BRAIN COMMUNICATIONS

Dynamic neurocognitive changes in interoception after heart transplant

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Heart–brain integration dynamics are critical for interoception (i.e. the sensing of body signals). In this unprecedented longitudinal study, we assessed neurocognitive markers of interoception in patients who underwent orthotopic heart transplants and matched healthy controls. Patients were assessed longitudinally before surgery (T1), a few months later (T2) and a year after (T3). We assessed behavioural (heartbeat detection) and electrophysiological (heartbeat evoked potential) markers of interoception. Heartbeat detection task revealed that pre-surgery (T1) interoception was similar between patients and controls. However, patients were outperformed by controls after heart transplant (T2), but no such differences were observed in the follow-up analysis (T3). Neurophysiologically, although heartbeat evoked potential analyses revealed no differences between groups before the surgery (T1), reduced amplitudes of this event-related potential were found for the patients in the two post-transplant stages (T2, T3). All these significant effects persisted after covariation with different cardiological measures. In sum, this study brings new insights into the adaptive properties of brain–heart pathways.

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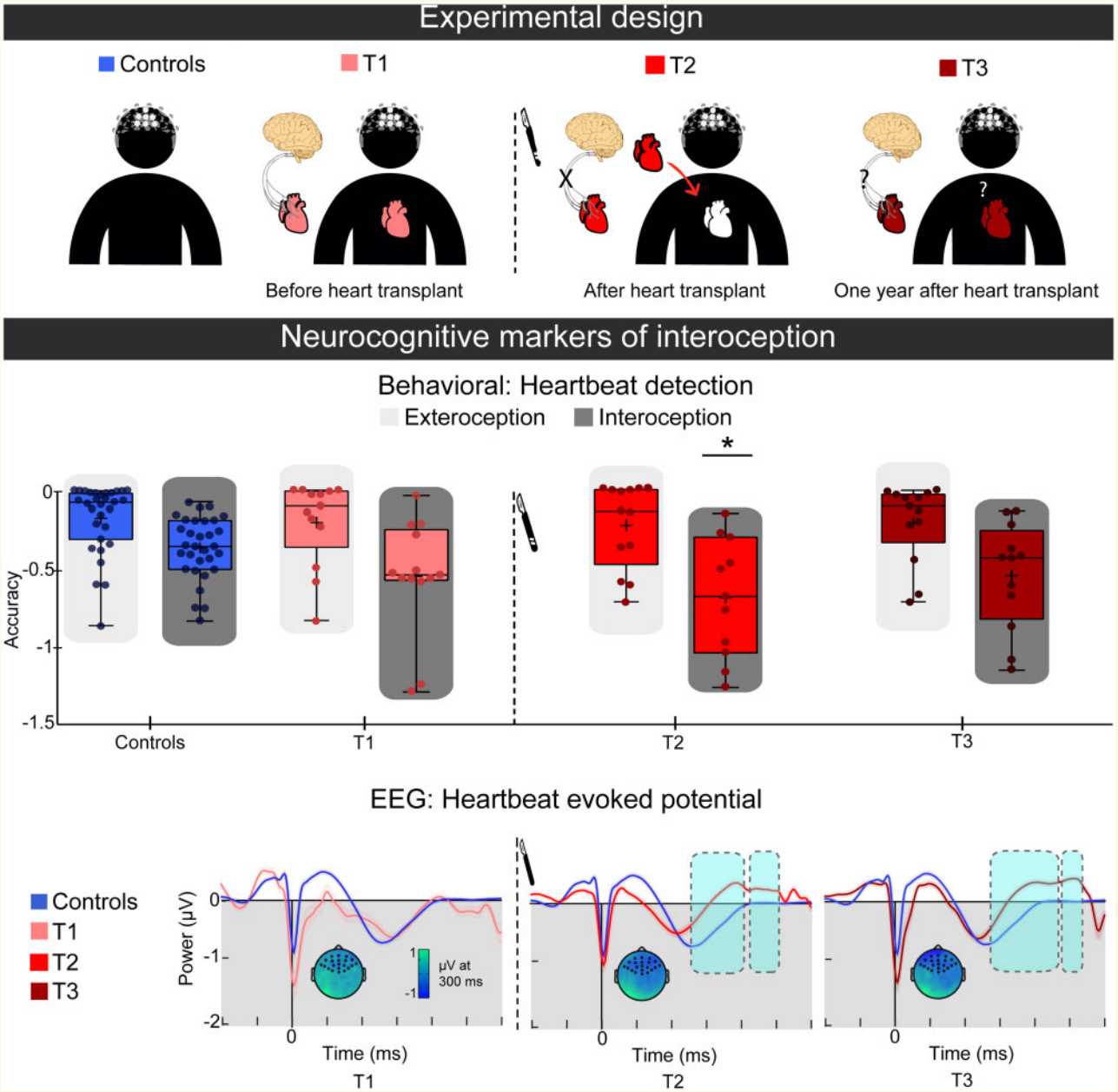
Keywords: interoception; heartbeat detection; heartbeat evoked potential; heart transplantation; vagus nerve denervation

Abbreviations: EA = exteroceptive accuracy; HBD = heartbeat detection; HEP = heartbeat evoked potential; IA = interoceptive accuracy; ROI = region of interest; RSA = respiratory sinus arrhythmia

Received February 19, 2020. Revised June 12, 2020. Accepted June 18, 2020. Advance Access publication July 8, 2020

Graphical Abstract



Introduction

Research on interoception, the sensing of body signals (Critchley and Harrison, 2013), has provided insights into the mechanisms supporting brain–body interactions (Craig, 2002; Ibanez and Manes, 2012; Critchley and Harrison, 2013; Ibanez *et al.*, 2017; Ibanez, 2018) while inspiring new clinical agendas for neuropsychiatric disorders (Muller *et al.*, 2015; García-Cordero *et al.*, 2016). In particular, cardiac interoception has been proposed to hinge on two main pathways: a central one running from the vagus nerve afferents to the brain (Craig, 2002), as supported by results from heartbeat evoked potential (HEP) source analyses (Couto *et al.*, 2015; Salamone *et al.*, 2018), and vagal stimulation (Villani *et al.*, 2019), as well as indirect EEG and neuroimaging evidence (Critchley *et al.*, 2004; Pollatos and Schandry, 2004; García-Cordero *et al.*, 2017), and a secondary one involving somatosensory afferents sensing heartbeats on the chest (Khalsa *et al.*, 2009). However, evidence for these pathways comes from single cases (Khalsa *et al.*, 2009; Couto *et al.*, 2014) and correlational studies (Craig, 2002; Critchley *et al.*, 2004; Schulz *et al.*, 2018), limiting the potential for mechanistic conceptualizations.

Moreover, the neuroplastic properties of these routes remain completely uncharted, casting major doubts on the dynamic adaptability of either pathway. To bridge these gaps, we aimed to test cardiac interoception with a novel approach by conducting the first-ever longitudinal assessment of behavioural and neurophysiological changes following the interruption and re-establishment of afferent vagus nerve signalling due to heart transplant.

Heart transplant recipients offer a critical model to address this issue, as the orthotopic procedure leads to afferent vagus nerve disconnection of the heart (Uberfuhr *et al.*, 2000) with the preservation of somatosensory information (having preserved chest sensations) (Stark *et al.*, 1991). Moreover, research on this population may illuminate neuroplastic mechanisms underlying the reinnervation process, which, despite controversies, might occur 1 year after transplant (Uberfuhr *et al.*, 2000). In a pioneering study, Barsky *et al.* (1998) reported indicators of interoception after heart transplant in only a third of patients. However, they evaluated interoception exclusively in post-surgically and behaviourally with a dual-discrimination task (Barsky *et al.*, 1998). Dual tasks have been criticized as they do not exclusively involve interoceptive processes and engage other (non-interoceptive) cognitive resources (Ring and Brener, 1996; Murphy *et al.*, 2018). Moreover, since this task does not require heartbeat tracking, the subjects' attention is not directed to inner signals, which constitutes a critical aspect of interoceptive tasks (Couto *et al.*, 2014). Also, this study did not provide brain measures and, more importantly, it failed to control for individual differences, as it performed only post-surgery assessments rather that pre/post-transplantation measures.

Thus, assessing these patients right before the transplant and a year later enabled us to analyse the potential effect of reinnervation associated with interoception. We predicted that denervation would modulate neurocognitive markers of interoception, indexed by decreased accuracy and altered HEP amplitude after transplant, and we further anticipated that this might be reversed by neural plasticity after a year.

Materials and methods

Participants

We evaluated neurocognitive markers of interoception in 13 patients who underwent orthotopic heart transplant and 30 matched healthy participants (Table 1 and Fig. 1A). Patients were assessed before the transplant (T1), roughly 4 months afterwards (T2: mean = 4.32, standard deviation = 2.0), and then ~1 year (T3: mean = 15.12, standard deviation = 2.78) after surgery. During the first assessment (T1), patients were hospitalized at University Hospital Favaloro Foundation in Buenos Aires, waiting for a heart donor, and they were assessed in a quiet room specially prepared for the experimental tasks with the hd-EEG equipment. Evaluations after surgery (T2 and T3) were performed with the same acquisition equipment and setting as in T1 (including the same computer screen, keyboard and headphones). For more details, see Supplementary Methods 1.1. All patients were receiving post-transplant medication (immunosuppressive therapy). However, to our knowledge, current evidence indicates that immunotherapy could have an effect over interoception via impacting the respiratory sinus arrhythmia (RSA; Rich *et al.*, 2016). To deal with cofounding biases, RSA as well as other ECG measurements were controlled for.

Thirty-one healthy volunteers without cardiological history were recruited as a control sample. Neither patients nor controls presented a history of psychiatric or neurological conditions, and both samples were matched for sex, age, education, and body mass index (Table 1). Heart period presented differences in patients regarding controls across all stages, including T1, where no group differences in interoception were observed. In addition, no heart period post-transplant changes were observed in patients. No within-group changes emerged in patients either [$F(2,34) = 0.44$, $P = 0.65$; *post hoc* effects in T1–T2 comparison: $F(1,24) = 0.66$, $P = 0.43$, T2–T3 comparison: $F(1,24) = 0.3$, $P = 0.59$]. Hence, there were no modifications in the patient's heart period across time. As our aim was to assess whether post-transplant changes affected interoception; we only implemented covariation analyses for those variables that changed after T1. Since an abrupt reduction in RSA is a robust measure of afferent vagal nerve disconnection (Lu *et al.*, 2016), we compared this measure for each time point (T1–T3) (see Table 1). No differences were found between patients in T1 respect to controls, but as expected after heart transplant, the RSA was significant lower in patients in T2 and T3 (see Supplementary Results 2.1 and Fig. 1). Considering these differences, we used the RSA as covariate to rule out its potential effects on our main significant results (which were reported with and without this covariation).

All participants signed an informed consent in accordance with the Declaration of Helsinki. The study was approved by the institutional ethics committee.

Task design and acquisition

Heartbeat detection task

To assess cardiac interoception, we applied a heartbeat detection (HBD) task (Couto *et al.*, 2014, 2015; Canales-Johnson *et al.*, 2015; García-Cordero *et al.*, 2016, 2017; Yoris *et al.*, 2017, 2018; Salamone *et al.*, 2018; Gonzalez Campo *et al.*, 2019) involving two interlaced conditions. First, an exteroceptive accuracy (EA) condition was used as control measure of external monitoring skills (Critchley *et al.*, 2004; García-Cordero *et al.*, 2016, 2017; Salamone *et al.*, 2018; Yoris *et al.*, 2018). This

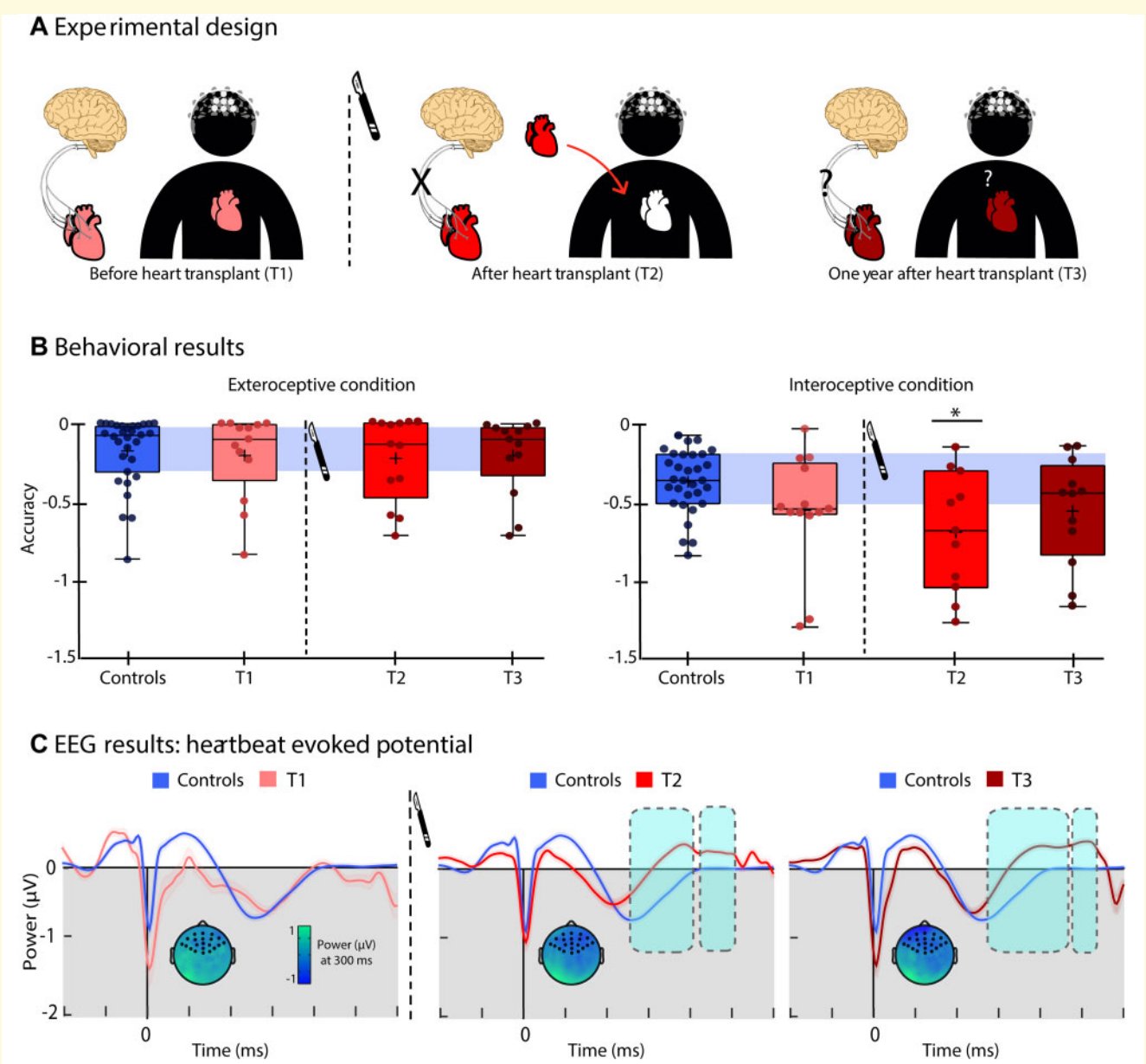


Figure 1 Study's design and results. (**A**) Experimental design. Patients were assessed at three time points, namely: first, before heart transplant (T1, pink); second, a few months afterwards (T2, red), when heart-brain communication had been disrupted; and finally, roughly 1-year post-surgery (T3, dark red). (**B**) Behavioural results. We evaluated the performance of patients in an HBD task across the three time points (T1–T3) and compared IA and EA to the control sample (blue) using repeated measures ANOVA separately for each group. Better performance is characterized by higher accuracy scores. Significant results are marked with an asterisk (*), at $P < 0.05$. Each group's mean is depicted with a cross. Each median is represented with a horizontal line. Blue shadowed horizontal bars indicate the lower (Q1) and upper (Q3) quartiles of the control sample. (**C**) EEG results: HEP. Columns show the results of the comparison between the control group and the patients' HEP modulation across the three time points (over a frontal ROI). Shadowed lines around the ERPs indicate standard error of the mean. Turquoise boxes show statistically significant differences at $P < 0.05$ for a minimum extension of five consecutive points of difference (Salamone *et al.*, 2018)—for T2, in 266–407 and 430.5–508.6 ms windows; for T3, in 282–466 and 481–536 ms windows. Scalp topographies show the electrodes used for the frontal ROI and the differences in amplitude (microvolts) between groups at 300 ms. For further details, see Supplementary Figs 1–5.

condition involved three blocks: (i) one where participants had to follow a recorded heartbeat presented at a regular frequency (60 bpm) (digitally constructed from a real ECG record) by tapping a key from a keyboard; (ii) another one that presented manipulated beats to have the same average frequency (60 bpm), but with irregular heartbeat intervals; and (iii) the last one, in which participants were requested to follow their own heartbeats using an stethoscope to have auditory feedback of their cardiac activity. Then, to evaluate inner signal monitoring, the

Table 1 Demographic measures

	Healthy participants	Patients	χ^2	P^a
Sex	M = 20/F = 11	(T1) M = 9/F = 4	(T1) 0.003	(T1) 0.95
		(T2) M = 8/F = 3	(T2) 0.18	(T2) 0.66
		(T3) M = 9/F = 4	(T3) 0.003	(T3) 0.95
			(DF) *F*	P^b
Age	40.45 (18.20)	(T1) 45.08 (15.19)	T1 (1,42) 0.65	(T1) 0.43
		(T2) 47.81 (14.49)	T1 (1,42) 2.55	(T2) 0.11
		(T3)45.92 (15.93)	T3 (1,42) 1.72	(T3) 0.19
Education	13.58 (2.80)	(T1) 12.54 (4.35)	T1 (1,42) 0.90	(T1) 0.35
		(T2) 11.81 (4.31)	T2 (1,40) 2.70	(T2) 0.10
		(T3) 13.00 (4.19)	T3 (1,42) 0.31	(T3) 0.57
RSA	1.53 (1.57)	(T1) 1.69 (1.61)	T1 (1,42) 0.09	(T1) 0.75
		(T2) 0.43 (0.63)	T2 (1,40) 5.18	(T2) 0.03[b]
		(T3) 0.31 (0.35)	T3 (1,42) 7.76	(T3) 0.01[b]
BMI	26.38 (3.58)	(T1) 25.48 (4.38)	T1 (1,42) 0.19	(T1) 0.66
		(T2) 25.46 (2.55)	T2 (1,40) 0.48	(T2) 0.49
		(T3) 26.27 (3.72)	T3 (1,42) 0.01	(T3) 0.93
HP	74.29 (9.88)	(T1) 93.91 (19.18)	T1 (1,42) 22.98	(T1) <0.01[b]
		(T2) 100.41 (17.56)	T2 (1,40) 36.85	(T2) <0.01[b]
		(T3) 97.60 (9.54)	T3 (1,42) 51.99	(T3) <0.01[b]

Mean and standard deviation are presented for each group.
BMI = body mass index; HP = heart period.
[a]Gender was analysed with the Pearson chi-squared ($\chi 2$) test.
[b]Demographic data were assessed through ANOVAs. For details on RSA analysis, see Supplementary Information 2.

participants completed an interoceptive accuracy (IA) condition, in which they were asked to follow their own heartbeats by tapping the same key but in the absence of any external feedback (e.g. pulse) (Melloni *et al.*, 2013; Couto *et al.*, 2014, 2015; Canales-Johnson *et al.*, 2015; Yoris *et al.*, 2015; García-Cordero *et al.*, 2016, 2017; Salamone *et al.*, 2018). This condition encompassed four blocks (all with the same instruction); two of them were administered before the feedback block from the exteroceptive condition (EC) and two were administered after this. All blocks lasted 2.5 min, and the total length of the HBD task was ~25 min (including instructions). During this task, high density EEG (hdEEG) and ECG signals were recorded.

Accuracy scores were based on a synchronization measurement to evaluate the oscillatory coupling between cardiac frequency and motor tapping over time across different time windows. Unlike other metrics, it captures the ability to adjust responses to cardiac changes irrespective of the total number of responses (de la Fuente *et al.*, 2019; Fittipaldi *et al.*, 2020). This kind of indexes has been used to assess both exteroceptive (Engel *et al.*, 2001; Buonomano and Laje, 2010; Arenas *et al.*, 2012) and interoceptive signals (Couto *et al.*, 2014; de la Fuente *et al.*, 2019). Specifically, this measure captures the ability to adjust responses to cardiac changes and it is not biased by the total number of responses. For this reason, participants with more responses will not necessarily present a higher accuracy and this will depend on how well they were able to synchronize their responses with their own (or the recorded) heartbeats. For more details on this measurement, see Supplementary Methods 1.2. Given that IA is a stable trait in healthy subjects, the control group was used as a normative sample to compare patients' performance over time (Ferentzi *et al.*, 2018). To confirm previous results of task stability over time (Couto *et al.*, 2014; Ferentzi *et al.*, 2018), we reported a supplementary study with healthy participants showing no significant variation in performance in a test/retest design (Supplementary Methods 1.3). Additional HBD analyses were performed to assess within-group associations. Regression analyses were performed between T1–T2 and T2–T3. This allowed us to test two possible outcomes consistent with the main hypothesis: as the surgery interrupts the brain–heart communication, T1 should not predict T2 and, given that partial plastic processes are expected from T2 to T3, the former should predict the latter.

Heartbeat evoked potential

We obtained EEG signals during the interoceptive block. We extracted the HEP, a reliable index of attention to body signals (Pollatos and Schandry, 2004; Muller *et al.*, 2015; García-Cordero *et al.*, 2016, 2017; Pollatos *et al.*, 2016; Yoris *et al.*, 2017, 2018). hd-EEG signals were acquired with a Biosemi Active-two 128 channel system at 1024 Hz. Data were resampled offline at 256 Hz and filtered at 0.5–30 Hz. The signal was re-referenced offline to the average reference. Eye movements or blink artefacts were corrected with independent component analysis (Kim and Kim, 2012) and with a visual inspection protocol, as done previously (Schandry and Montoya, 1996; Dirlich *et al.*, 1997; Pollatos and Schandry, 2004; Terhaar *et al.*, 2012; García-Cordero *et al.*, 2016, 2017; Yoris *et al.*, 2017, 2018; Salamone *et al.*, 2018). To avoid cardiac field artefacts (Kern *et al.*, 2013), we analysed a time frame

between the 200 and 550 ms, proposed to be less affected by cardiac field artefacts (Dirlich *et al.*, 1997; Kern *et al.*, 2013; Park *et al.*, 2014). As the cardiac field artefacts effect over early HEP windows cannot be ruled out, windows after 200 ms are robust and standard to evaluate HEP modulations (Gray *et al.*, 2007; Yoris *et al.*, 2018). Moreover, independent component analysis (ICA) elimination may have two sources of confounding. First, artefact survival is uncertain, as it is not clear whether their removal fully eliminates their effects over the signal; second, there is no assurance that deletion may not result in (non-artefactual) signal loss (Castellanos and Makarov, 2006). A better approach to control for artefacts is to report results after 200 ms (García-Cordero *et al.*, 2016, 2017; Yoris *et al.*, 2017, 2018; Salamone *et al.*, 2018). In any case, there are no reasons to assume that the artefact is not randomly distributed between groups and measurements. R-wave values from the ECG signal were identified with a peakfinder function on Matlab and used to segment continuous hd-EEG data for HEP analysis (Canales-Johnson *et al.*, 2015; García-Cordero *et al.*, 2016, 2017; Yoris *et al.*, 2017; Salamone *et al.*, 2018). These EEG epochs were delimited between −300 and 600 ms and baseline-corrected from −300 to 0 ms. Low drifts were removed by linear trend corrections (Delorme and Makeig, 2004).

Statistical analysis

Heartbeat detection task

Performance on the HBD task was scrutinized via repeated measures ANOVA, with a within-subject factor (condition: IA and EA) and a between-subjects factor (group: patients and controls). This analysis was repeated for each time point (before the heart transplant: T1; after the surgery: T2; and in the 1-year follow-up: T3) using always the same control group as a normative data, given that interoceptive performance with this task has proven stable over time (Couto *et al.*, 2014; Ferentzi *et al.*, 2018). This strategy was implemented to evaluate the patients' change after the intervention considering the performance of the healthy controls as a reference point of normal behaviour (this group presented no neurological, psychiatric or cardiological conditions). For each significant ANOVA, a Tukey's *post hoc* analysis was performed to explore the differences between conditions (the alpha level was set at $P < 0.05$). We excluded one subject who presented results that were 2.5 SD above the individual group average (one T3 patient on the average of the IA condition).

The effect sizes of ANOVA results were calculated via partial eta squared. To assess whether significant differences in interoceptive results were affected by RSA differences between groups, we performed ANCOVA tests and correlations.

Heartbeat evoked potential

ERP modulations across all conditions of the HBD task were compared through a point-by-point Monte Carlo permutation test with bootstrapping (Manly, 2006). As for the behavioural data, the control group was used as normative data to compare the patients' HEP modulations at each time point. The permutation analysis constitutes a robust approach to compare EEG data, as seen in several studies assessing modulations in the HEP (Couto *et al.*, 2014, 2015; Canales-Johnson *et al.*, 2015; García-Cordero *et al.*, 2016, 2017; Yoris *et al.*, 2017, 2018; Salamone *et al.*, 2018) and other ERPs (Chennu *et al.*, 2013; Ibanez *et al.*, 2013; Amoruso *et al.*, 2014; Gonzalez-Gadea *et al.*, 2015; Melloni *et al.*, 2015, 2016). This method overcomes the multiple comparisons problem and does not depend on multiple comparison corrections or Gaussian distribution assumptions (Nichols and Holmes, 2002). Moreover, given that it is a point-by-point approach, it avoids the selection of narrow *a priori* windows, preventing circularity biases and allowing the analysis of each point of the signal comprised within the HEP latency (Montoya *et al.*, 1993; Pollatos and Schandry, 2004; Canales-Johnson *et al.*, 2015; Salamone *et al.*, 2018). It allowed us to compare the complete signal within 200–500 ms, which constitutes a typical HEP latency (Pollatos and Schandry, 2004; Canales-Johnson *et al.*, 2015; Muller *et al.*, 2015; Pollatos *et al.*, 2016). The main HEP analyses were based on a frontal region of interest (ROI) associated with this ERP modulation (Couto *et al.*, 2014; García-Cordero *et al.*, 2016; Marshall *et al.*, 2017). The ROI was composed of 20 electrodes: C3 C4 C5 C10 C11 C12 C13 C14 C19 C20 C21 C22 C24 C25 C26 C27 C32 D3 D4 D5. Additional analysis with three frontal ROIs (left-frontal, central-frontal and right-frontal ROIs) was performed to evaluate the modulation in different locations (Supplementary Results 2.4). To assess whether significant differences in interoceptive results were affected by ECG differences between groups, we performed ANCOVA tests and correlations.

Data availability statement

In house scripts and anonymized results that support the study findings are available from the corresponding author upon reasonable request. Task is available online at: http://bit.ly/2EpfGrq.

Results

In the HBD task, before the surgery (T1), we found a significant effect of condition [$F(1,42) = 55.68$, $P < 0.01$, $np2 = 0.57$] reflecting better outcomes on EA over IA (García-Cordero *et al.*, 2017; Yoris *et al.*, 2017). In addition, a significant interaction between group and condition was observed [$F(1,42) = 5.05$, $P = 0.03$,

np2 = 0.11]. However, Tukey's *post hoc* tests did not show differences between groups in either condition (EA: $P = 0.99$, IA: $P = 0.12$), indicating that both patients and controls presented a similar performance across the task in this first assessment.

Roughly 4 months after heart transplant (T2), there was a significant group effect [$F(1,40) = 6.86$, $P = 0.01$, np2 = 0.15] showing better performance in controls than patients, alongside a significant condition effect [$F(1,40) = 69.50$, $P < 0.01$, np2 = 0.63], as in the previous comparison (EA > IA). We also found a significant interaction between group and condition ($F = 10.82$, $P < 0.01$, np2 = 0.21). Tukey's *post hoc* tests revealed that patients were outperformed by controls in the IA condition ($P < 0.01$) but not in the EA condition ($P = 0.73$).

In the follow-up analysis (T3), there was a significant effect of condition [$F(1,41) = 49.66$, $P < 0.01$, np2 = 0.55], with EA yielding higher accuracy. However, no between-group differences were found [$F(1,41) = 2.70$, $P = 0.11$, np2 = 0.06]. A trend was found in the interaction between group and condition [$F(1,41) = 4.12$, $P = 0.05$, np2 = 0.09]. Tukey's *post hoc* tests revealed no between-group differences in any of the conditions (EA: $P = 0.92$; IA: $P = 0.10$) (see Fig. 1B and Supplementary Results 2.2.1 and Fig. 2).

All the significant effects above persisted after covariation with RSA (see Supplementary Results 2.1.2). As a complementary approach, and to compare individual changes after the heart transplant in patients, we analysed IA outcomes in T2 and T3 relative to T1. Regarding T1, patients presented a significant poorer performance 4 months after the surgery (T2), with no differences 1 year after (T3, Supplementary Results 2.3 and Fig. 3). Finally, regression analyses were performed to compare pre–post behavioural changes (see Supplementary Results 2.2.2). As expected, while T1 did not predict outcome of T2, the latter predicted T3 results.

HEP analyses revealed no differences between patients and controls before the surgery (T1), alongside reduced amplitudes for the patients in the two post-transplant stages (T2 and T3) (Fig. 1C and Supplementary Fig. 4). These differences emerged within the canonical latency of the HEP, starting at 200 ms after the R peak (Pollatos and Schandry, 2004). Before the surgery (T1), in the larger frontal ROI, no HEP differences were found between patients and controls (Fig. 1C). However, in both post-transplant time points (T2 and T3), patients showed less negative HEP amplitude compared to controls (significant differences emerged in the 266–407 ms window and a 430.5–508.6 ms window in T2 and in a 282–466 ms window and a 481–536 ms window in T3). The same pattern of results was observed for the left, right and central-frontal ROIs (Supplementary Results 2.4 and Fig. 4). The larger differences between groups (262.5–407.2 ms window in T2 and 274.2–469.5 ms window in T3) remained significant even after controlling for RSA and ECG differences (Supplementary Results 2.1.3 and 2.5 and Fig. 5, respectively).

Discussion

This is the first longitudinal study to assess the critical role of heart transplant on neurocognitive markers of interoceptive. The interruption of one of the key pathways allows drawing inferences about heart-brain integration dynamics. Approximately 4 months after vagal disconnection (T2), patients presented alterations in neurocognitive (behavioural and electrophysiological) interoceptive markers. Previous studies have suggested that both the vagal and the somatosensory pathways play complementary roles in interoceptive processing, but they did not directly test whether one pathway predominates over the other (Craig, 2002; Critchley *et al.*, 2004; Pollatos and Schandry, 2004; Couto *et al.*, 2014). The only prior evidence of behavioural interoceptive alterations in transplant patients (Barsky *et al.*, 1998) suggests that only one-third of them had preserved cardiac awareness. Here, we found that most individual trajectories of patients indicated impaired interoception post-transplant. Importantly, we overcame some limitations of the Barsky *et al.* (1998) study. First, our pre/post-design allowed controlling for individual differences before the transplant. Second, our design complemented behavioural assessments with electrophysiological measures of interoceptive changes. In addition, our task minimizes other (non-interoceptive) cognitive processes and is based on synchronization measurements, which have proven more reliable than traditional indexes (de la Fuente *et al.*, 2019; Fittipaldi *et al.*, 2020). Finally, we showed both early and late changes post-transplant. In this sense, our results highlight the potential role of afferent vagus nerve dynamics in the domain. Indeed, given that heart transplant involves disconnection of vagal afferents with preserved somatosensory information, the latter does not seem to be self-sufficient for proper interoceptive function in this context of neuroadaptation after surgery. Notably, the observed deficits occurred even when controlling for predictable changes in RSA or other ECG measurements, highlighting their dependence on neurovisceral (rather than only peripheral) disturbances. Therefore, although both neural pathways may well afford complementary interoceptive mechanisms, vagal nerve integrity seems distinctively crucial for this domain. This finding, in fact, aligns with correlational studies underscoring the predominant role of the (vagal) pathway in neurovisceral integration (Craig, 2002; Critchley *et al.*, 2004; Pollatos and Schandry, 2004; Couto *et al.*, 2014).

Regarding T3, behavioural results showed no difference between patients and controls. However, reduced HEP modulations remained significant, suggesting a partial impact of vagal reinnervation. This lack of correspondence between behavioural and neurophysiological interoceptive

markers could be partly driven by a reorganization of the somatosensory route, which might contribute to the re-adjustment of behavioural outcomes even despite dysfunctional signalling from the direct route. This reorganization is also observed in phantom limb syndrome, characterized by somatosensory plasticity of brain function (Flor *et al.*, 1995). Moreover, considering that reinnervation varies widely across time (actually proving absent in some clinical cases; Uberfuhr *et al.*, 2000) and while the HEP modulations might be capturing subtler changes remaining after 1 year, the behavioural measure could be a signal of compensatory plasticity processes (Khalsa *et al.*, 2009). Therefore, although longer follow-up research is needed, our findings suggest that the neural markers (HEP) may be a candidate for tracking the variability in brain–heart connection. Although there is no direct evidence that HEP modulations are vagally mediated, previous correlational studies suggested that afferent information impacts the HEP, including interoceptive HEP modulations (Pollatos and Schandry, 2004; García-Cordero *et al.*, 2017), source reconstruction analysis (Couto *et al.*, 2015; Salamone *et al.*, 2018) and association between vagal stimulation and HEP activity (Villani *et al.*, 2019). This study complements such results by pointing to a potential role of the vagal pathway over HEP modulations in heart transplant recipients. Future investigations should target this issue, ideally contemplating additional interoceptive sources and indicators of reinnervation.

Limitations and future studies

This report presents some limitations that could be addressed in future studies. For instance, results in transplant patients are mainly an effect of surgery seen in our pre/post-design. Although controls were not assessed longitudinally, we have provided empirical evidence (Supplementary Methods 1.3) attesting to the temporal stability of task outcomes. Nevertheless, future studies could better tackle this potential limitation via longitudinal follow-up tests in healthy controls. Heart transplant patients have preserved heartbeat-related chest sensations (Stark *et al.*, 1991), attesting to the specificity of vagal (as opposed to somatosensory) disruptions. However, future studies could incorporate patients with sternotomy surgery and lung transplantation (without heart transplant) to test the specific compromise of somatosensory pathways in the present population. Although our study included comparisons with healthy participants, future studies could include other control samples featuring contrastive medical conditions (Barsky *et al.*, 1998). Future experiments could also control for differences in the administration of medication over interoceptive mechanisms. As afferent and efferent vagal innervation is compromised after heart transplant surgery, the potential role of sympathetic efferents influencing brain–body signalling communication should still be further investigated.

Conclusions

In conclusion, this study reveals for the first time the dynamical neurocognitive changes in interoception after heart transplant. By providing novel evidence of the differential role of the vagal pathway during interoception, our framework unveils hitherto unknown aspects of this pathway's adaptive properties. Future research along these lines could usefully further our understanding of neurovisceral interactions.

Supplementary material

Supplementary material is available at *Brain Communications* online.

Acknowledgements

We sincerely thank the participants and patients for their invaluable time and commitment to our study.

Funding

This work has been partially supported by CONICET, CONICYT/FONDECYT (1170010), FONCyT-PICT (2017-1818 and 2017-1820), FONDAP (15150012) and the Global Brain Health Institute (GBHI).

Competing interests

The authors report no competing interests.

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