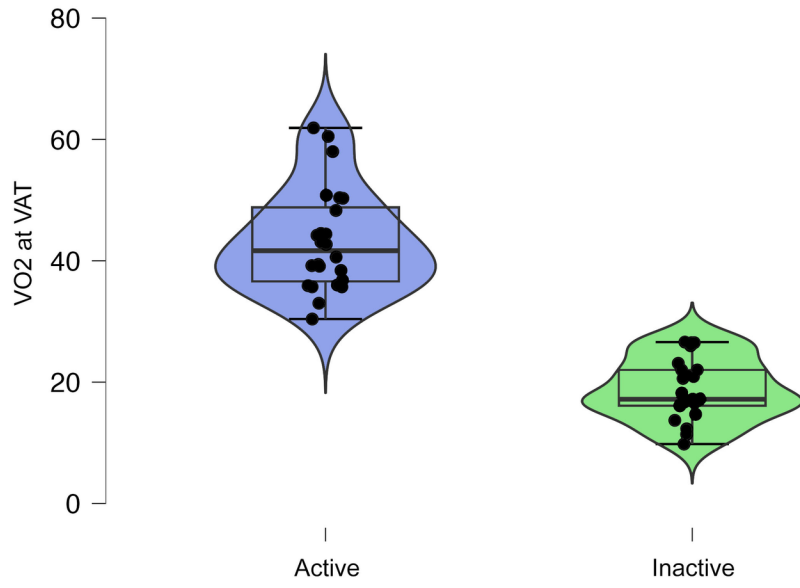
<sup>7</sup>Complutense University of Madrid

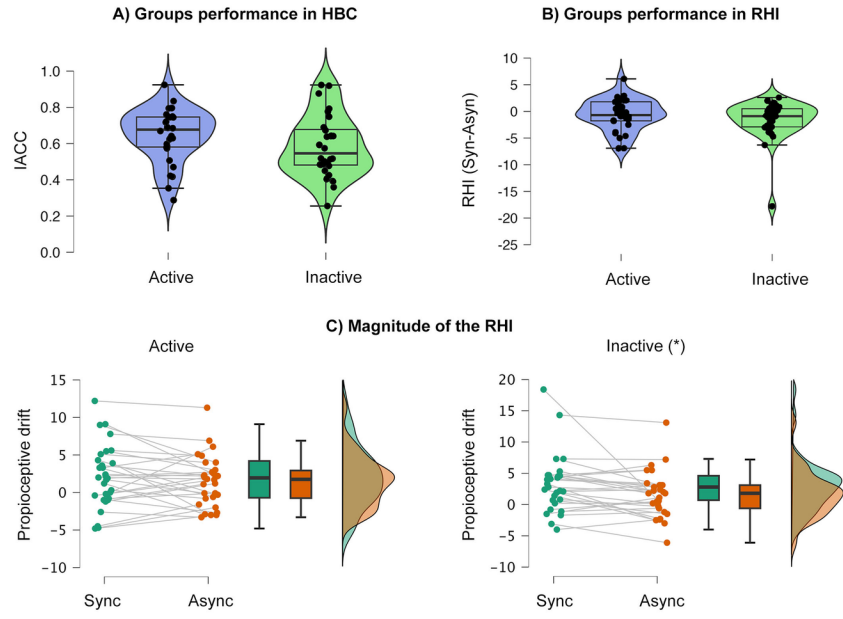
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## Abstract

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 and neural activity associated with the processing of afferent cardiac signals. In this study, we aimed to investigate this hypothesis through two cross-sectional studies, categorizing participants as high or low fit based on physical fitness (Study I) or self-reported physical activity levels (Study II). Interoception was assessed using the Heart-Evoked Potential (Studies I and II), the Heartbeat Counting task (Study II), and the Rubber Hand Illusion (Study II). Despite consistent between-group differences in electrocardiogram recordings in both studies, there were not statistically significant between-group differences in any of the measures of interoception. Consequently, our results do not provide evidence to support the notion that regular physical exercise leads to an increase in cardiac interoception.

### Cardiorespiratory Fitness Test





*No evidence of a relationship between regular physical exercise and cardiac interoception.*

*Adrián E. Yoris<sup>1,2\*</sup>, Luis F. Cira<sup>1,2</sup>, Antonio Luque-Casado<sup>3</sup>, Caterina Salvotti<sup>4</sup>, Ana Tajadura-Jiménez<sup>5,6</sup>, Chiara Avancini<sup>1,2</sup>, Daniel Sanabria<sup>1,2</sup>, Pandelis Perakakis<sup>7</sup>.*

<sup>1</sup> Mind Brain and Behavior Research Center (CIMCYC), University of Granada, Spain.

<sup>2</sup> Departamento de Psicología Experimental, Universidad de Granada.

<sup>3</sup> Sport Sciences Research Centre, Rey Juan Carlos University, Madrid, Spain

<sup>4</sup> Department of Performance Psychology, Institute of Psychology, German Sport University Cologne

<sup>5</sup> Department of Computer Science and Engineering, Universidad Carlos III de Madrid, Spain

<sup>6</sup> UCL Interaction Centre, University College London, United Kingdom

<sup>7</sup> Department of Social, Work, and Differential Psychology, Faculty of Psychology, Complutense University of Madrid, Spain.

***\*Corresponding author***

Dr. Adrián Yoris

E-mail: aeyoris@ugr.es

Departamento de Psicología Experimental, Universidad de Granada.

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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 and neural activity associated with the processing of afferent cardiac signals. In this study, we aimed to investigate this hypothesis through two cross-sectional studies, categorizing participants as high or low fit based on physical fitness (Study I) or self-reported physical activity levels (Study II). Interoception was assessed using the Heart-Evoked Potential (Studies I and

II), the Heartbeat Counting task (Study II), and the Rubber Hand Illusion (Study II). Despite consistent between-group differences in electrocardiogram recordings in both studies, there were not statistically significant between-group differences in any of the measures of interoception. Consequently, our results do not provide 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 period of time, physical exercise induces physiological adaptations at, for example, metabolic, muscular, and cardiovascular levels (Garber et al., 2011; Hellsten & Nyberg, 2015). 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 behavioural 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.

To address that gap in the literature, and to add further to the framework described in Wallman-Jones et al. (2021), here we investigate the potential link between regular physical exercise and cardiac interoception in two cross-sectional studies comparing physically active with physically inactive individuals. In Study I, participants were also characterized on the basis of 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 presented here, 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 own 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).

Following on Wallman-Jones et al. (2021), and on the basis of the extant evidence from the fields of exercise physiology and interoception, in Study I, it is expected that individuals with higher levels of chronic physical exercise would show: a) enhanced cardiovascular fitness, b) group differences in the cardiac signal measured by means of electrocardiography, and c) group differences in HEP amplitude. In Study II, we expected: a) enhanced interoception in the active group, indexed by better IAcc, b) reduced strength of the RHI, and c) group differences in HEP amplitude.

## **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 the original article for more details of this protocol).

Note that only males participated in the study, due to limitations in recruiting very active female participants in the Granada area. 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 corresponded to open and closed eyes conditions, respectively. 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 downsampled 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, 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. This decision was made based on the challenges associated with identifying heart-related components using automatic classifiers, summarized in Pion-Tonachin (2019). Finally, each of the components automatically marked for rejection was verified by visual inspection, drawing on previous experience in HEP analysis (Yoris et al., 2018; Yoris et al., 2017).

#### 2.1.4 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). 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).

## *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 VO<sub>2</sub> consumption and relative power output at VAT ( $p < 0.001$ ).

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

	Active	Inactive
<b>Demographic and anthropometric characteristics</b>		
<b>Sample (n)</b>	24 Male	21 Male
<b>Age (years)</b>	22.52 ( $\pm$ 3.74)	23.23 ( $\pm$ 2.46)
<b>Weight (kg)</b>	69.92 ( $\pm$ 6.51)	77.70 ( $\pm$ 20.14)
<b>Height (cm)</b>	177 ( $\pm$ 5.1)	178 ( $\pm$ 7.0)
<b>BMI (kg/m<sup>2</sup>)</b>	22.32 ( $\pm$ 1.78)	24.28 ( $\pm$ 4.89)
<b>Cardiorespiratory fitness</b>		
<b>VO<sub>2</sub> at VAT (mL·min<sup>-1</sup>·kg<sup>-1</sup>) *</b>	43.30 ( $\pm$ 8.50)	18.81 ( $\pm$ 5.11)

\* Indicates statistically significant differences ( $p < 0.001$ ); VAT, ventilatory anaerobic threshold.

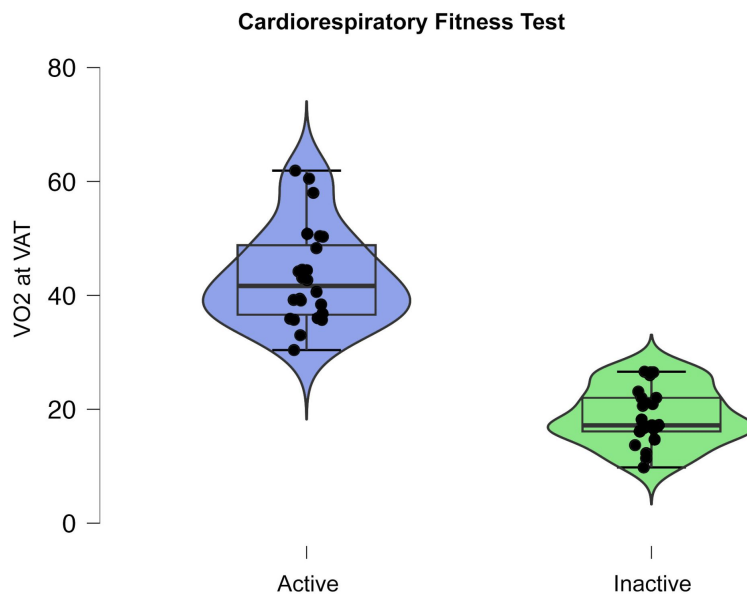


Figure 1: Group performance in the cardiorespiratory fitness test. An independent t-test revealed significant group differences ( $p < 0.001$ ). A complementary Bayesian t-test for independent samples produced a  $BF_{10}$  of  $3.740 \times 10^{11}$ , indicating extreme evidence in favor of the alternative hypothesis ( $H_1$ ).

Participants' performance was measured as  $VO_2$  at VAT ( $\text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ). 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  $p_s > 0.05$ ). 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  $2.161 \times 10^6$ , 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.

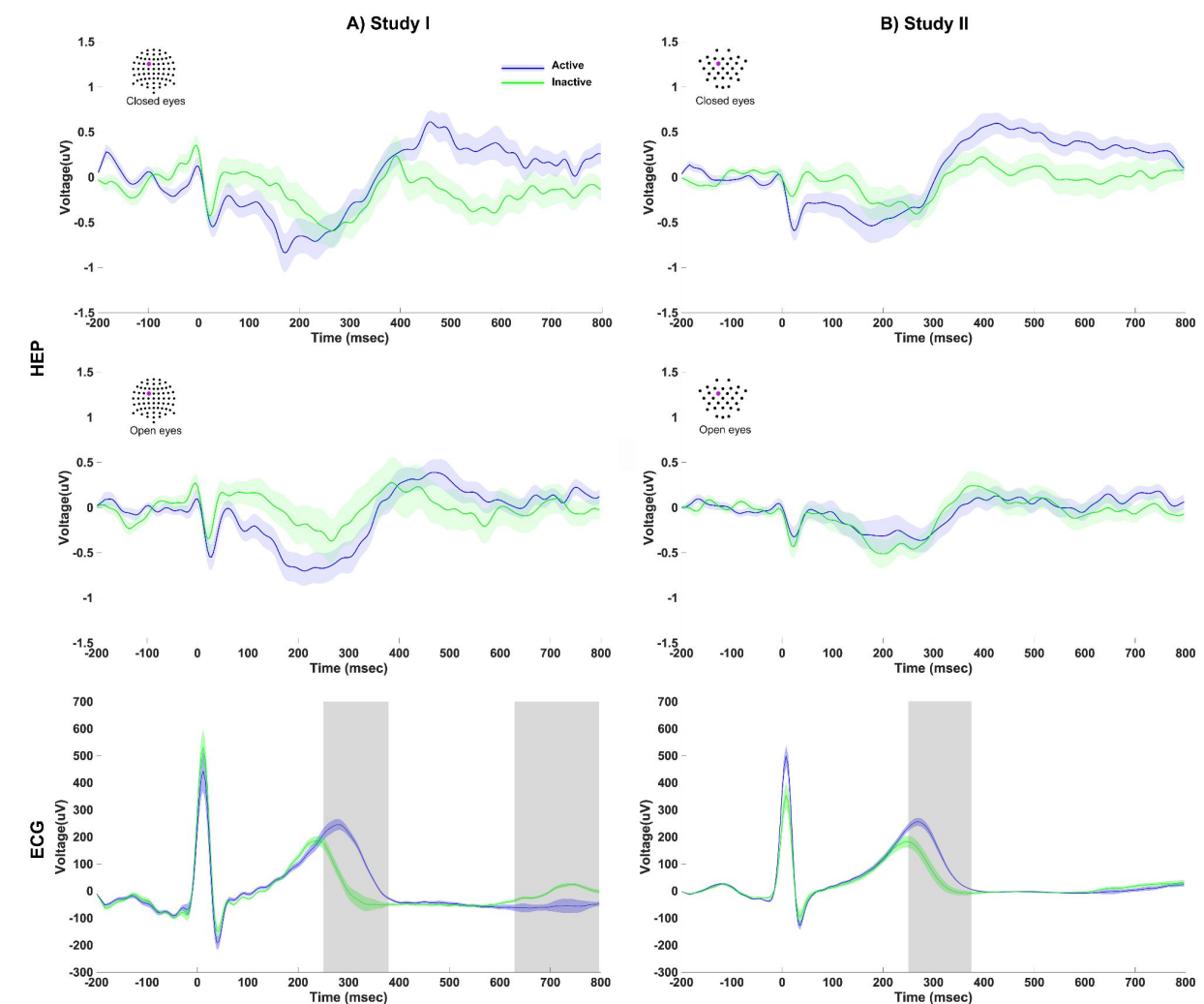


Figure 2: HEP and ECG results in Study I and Study II. In the top panel (A) and (B), for illustrative purposes, we compare the HEP amplitude at electrode FC1 in the closed

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 Supplementary Materials I and II. 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:

$$IAcc = 1/6 * \sum (1 - (|recorded\ heartbeats - counted\ heartbeats|) / recorded\ heartbeats).$$

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 180° 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. 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 in each group.

	<b>Active</b>	<b>Inactive</b>
<b>Sample (n)</b>	15 (male) 15 (female)	9 (male) 21 (female)
<b>Age(years)</b>	23.8 ( $\pm$ 3.47)	24.47 ( $\pm$ 4.79)
<b>Weight (kg)</b>	64.21 ( $\pm$ 10.68)	62.08 ( $\pm$ 11.16)
<b>Height (cm)</b>	169.2 ( $\pm$ 9.19)	168.92 ( $\pm$ 8.73)
<b>BMI (kg/m<sup>2</sup>)</b>	22.31 ( $\pm$ 2.43)	21.64 ( $\pm$ 2.55)
<b>Profile of exercise practice (hours)</b>	8.37 ( $\pm$ 2.36)	0.38 ( $\pm$ 0.67)

Values represent the mean and standard deviation.

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  $p$ s > 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's worth noting that Study I and Study II utilized different scalp configurations, with 64 and 23 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 ( $H_0$ ) 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  $H_0$ .

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 ( $H_1$ ).

### IACC results

Independent t-tests showed no-significant difference ( $t(56) = 1.442$ ,  $p = .155$ ) in IACC between active and inactive individuals (Figure 2, A). The Bayesian t-test for independent samples produced a  $BF_{10}$  of 0.631, indicating anecdotal evidence in favor of the null hypothesis ( $H_0$ ).

### 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 2, B). Additionally, as an

exploratory analysis, we assessed the magnitude of the RHI effect per group (Figure 2, 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 ( $t(28) = -2.34, p = 0.02$ ). This difference in the inactive group persisted ( $t(27) = -2.46, p = 0.02$ ) after removing a participant showing an extremely large effect (see Figure 2, 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 ( $H_0$ ).

### Correlations between IACC and RHI

To explore potential associations between IACC and RHI, we conducted a Pearson correlation. No significant association was found between the variables before ( $r = -0.168, p = 0.20$ ) and after removing that individual with a very large RHI ( $r = -0.184, p = 0.17$ ).

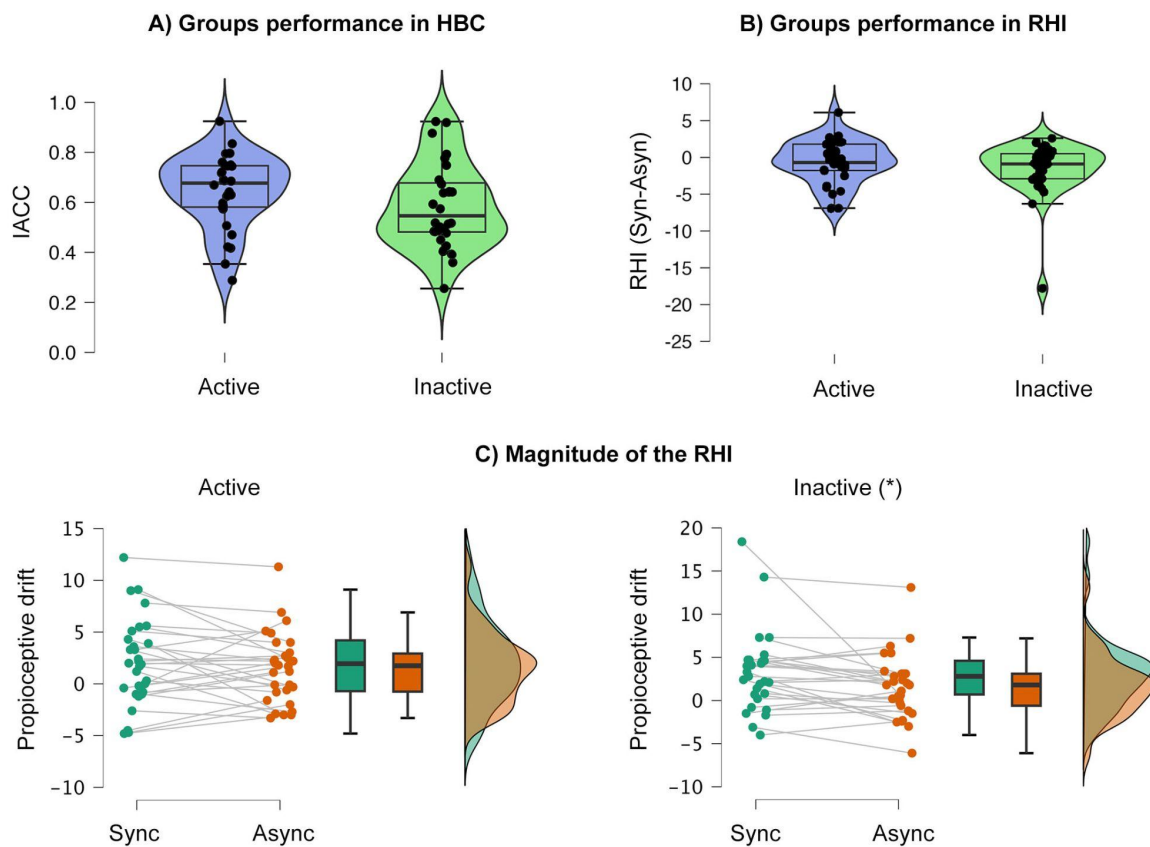


Figure 3. Results in HBC and RHI. A) Participants' Performance in HBC as a measure of IAcc. B) Participants' Performance in RHI was obtained by subtracting Syn-Asyn conditions. C) RHI Magnitude Across Groups is presented by comparing intragroup conditions. Violin plots depict the distribution of participants 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***

The aim of the present study was to investigate the possible relationship between regular physical exercise and cardiac interoception, comparing physically active and inactive individuals with respect to HEP (Study I and II), IAcc, and propensity to the RHI (Study II). Contrary to our initial expectations, our findings do not provide substantial evidence for a significant relationship between regular exercise and interoception, both behaviorally and neurally. However, it is worth noting that the cumulative impact of regular physical exercise on the cardiovascular system was clear in the data from both studies, as indicated by the observed group differences in cardiorespiratory fitness testing (Study I) and ECG waveforms (Study I and II).

To address the lack of consensus on the particular region of interest and latency range where HEP differences have been traditionally reported (Coll et al., 2021), we opted for a data-driven cluster-based analysis making no a-priori decisions on regions or time windows of interest. This methodologically rigorous approach, while revealing no differences between the well-differentiated groups in terms of physical fitness and cardiovascular activity, does not definitively rule out the hypothesis of a positive effect of regular physical exercise on HEP. It is important to note that the cluster-based approach can inflate the probability of type II errors. Narrowing down the number of electrodes and latency range for the analyses might lead to statistically significant differences. However, given the current state of the HEP literature, we believe that such an option is not justified at this point. Thus, although the absence of evidence is not evidence of absence, the fact that our analysis did not reveal any spatiotemporal clustering between well-differentiated groups (in terms of cardiovascular fitness)

suggests that the effect of regular exercise on the neural processing of cardiac afferent signals may not be as robust as previously suggested.

To further investigate the strength of evidence for our proposed hypotheses, we conducted complementary Bayesian analyses. Regarding group differences in HEP within Study I, the cluster identified as having the highest significance in terms of frequentist statistics (cluster 1,  $p = 0.051$ ) during the 'eyes closed' condition demonstrated 'strong' evidence in favor of H1, with 'anecdotal' evidence supporting the differences in the 'open eyes' condition. It is worth noting that this cluster, although consistent with the electrodes reported in the literature in terms of spatial distribution (Coll et al., 2021), falls within a late temporal window (750-800 ms) that, according to some studies (Petzschner et al., 2019), may be influenced by the cardiac activity (CFA) of the subsequent heartbeat. Additionally, these variations in HEP temporally coincide with differences in the averaged EEG waveform, raising again questions about the possibility cardiac activity influencing the HEP. Apart from HEP results in Study I, none of the measures analyzed to examine the impact of regular physical exercise on interoception provided Bayesian factors (BF) supporting our hypothesis.

Regarding the HBC and the RHI results. Although these two tasks have been widely used as proxies for IAcc (Schulz et al., 2021) and proprioceptive drift (Tsakiris & Haggard, 2005) respectively, in our study they revealed no differences between groups. Nevertheless, our exploratory analyses showed a statistically significant RHI effect solely in the inactive group of Study II, which could imply an enhanced body representation in the active group. However, it is crucial to note that the lack of differences in IAcc and HEP and the absence of a significant correlation between IAcc and the magnitude of the RHI prevents us from drawing any definite conclusions regarding these RHI exploratory findings. As mentioned earlier, these results do not dismiss the possibility of potential differences in interoception related to regular exercise. However, they suggest that if such differences exist, their effect size might be small, raising concerns about the sensitivity and validity of these measures in capturing exercise-induced cardiac interoception adaptations.

Finally, it is important to acknowledge that we did not control for the specific type of physical exercise in either of the two studies. Participants in Study I were endurance

cyclists and triathletes, while the type of exercise in Study II was not assessed. It is likely that specific types of exercise have a different impact on interoceptive processing. However, our aim here was to focus on the potential modulating effect of regular exercise through alterations in the afferent cardiovascular signal as a bottom-up mechanism, in line with the hypothesis proposed by Wallman-Jones et al. (2021). Physical activity may still elicit interoceptive changes through alternative mechanisms that are not directly related to cardiovascular fitness. Further studies are therefore warranted to investigate specific types of exercise that may have differential effects on interoceptive processing.

In conclusion, our findings do not provide support for regular practice physical exertion as a bottom-up process that could reliably influence interoception. Although they do not rule out the existence of an interaction, they point out that the relationship is not as strong as 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 Materials**

*Suppl. Material Study I. HEP results per channel.*

*Suppl. Material Study II. HEP results per channel.*

### **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.

### **References**

- Antony, M. M., Brown, T. A., Craske, M. G., Barlow, D. H., Mitchell, W. B., & Meadows, E. A. (1995). Accuracy of heartbeat perception in panic disorder, social phobia, and nonanxious subjects. *Journal of Anxiety Disorders*, 9(5), 355-371. [https://doi.org/10.1016/0887-6185\(95\)00017-1](https://doi.org/10.1016/0887-6185(95)00017-1)
- Botvinick, M., & Cohen, J. (1998). Rubber hands 'feel' touch that eyes see. *Nature*, 391(6669), 756. <https://doi.org/10.1038/35784>
- Canales-Johnson, A., Silva, C., Huepe, D., Rivera-Rei, A., Noreika, V., Garcia Mdel, C., Silva, W., Ciraolo, C., Vaucheret, E., Sedeno, L., Couto, B., Kargieman, L., Baglivo, F., Sigman, M., Chennu, S., Ibanez, A., Rodriguez, E., & Bekinschtein, T. A. (2015). Auditory Feedback Differentially Modulates Behavioral and Neural Markers of Objective and Subjective Performance When Tapping to Your Heartbeat. *Cereb Cortex*, 25(11), 4490-4503. <https://doi.org/10.1093/cercor/bhv076>
- Coll, M. P., Hobson, H., Bird, G., & Murphy, J. (2021). Systematic review and meta-analysis of the relationship between the heartbeat-evoked potential and interoception. *Neurosci Biobehav Rev*, 122, 190-200. <https://doi.org/10.1016/j.neubiorev.2020.12.012>
- Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of neuroscience methods*, 134(1), 9-21. <https://doi.org/10.1016/j.jneumeth.2003.10.009>
- Filippetti, M. L., & Tsakiris, M. (2017). Heartfelt embodiment: Changes in body-ownership and self-identification produce distinct changes in interoceptive accuracy. *Cognition*, 159, 1-10. <https://doi.org/10.1016/j.cognition.2016.11.002>

- Garber, C. E., Blissmer, B., Deschenes, M. R., Franklin, B. A., Lamonte, M. J., Lee, I. M., Nieman, D. C., Swain, D. P., & American College of Sports, M. (2011). American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med Sci Sports Exerc*, 43(7), 1334-1359. <https://doi.org/10.1249/MSS.0b013e318213fefb>
- Garfinkel, S. N., Seth, A. K., Barrett, A. B., Suzuki, K., & Critchley, H. D. (2015). Knowing your own heart: distinguishing interoceptive accuracy from interoceptive awareness. *Biol Psychol*, 104, 65-74. <https://doi.org/10.1016/j.biopsycho.2014.11.004>
- Georgiou, E., Matthias, E., Kobel, S., Kettner, S., Dreyhaupt, J., Steinacker, J. M., & Pollatos, O. (2015). Interaction of physical activity and interoception in children. *Front Psychol*, 6, 502. <https://doi.org/10.3389/fpsyg.2015.00502>
- Hellsten, Y., & Nyberg, M. (2015). Cardiovascular Adaptations to Exercise Training. *Compr Physiol*, 6(1), 1-32. <https://doi.org/10.1002/cphy.c140080>
- JASP Team. In. (2023). JASP Team (Version 0.17. 2).
- Jones, G. E., & Hollandsworth, J. G. (1981). Heart rate discrimination before and after exercise-induced augmented cardiac activity. *Psychophysiology*, 18(3), 252-257. <https://doi.org/10.1111/j.1469-8986.1981.tb03029.x>
- Khalsa, S. S., Adolphs, R., Cameron, O. G., Critchley, H. D., Davenport, P. W., Feinstein, J. S., Feusner, J. D., Garfinkel, S. N., Lane, R. D., Mehling, W. E., Meuret, A. E., Nemeroff, C. B., Oppenheimer, S., Petzschner, F. H., Pollatos, O., Rhudy, J. L., Schramm, L. P., Simmons, W. K., Stein, M. B., . . . Interoception Summit, p. (2018). Interoception and Mental Health: A Roadmap. *Biol Psychiatry Cogn Neurosci Neuroimaging*, 3(6), 501-513. <https://doi.org/10.1016/j.bpsc.2017.12.004>
- Luque-Casado, A., Perakakis, P., Hillman, C. H., Kao, S.-C., Llorens, F., Guerra, P., & Sanabria, D. (2016). Differences in sustained attention capacity as a function of aerobic fitness. *Medicine & Science in Sports & Exercise*, 48(5), 887-895. <https://doi.org/10.1249/MSS.0000000000000857>
- Maris, E., & Oostenveld, R. (2007). Nonparametric statistical testing of EEG-and MEG-data. *Journal of neuroscience methods*, 164(1), 177-190. <https://doi.org/10.1016/j.jneumeth.2007.03.024>
- Montgomery, W. A., Jones, G. E., & Hollandsworth Jr, J. G. (1984). The effects of physical fitness and exercise on cardiac awareness. *Biological psychology*, 18(1), 11-22. [https://doi.org/10.1016/0301-0511\(84\)90022-x](https://doi.org/10.1016/0301-0511(84)90022-x)
- Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J. M. (2011). FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput Intell Neurosci*, 2011, 156869. <https://doi.org/10.1155/2011/156869>

- Park, H. D., & Blanke, O. (2019). Heartbeat-evoked cortical responses: Underlying mechanisms, functional roles, and methodological considerations. *Neuroimage*, *197*, 502-511. <https://doi.org/10.1016/j.neuroimage.2019.04.081>
- Perakakis, P. (2021). *HEPLAB: a Matlab graphical interface for the preprocessing of the heartbeat-evoked potential (v1.0.2)*. <https://doi.org/https://doi.org/10.5281/zenodo.4889450>
- Petzschner, F. H., Weber, L. A., Wellstein, K. V., Paolini, G., Do, C. T., & Stephan, K. E. (2019). Focus of attention modulates the heartbeat evoked potential. *Neuroimage*, *186*, 595-606. <https://doi.org/10.1016/j.neuroimage.2018.11.037>
- Pion-Tonachini, L., Kreutz-Delgado, K., & Makeig, S. (2019). ICLabel: An automated electroencephalographic independent component classifier, dataset, and website. *Neuroimage*, *198*, 181-197. <https://doi.org/10.1016/j.neuroimage.2019.05.026>
- Pion-Tonachini, L., Makeig, S., & Kreutz-Delgado, K. (2017). Crowd labeling latent Dirichlet allocation. *Knowl Inf Syst*, *53*(3), 749-765. <https://doi.org/10.1007/s10115-017-1053-1>
- Schandry, R. (1981). Heart beat perception and emotional experience. *Psychophysiology*, *18*(4), 483-488. <https://doi.org/10.1111/j.1469-8986.1981.tb02486.x>
- Schulz, A., Back, S. N., Schaan, V. K., Bertsch, K., & Vogele, C. (2021). On the construct validity of interoceptive accuracy based on heartbeat counting: Cardiovascular determinants of absolute and tilt-induced change scores. *Biol Psychol*, *164*, 108168. <https://doi.org/10.1016/j.biopsycho.2021.108168>
- Suksasilp, C., & Garfinkel, S. N. (2022). Towards a comprehensive assessment of interoception in a multi-dimensional framework. *Biol Psychol*, *168*, 108262. <https://doi.org/10.1016/j.biopsycho.2022.108262>
- Tsakiris, M., & Haggard, P. (2005). The rubber hand illusion revisited: visuotactile integration and self-attribution. *J Exp Psychol Hum Percept Perform*, *31*(1), 80-91. <https://doi.org/10.1037/0096-1523.31.1.80>
- Tsakiris, M., Tajadura-Jiménez, A., & Costantini, M. (2011). Just a heartbeat away from one's body: interoceptive sensitivity predicts malleability of body-representations. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1717), 2470-2476. <https://doi.org/10.1098/rspb.2010.2547>
- Wallman-Jones, A., Palser, E. R., Benzing, V., & Schmidt, M. (2022). Acute physical-activity related increases in interoceptive ability are not enhanced with simultaneous interoceptive attention. *Sci Rep*, *12*(1), 15054. <https://doi.org/10.1038/s41598-022-19235-z>



- Wallman-Jones, A., Perakakis, P., Tsakiris, M., & Schmidt, M. (2021). Physical activity and interoceptive processing: Theoretical considerations for future research. *Int J Psychophysiol*, 166, 38-49. <https://doi.org/10.1016/j.ijpsycho.2021.05.002>
- Yoris, A., Abrevaya, S., Esteves, S., Salamone, P., Lori, N., Martorell, M., Legaz, A., Alifano, F., Petroni, A., Sanchez, R., Sedeno, L., Garcia, A. M., & Ibanez, A. (2018). Multilevel convergence of interoceptive impairments in hypertension: New evidence of disrupted body-brain interactions. *Hum Brain Mapp*, 39(4), 1563-1581. <https://doi.org/10.1002/hbm.23933>
- Yoris, A., Garcia, A. M., Traiber, L., Santamaria-Garcia, H., Martorell, M., Alifano, F., Kichic, R., Moser, J. S., Cetkovich, M., Manes, F., Ibanez, A., & Sedeno, L. (2017). The inner world of overactive monitoring: neural markers of interoception in obsessive-compulsive disorder. *Psychol Med*, 47(11), 1957-1970. <https://doi.org/10.1017/S0033291717000368>