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Bilateral Rolandic Operculum processing underlying heartbeat awareness reflects changes in bodily self-consciousness

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Two hearts and one self: integrating cardio-visual stimulation

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Abstract

Exteroceptive bodily signals (including tactile, proprioceptive, and visual signals) are important information contributing to self-consciousness. Moreover, prominent theories proposed that visceral signals about internal bodily states are equally or even more important. Neuroimaging studies have described several brain regions which process signals related to bodily self-consciousness (BSC) based on the integration of exteroceptive signals (e.g. the premotor cortex, the angular gyrus, the supramarginal gyrus and the extrastriate body area), and that another brain region, the insula/operculum which is involved in interoception and interoceptive awareness, processes signals critical for self-awareness. Providing evidence for the integration of exteroceptive and interoceptive bodily signals, recent behavioral experiments have demonstrated that the manipulation of interoceptive (i.e. cardiac) signals, coupled with that of exteroceptive (i.e. visual) signals, also modulates BSC. Does this integration occur within or outside the structures described above? To this end, we adapted a recently designed protocol that uses cardio-visual stimulation to induce altered states of BSC to fMRI. Additionally, we measured neural activity in a classical interoceptive task. We found six brain regions (the bilateral Rolandic operculum, the bilateral supramarginal gyrus, the right frontal inferior operculum, and the left temporal superior

gyrus) that were activated differently during the interoception task as opposed to a control task. The brain regions which showed the highest selectivity for BSC based on our cardio-visual manipulation were found in the bilateral Rolandic operculum. Given our findings, we propose that the Rolandic operculum processes integrated exteroceptive-interoceptive signals that are necessary for interoceptive awareness as well as BSC.

Introduction

Self-consciousness has traditionally been the remit of philosophical inquiry, which has produced an expansive body of work containing a plethora of divergent theories about the nature of the self and self-consciousness which are not data-driven (Bermúdez, 1998, Gallagher and Shear, 1998). Recently, experimental approaches have been developed to investigate self-consciousness, and have revealed the brain mechanisms underpinning multisensory bodily signals (i.e., bodily self- consciousness, BSC) (Knoblich, 2002, Jeannerod, 2003, Legrand, 2007, Blanke and Metzinger, 2009, Damasio and Meyer, 2009, Christoff et al., 2011, De Vignemont, 2011, Ehrsson, 2012, Serino et al., 2013, Blanke et al., 2015). Evidence has denoted that BSC is related to the processing and integration of specific exteroceptive signals, including somatosensory, vestibular and visual signals. Moreover, neurological findings have shown that electrical interference or damage to critical multisensory integration areas leads to alterations of BSC, such as the feeling that an artificial body is one's own (altered self-identification) and that the self is located in a spatial position other than that of the physical body (altered self-location) (Blanke et al., 2004). Weaker, but controlled and replicable alterations of BSC in healthy subjects can be experimentally induced by manipulating multisensory cues, such as with the visual-tactile Full Body Illusion (FBI) paradigms (Ehrsson, 2007, Lenggenhager et al., 2007, Petkova and Ehrsson, 2008, Aspell et al., 2009, Palluel et al., 2011, Aspell et al., 2013, Salomon et al., 2013).

Exteroceptive bodily signals are not the sole contributors to BSC, and prominent theories have emphasized that internal states and visceral signals provide critical signals for self-consciousness (Damasio, 2000, Craig, 2002). Based on these proposals, recent behavioral experiments (Aspell et al., 2013, Seth, 2013, Suzuki et al., 2013, Ronchi et al., 2015) have demonstrated that the manipulation of interoceptive signals (i.e. cardiac) coupled with that of exteroceptive signals (i.e. visual), also modulates BSC (Aspell et al., 2013, Suzuki et al., 2013, Ronchi et al., 2015). Several findings suggest that the insular cortex is most likely the structure which mediates cardio-visual effects on BSC (Tsakiris et al., 2007, Ronchi et al., 2015). Moreover, neuropsychological studies show that insular lesions are associated with deficits in BSC, such as somatoparaphrenia (Levine et al., 1991, Cereda et al., 2002, Vallar and Ronchi, 2009, Karnath and Baier, 2010) or heautoscopy (Heydrich and Blanke, 2013). In addition, a cortical region in the frontal operculum/insular cortex has been repeatedly associated with the processing of cardiac signals and interoceptive awareness (Damasio, 2000, Craig, 2002, Critchley et al., 2004, Jabbi et al., 2007, Craig, 2009, Wiebking et al., 2014, Garfinkel et al., 2015). Yet, little is known about which brain areas process the integration of interoceptive and exteroceptive bodily signals that mediate BSC.

In this study, we investigated the brain mechanisms of BSC in relation to cardio-visual stimulation while participants were exposed to our recent cardio-visual stimulation paradigm (Aspell et al., 2013) inside the MRI scanner. The main focus was to identify the brain regions involved in processing cardio-visual integration and how these areas compare with those involved in interoceptive (cardiac) awareness.

Methods

Participants

Sixteen healthy right-handed participants took part in the study (11 males, five females, mean age=24.5 years; SD=2.7 years). All participants signed the informed consent and were compensated 20 Swiss Franc per hour for their participation. The study was approved by the

Ethics Committee of the Faculty of Biology and Medicine of the University of Lausanne Switzerland and conducted in agreement with the ethical standards of the Declaration of Helsinki.

Experimental procedure

Participants completed the study on two consecutive days. On *Day 1* we measured participant's pulse transit time – i.e. the lag between the electrocardiography (ECG) R-wave and the peak of the photoplethysmography (PPG) signal (Fig. 1) and took a photograph of the participant's back to personalize the cardio-visual feedback to each subject. On *Day 2* participants carried out the experiment inside the MRI scanner. They were kept ignorant of the aims of the study until after the experiment.

Simultaneous ECG and PPG for heartbeat detection outside the MRI scanner (Day 1)

A PPG sensor was used to provide feedback during FBI-CV (*see cardio-visual stimuli*). To ensure that the feedback was in line with the ejection of blood from the heart, we had to estimate the temporal delay between the PPG peak and the R-wave and modify the feedback computation accordingly. Hence, on *Day 1* participants were asked to rest supine on a mattress to record ECG and PPG signals simultaneously for 2 minutes. ECG was acquired with a Biosemi Active II system (Biosemi, The Netherlands) at a sampling rate of 2048 Hz using two electrodes attached to the left side of each participant's chest at the midclavicular line in the third and fifth intercostal space respectively. The MRI-compatible PPG sensor, an infrared light emitter and the silicon infrared phototransistor (Fairchild™ QSE113), was attached to each participant's left index finger. The PPG signal was sampled at a frequency of 100 Hz and transmitted to a custom-developed software (ExpyVR development environment, freely available from http://lnco.epfl.ch/expyvr) via a USB connection using a dedicated microcontroller (Arduino™ UNO, ATmega328). Upon PPG peak detection, the software sent a marker through a parallel port to the Biosemi Active II system that was recorded together with the ECG tracks (Fig. 1). The mean pulse transit time was defined as the average lag between the peak of the ECG R-wave and the marker of the

peak of the PPG signal (Fig. 1). In agreement with other research (Suzuki et al., 2013) and unpublished protocols by our group, we estimated the cardiac R-wave occurs on average 350 ms (SD=±50 ms) before the PPG signal peak. The choice to synchronize the stimulus to the R-wave (i.e. the ejection of the blood from the heart) was made to mimic as closely as possible the original protocol used by (Aspell et al., 2013).

Experimental setup and design inside the MRI scanner (Day 2)

Following *Day 1*, we acquired brain imaging data. Participants were supine in the MRI scanner while their heart rate was monitored using a PPG sensor MRI-compatible attached to their left index finger (the same sensor used on *Day 1*). We chose this solution because no MRI-compatible ECG sensor capable of real-time data streaming was available and because the PPG-based solution was entirely satisfying for the purpose of the experiment (i.e. correction of timing between PPG and R peak showed reliable results). A response box (Current Designs Philadelphia, PA) with two buttons was provided to the participants who held it with their right hand. The experimental protocol started with the acquisition of structural images, followed by two FBI under CV stimulations (FBI-CV; see below), a questionnaire measuring subjectively bodily-self experience, and a Heart Awareness Task (HAT; see below).

Cardio-visual stimuli

During FBI-CV stimulations participants were presented with an image of either their back (called Body) or an image of an inanimate object (called No-body) (Fig. 2). During the stimulation period, participants were asked to look at an image of their Body (B) (i.e. a picture of their body viewed from behind which was taken on *Day 1*) or at a control No-body Object (O) (i.e. a picture of an inanimate, but body-sized, rectangular object) (Fig. 2) while the white outlined contour flashed synchronously (S) or asynchronously (A) with the participant's heartbeat recorded in real-time. ExpyVR software was used to show the white outlined contour around the body or the object, flashing synchronously or asynchronously,

with respect to the real-time detection of participant's heartbeat using the PPG sensor. In the synchronous condition, the white outlined contour was displayed at the peak of the PPG signal corrected for the mean pulse transit time (t_1'; see Fig. 1), thus at the cardiac R-wave. This procedure was carried out to generate approximate synchrony between the outlined contour and the R peak of the QRS complex of the next heartbeat (Fig. 1). This anticipation of the R peak of the next heartbeat was based on the hypothesis that the lag between the R peak and the PPG peak is constant under identical measurement conditions and over 24h (Drinnan et al., 2001). For the asynchronous condition, the participant's heart signal was replaced with a pre-recorded ECG (30 s template at 67 beats per minute (BPM) from a healthy subject) stretched or compressed in time to match the 80% of the continuously measured participant's heart rate. For example, replaying the template at x1.07 speed generated a signal at 72 BPM, which is 80% of a participant's heart rate of 90 BPM (Salomon et al., 2016). Thus, during asynchronous cardio-visual stimulation, the outlined white contour was dynamically desynchronized (i.e. there was a constantly updated change in the frequency domain) from the detected heartbeat (Salomon et al., 2016). In both synchronous and asynchronous conditions, the outlined contour progressively changed its illumination from total transparency (0%) to full opacity (100%) and back to transparent (0%) following the positive section of a sinusoidal function for a total duration of 500 ms.

Cardio-visual stimulation and self-location task

All participants performed two FBI-CV runs separated by 3-5 min of rest. Each run consisted of 16 trials randomly ordered that reflected the 2x2 factorial design with *stimulation* (Synchronous (S) and Asynchronous (A)) and *visual stimulus* (Body (B) and No-body (O)) as the main factors (Fig. 2). Each trial included 40 s of stimulation, 5 s for the self-location task (i.e. Mental Ball Drop task, MBD) (Lenggenhager et al., 2009, Ionta et al., 2011, Pfeiffer et al., 2013), and 12 s of rest. The choice to perform only a few trials (with a long duration) was dictated by the necessity that illusion induction requires an extended duration of cardio-visual stimulation. As we also carried out other tasks (i.e. subjective ratings on BSC and

Heart Awareness Task; see next sections respectively), any further addition of trials or conditions would have increased the risk of inducing discomfort (or excessive movement) to participants due to a prolonged time in the MRI scanner. Accordingly, we made a trade-off between length and number of trials as in previous FBI-like fMRI experiments (Gentile et al., 2011, Ionta et al., 2011). To perform the MBD task, participants had to imagine holding and then releasing a ball and to estimate when the ball would hit the ground. The MBD task began with the appearance on the screen of a virtual arm dropping a red ball to remind participants of the instructions. Participants were required to indicate the moment of the ball release by pressing a button on the response box and keeping it pressed until they imagined the impact of the ball on the ground. Response times were recorded and calculated as the time difference between the button press and the release. Participants were instructed to operate the response button with their index finger or middle finger (fingers were counterbalanced across participants). Participants were not informed about the synchrony manipulation of the white outlined contour on the image or that it was related to their heartbeat. We used the MBD task to measure participants' self-location in space implicitly. The FBI-CV task was always performed before the HAT to rule out the possibility that participants could guess the experimental manipulations by being exposed to an explicit heartbeat task.

Subjective responses

After the completion of the FBI-CV runs, we displayed each of the four different experimental conditions (BS, BA, OS, OA) in a randomized order for 40 s while participants were in the MRI scanner, without acquiring any functional data. After each condition, subjective ratings on BSC were assessed by a nine-item questionnaire (Table 1; adapted from (Ionta et al., 2011, Pfeiffer et al., 2013)). The items were shown randomly on the same screen and rated on a 7-point Likert scale (1 to 7; where the lowest number indicated complete disagreement and the highest number indicated complete agreement). This was then consequently analyzed using an ipsatization procedure (Cattell, 1944, Broughton and Wasel, 1990).

Questionnaire items are reported in Table 1. Critical questions for the aim of the present study are Q1 assessing self-identification with the virtual body; Q2, Q5, and Q6 assessing changes in self-location; and Q7 and Q8 assessing changes in heart perception.

Heartbeat Awareness Task (HAT)

Following the questionnaire, all participants performed a Heartbeat Awareness Task (HAT). The HAT was adapted from the protocol described in (Critchley et al., 2004) using visual, instead of auditory stimulation. Twenty-four different trials were presented in a random order. In each trial, we manipulated participants' *attentional focus*, the *stimulation* and the *color* of the visual stimulus. Each trial began with the word HEART or COLOR, appearing for 5 s followed by 10 s of a flashing rectangular stimulus. In the HEART trials, participants were asked to judge whether the rectangular flashed synchronously with their heartbeat. While in the COLOR control task, they were asked to decide whether the flashing rectangle changed its color. The stimulation was manipulated in two ways. In the synchronous condition, the appearance of the stimulus was aligned with the detection of the PPG peak corrected for the mean pulse transit time (as for the same condition in the FBI-CV), thus at the estimated R peak. Conversely, in the asynchronous condition, the appearance of the stimulus was delayed 500 ms from the detection of the estimated R peak. Although this was different from the asynchronous condition in the FBI-CV, we chose this delay to be consistent with the heartbeat sensitivity protocol described in (Critchley et al., 2004). In half of the trials, the color of the rectangular stimulus was altered. Specifically, the rectangular changed color for one second from white to cream. We modeled the whole run with a 2x2x2 factorial design with attention (Heart or Color) stimulation (Synchronous (S) or Asynchronous (A)), and color (Color Change or No Color Change). At the end of each trial, participants were prompted to respond the question appearing on the screen by pressing the button either with the index or with the middle finger of their right hand. One button was associated with the answer YES (i.e. the rectangular stimulus was flashing synchronously with their heartbeat or, in the control task the rectangular changed its color) and the other with the NO answer (the

rectangular stimulus was not flashing in synchrony with the participants' heartbeat or, in the control task the rectangular stimulus did not change its color). The association between the finger (index or middle) and answer (YES or NO) was counterbalanced amongst participants.

Analysis of subjective responses

Individual questionnaire ratings were standardized using ipsatization procedure (Cattell, 1944, Broughton and Wasel, 1990). For each subject individually, each response score was subtracted from the average score of that subject in all questions/conditions and then divided by the standard deviation of the subject in all questions/conditions (Romano et al., 2014, Ronchi et al., 2015). Standardized responses to each question were analyzed using a two-way repeated measures ANOVA, with stimulation (Synchronous and Asynchronous) and visual stimulus (Body and No-body) as the main factors.

Implicit self-location in space task analysis

Mental Ball Drop response times were also analyzed using a two-way repeated measures ANOVA, with stimulation (Synchronous and Asynchronous) and visual stimulus (Body and No-body) as the main factors. Trials, where participants took more than 5 s to respond, were excluded from the analysis.

fMRI acquisition and preprocessing

Imaging was performed using a 7T Magnetom Siemens scanner equipped with a 32-channel head coil with a mirror for back-projected visual feedback. Functional images were acquired using Echo Planar Imaging (EPI) comprising of 46 oblique slices (matrix size 140×140, FOV=210 mm, TR = 2.5 s, TE = 26 ms, voxel size 1.5×1.5×1.5 mm^3, no gap). We placed the slices over the insular cortex including anteriorly the superior frontal cortex, posteriorly the superior parietal lobe and inferiorly the occipital lobe. Functional MRI data was analyzed using SPM12 (Wellcome Department of Cognitive Neurology, Institute of Neurology, UCL,

London, UK). For each subject, functional images were corrected for head movements by spatially realigning all volumes to their mean. For precise coregistration, a single whole-brain EPI volume with 81 slices was acquired. T1-weighted anatomical image was collected using MP2RAGE (Marques et al., 2010) sequence (TR = 5.5 s; TE = 2.8 ms; TI1 = 750 ms, TI2 = 2350 ms). The anatomical images from all participants were segmented into GM, WM, CSF using the DARTEL-template (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra) procedure (Ashburner, 2007). The realigned and coregistered EPI volumes were subsequently normalized to MNI space using the DARTEL-generated flow fields (voxel size 1.5 mm) and spatially smoothed (5 mm FWHM).

fMRI data analysis

Statistical analysis at a single subject level was implemented using a first-level fixed effects analysis. The effects of the cardio-visual FBI and HAT were estimated on a voxel-by-voxel basis using the General Linear Model (Friston et al., 1994). Each experimental condition was modeled using the canonical hemodynamic response function (HRF) and its time derivative as the basis function. In the cardio-visual FBI, for each functional run, the model included ten regressors (the HRF for SB, AB, SO, AO, MBD and their temporal derivatives) plus the six motion parameters as nuisance regressors. In the HAT, the model included 20 regressors (one HRF for the eight experimental conditions, one for the button press and their temporal derivatives), plus six motion parameters as nuisance regressors. In both models, the cut-off value of the high-pass filter was set to 500 s. We applied a common choice to change the default value in SPM12 to 3x the period at which the task-related activations were expected to repeat (Ashby, 2011). A second-level flexible factorial analysis was performed to reveal significant regional effects for cardio-visual FBI and HAT separately. Specifically, in the cardio-visual FBI design matrix, we submitted the four contrasts images (e.g. SB > baseline, AB > baseline, SO > baseline, AO > baseline) to a two-way repeated measures ANOVA with two within-subject factors (i.e. stimulation (S, A) and visual stimulus (B, O)). To account for the within-subject correlations between each measurement level and thus handle non-

sphericity correctly, we also included the factor "participants" in the model. To control for multiple testing a family-wise error (FWE) correction at the voxel level was applied to the contrasts of interests (e.g. the main effect of stimulation, the main effect of visual stimulus and the stimulation X visual stimulus interaction). Similarly, in the HAT design matrix, we submitted the eight contrasts images (e.g. Heart - Sync - Color Change > baseline) to a three-way repeated measures ANOVA with three within-subject factors (i.e. attention, stimulation, and color). The design matrix included the factor "participants" and FWE correction was applied to control for multiple testing. For completeness, when no activation survived to FWE correction, a topological FDR on the cluster level was used at q = .05 corrected, based on an initial feature-defining voxel threshold set to p = .001 uncorrected.

Region of interest analysis (ROI)

The HAT aimed to map the areas of the brain which respond to heartbeat awareness (Critchley et al., 2004). To examine whether the same brain regions activated during heartbeat awareness were sensitive to cardio-visual FBI stimulations, we used the differential activity Heart > Color to define functional *a priori* regions of interests for ROI analysis. From those *a priori* brain regions, we extracted the average parameter estimates (beta values) during cardio-visual manipulation and examined their responses using a two-way repeated measures ANOVA with stimulation (S, A) and visual stimulus (B, O) as within-subject factors. Bonferroni correction was used (i.e. 0.05/nr of ROIs) to control for multiple testing.

Results

Heart awareness task: accuracy

Performance in the interoceptive task (mean percentage of correct response= 59%; s.e.m.=6%) was smaller than in the exteroceptive task (94%; s.e.m.=3%). Paired t-test showed that, overall, participants were more successful on the COLOR relative to HEART trials ($t_{(15,1)}$=-5.58, p<p=5.23e-05).

Questionnaire data: subjective changes during FBI-CV stimulation

The analysis of questionnaire responses revealed that cardio-visual synchrony during FBI-CV stimulations changed BSC. As expected, the ANOVA revealed a significant main effect of visual stimulus for Q1 (self-identification, $F_{(1,15)=}6.54$, p=0.02, Table 2-a) with participants reporting a higher self-identification when viewing a Body than a No-body stimulus (see Fig. 3). The two-way interaction visual stimulus X stimulation was not significant (p=0.08, Table 2-a), although subjects' responses suggest that participants self-identified more strongly with the virtual body when the stimulation was synchronized with their heartbeat as compared to asynchronous condition ($t_{(1,15)}$=2.39, p=0.03). No stimulation-dependent modulation was present for the object condition ($t_{(1,15)}$=1.11, p=0.28). These changes in self-identification were associated with changes in subjective bilocation and body reduplication. Indeed, for question Q5 (bilocation) a significant main effect of visual stimulus was found ($F_{(1,15)}$ = 5.91; p=0.02, Table 2-a), with participants reporting a higher sense of bilocation when viewing a body than when viewing the object stimulus (Fig. 3). A significant effect of visual stimulus was also found for Q6 (body reduplication, ($F_{(1,15)}$=4.78, p=0.04, Table 2-a), again with higher ratings for the body than for the object stimulus. For Q6, the stimulation X visual stimulation interaction was significant ($F_{(1,15)}$=4.78, p=0.04), showing a higher difference in ratings between synchronous and asynchronous for the Body condition, but not for the No-body object condition. Finally, we also found subjective changes in questions related to heart perception. A stimulation X visual stimulus interaction was found on question Q8 (heart reduplication, $F_{(1,15)}$=10.34, p=0.005, Table 2-a), with a positive difference between ratings in the Body synchronous and Body asynchronous conditions and a negative difference for the two No-body object conditions. For Q7 (heart mislocalization), a main effect of stimulation was found ($F_{(1,15)}$=7.75, p=0.01, Table 2-a) with higher ratings when the outlined contour flashed synchronously (Fig. 3) with their heartbeat. No significant effects were found for other items (see Table 2-a). The results revealed that the manipulation of the spatiotemporal

congruence between interoceptive and exteroceptive bodily signals induce subjective changes in BSC, specifically body and heart reduplication, similarly as those reported in a patient with a lesion in the insula.

Implicit self-location in space task during FBI-CV

The ANOVA on Mental Ball Drop response times revealed no significant main effect of visual stimulus ($F_{(1,14)}$=0.08, p=0.77) (Table 2-b) or stimulation ($F_{(1,14)}$=0.11, p=0.74) (Table 2-b) and no significant stimulation X visual stimulus interaction ($F_{(1,14)}$=0.41, p=0.52) (Table 2-b).

Brain activation during HAT

Firstly, we mapped the brain regions involved in heartbeat awareness. At the group level, the comparison between heart and control condition (T-contrast, Heart>Color, FWE=.05) revealed a significant difference in activity in the following six brain regions: including the bilateral Rolandic operculum, the bilateral supramarginal gyrus, the right frontal inferior operculum, and the left temporal superior gyrus. Activation of these brain areas was stronger for the heart as compared to the control condition (Fig. 4, see Table 3-a). Conversely, the perceptual control task (T-contrast, Color>Heart, FWE=.05) activated mostly visual brain areas, such as the bilateral occipital middle gyrus, the left calcarine fissure and the right fusiform gyrus (Fig. 4, see Table 3-b).

Region of interest analysis

Once we identified the six interoceptive awareness regions, we explored whether activity in these areas was differentially modulated by the four FBI-CV experimental conditions. To this end, for each of the six ROIs, a two-way repeated measures ANOVA was run with visual stimulus and stimulation during the cardio-visual FBI as within factors. Below we describe the results for each ROI separately.

In the right Rolandic operculum, a significant interaction was observed ($F_{(1,15)}$=31.89, p=4.64e-04) (Fig. 5-a, Table 4-a). A paired t-test (Bonferroni corrected) revealed a statistical difference ($t_{(1,15)}$=-4.46, p=4.52e-04) (Table 4-b) with higher beta values in the AB condition (Table 4-a) than the SB condition (Table 4-a). In the left Rolandic operculum, we also observed a significant interaction ($F_{(1,15)}$=24.21, p=1.84e-04) (Fig. 5-b, Table 4-a). A paired t-test (Bonferroni corrected) revealed a statistical difference ($t_{(1,15)}$=4.23, p=7.21e-04) (Table 4-b) with higher beta values in the in the SO condition (Table 4-a) than the AO condition (Table 4-a).

No other main effect or interaction in the other four ROIs survived Bonferroni correction (Table 4-b). In agreement with previous interoceptive investigations (Critchley et al., 2004, Salomon et al., 2016), our data suggests that the bilateral operculum is modulated during a classical interoceptive awareness task (HAT) and that it processes signals that are of relevance for BSC manipulated by cardio-visual stimulation.

Brain activation during FBI-CV stimulations

Finally, we analyzed changes in brain activity during cardio-visual stimulations at the whole brain level. The two-way repeated measures ANOVA at a group-level analysis indicated a significant positive interaction in the anterior cingulate cortex surviving to a topological FDR (q=.05 topological FDR based on an initial feature-defining voxel threshold set to p = .001 uncorrected, Fig. 6-c, Table 5-c). A significant main effect of stimulation was found in the middle cingulate cortex and the right thalamus (T contrast, FWE=.05, Fig. 6-a, Table 5-a) with higher activation in conditions of synchronous stimulation as compared to asynchronous stimulation for the middle cingulate cortex and the opposite modulation for the right thalamic activation. Additionally, three visual brain regions in striate and extrastriate cortex showed a significant main effect of visual stimulus (T contrast, FWE=.05, Fig. 6-b, Table 5-b), with a higher response to the body stimulus as compared to the No-body object stimulus (in the right middle temporal gyrus and left inferior occipital gyrus; the opposite effect was found in the right middle occipital gyrus).

Discussion

In the present study, we applied a cardio-visual stimulation paradigm to healthy participants to reveal the brain mechanisms that are involved in the integration of interoceptive-exteroceptive bodily signals that are relevant for BSC (i.e. the brain regions involved in processing cardio-visual information). In particular, we tested how and whether they overlap with the brain mechanisms of interoceptive (cardiac) awareness. Viewing a virtual body being illuminated in synchrony with one's heartbeat, induced changes in BSC, characterized by bilocation and body reduplication and stronger self-identification with the virtual body. These altered states of BSC were absent in the control conditions (i.e. asynchronous stimulation and viewing a non-bodily stimulus). Although the present behavioral data does not fully replicate previous behavioral BSC findings, we note that the changes in BSC induced during fMRI acquisitions were comparable (yet weaker and less selective) with respect to those reported in previous behavioral investigations using virtual reality and cardio-visual stimulation and carried out in the standing position using ECG recordings (Aspell et al., 2013, Suzuki et al., 2013, Ronchi et al., 2015). Importantly, our fMRI results reveal that the bilateral Rolandic operculum is activated not only during the experimental condition inducing these changes in BSC but also in a task testing cardiac awareness, showing the involvement of this structure in both tasks: mental states that have been associated with self-consciousness and mental states associated with interoceptive awareness. In the following, we discuss the present findings for cardiac awareness, for BSC, as well as their common and distinct brain mechanisms.

We applied a modified version of a well-established heartbeat awareness task and compared brain activity induced by the monitoring of visual stimuli in relationship to heartbeat signals with brain activity associated with the processing of the same visual stimuli, but unrelated to cardiac signals (Critchley et al., 2004, Pollatos et al., 2007). In agreement with previous studies on interoceptive processing and awareness, we identified six brain regions - namely the right and left Rolandic operculum, the left and right

supramarginal gyrus, the left superior temporal gyrus and the right frontal inferior operculum - where neural activity increased in the heartbeat perception task as compared to the control task (Brooks et al., 2002, Critchley et al., 2004, Pollatos et al., 2007, Craig, 2009, Zaki et al., 2012). We note that these previous studies used cardio-auditory matching tasks (Critchley et al., 2004, Zaki et al., 2012) or heartbeat couting paradigms (Pollatos et al., 2007) to investigate heartbeat awareness (for review see (Craig, 2010, Barrett and Simmons, 2015). Our protocol was based on a visual interoceptive task (and a visual control task without an interoceptive component). This visual mode of stimulation was chosen because the paradigm used to manipulate BSC also used visual stimulation. Despite these sensory differences, our protocol still activated the networks described in earlier works (Craig et al., 2000, Brooks et al., 2002, Critchley et al., 2004, Herbert et al., 2007, Pollatos et al., 2007, Zaki et al., 2012, Salomon et al., 2016). We note that we chose to perform the Heart Awareness Task *after* the cardio-visual FBI paradigm to rule out the possibility that participants would guess the experimental manipulations by being exposed to an explicit heartbeat task. That our participants were naïve with respect to the heartbeat-based experimental manipulation is supported by the fact that their performance in the heartbeat detection task was very low and in their measured subjective heartbeat awareness (Q3: *It seemed as if the flashing was my heartbeat*) we observed no significant differences across conditions. The data shows that they were not able to infer a clear link between their heartbeat and the illumination pattern on the screen.

Prominent evidence affirms that the brain's representations of internal bodily states (i.e. interoceptive processing) are relevant signals for the self and related aspects of consciousness (Damasio, 2000, Craig, 2002). Moreover, recent patient work has demonstrated that exteroceptive and interoceptive signals can be integrated for visceral perception (Khalsa et al., 2009). Another line of work has shown that the interaction of exteroceptive and interoceptive signals affects bodily aspects of self-consciousness, such as hand ownership (Suzuki et al., 2013), self-identification, and self-location (Aspell et al., 2009, Adler et al., 2014, Ronchi et al., 2015). Based on these findings, it has been argued

that such cardiac or respiration-related interoceptive effects on BSC are based on the convergence of visceral and somatosensory signals, processed in the spinal cord, brain stem, thalamus (Takahashi and Yokota, 1983, Foreman et al., 1984), and cortical visual signals, especially in the insula (Aspell et al., 2009, Craig, 2009). The present investigation extends these changes in BSC, as induced by sensory conflicts between exteroceptive and interoceptive stimulation, to fMRI. We presented cardio-visual illumination of the virtual body so that a flashing silhouette was either temporally synchronous or asynchronous with respect to the participant's heartbeats. Our findings show that in the constraining and supine body position during fMRI, it is possible to induce changes in BSC similarly to earlier subjective, behavioral and neuropsychological data on BSC using cardio-visual stimulations (Aspell et al., 2009, Suzuki et al., 2013, Ronchi et al., 2015). We found greater self-identification with the virtual body as compared to the other condition, as well as sensations of bilocation and body reduplication. Importantly, the data shows that bilateral Rolandic operculum contiguous to the insula is not only involved in the processing and awareness of interoceptive signals, but also in BSC-related processes based on cardio-visual stimulation. In particular, we found that the right operculum was significantly active when stimulation was synchronized with participants' heartbeat and only when cardio-visual stimulation was projected on a human body, reflecting subjective responses for illusory self-identification and body reduplication. This data also confirms the role of the Rolandic operculum and neighboring regions such as the insula in the processing of interoceptive signals that are of relevance for other manipulations of consciousness, such as visual consciousness (Salomon et al., 2016). More work is necessary to investigate how the Rolandic operculum and/or insula mediates these effects on BSC and visual consciousness and how specific such processing is for cardiac as opposed to other interoceptive signals activating these regions such as respiratory signals (von Leupoldt et al., 2009, Adler et al., 2014). Moreover, the present data points to potential differences in the right and left hemispheres, requiring further study.

We note that these opercular regions largely overlap with those found during focused attention towards one's heartbeat and that have been referred to as insula/operculum region by the prominent work of (Critchley et al., 2004). We know that the more stringent statistical threshold (i.e. FWE=0.05) used here is responsible for not revealing activations in the insula cortex as previously reported using FDR correction (Critchley et al., 2004). In addition, meta-analytic co-activation data (entering the MNI coordinates of the present significant opercular clusters obtained from the ROI analysis into a large-scale fMRI database (www.neurosynth.org) (Yarkoni et al., 2011)), revealed more than 40 studies reporting task-related co-activations between the frontal operculum and the nearby insula within a radius of 10 mm. Accordingly, we suggest that the present activations may constitute a bilateral opercular-insula network involved in interoceptive awareness and cardio-visual mediated changes in BSC.

The role of the operculum in integrating cardio-visual signals relevant for BSC is in line with previous reports in a neurological patient suffering from a right insular neoplastic damage (Ronchi et al., 2015). In this patient, the same aspects of BSC (self-identification, bilocation, body reduplication) were altered by the same pattern of cardio-visual stimulation implemented in the present study. Indeed, in this case-study, cardio-visual stimulation not only modulated BSC but also pathologically enhanced the level of BSC. Further evidence for the involvement of the opercular-insular cortex in BSC comes from patients suffering from heautoscopy. These patients (Brugger, 2002, Blanke et al., 2004, Heydrich and Blanke, 2013) report seeing a double of their body and experience strong bilocation and body reduplication, leading to serious troubles in perceiving the self as localized within the physical body or at a far distant position (i.e. within the illusory body) (Devinsky et al., 1989, Brugger, 2002, Blanke et al., 2004, Blanke and Mohr, 2005), even when the illusory body has a different appearance than the patient's body (Brugger, 2002, Blanke and Mohr, 2005). Lesion mapping linked heautoscopy to damage of the insular cortex, with a maximal overlap in the posterior insula (Heydrich and Blanke, 2013) and thus in proximity, but somewhat

more posterior compared to the present data in healthy subjects. Of relevance for interoceptive awareness and the role of cardio-visual signals in BSC is the finding that such patients often suffer from abnormal visceral (cardiac, respiratory, gastrointestinal) sensations. In the present study, we showed that exposing healthy subjects to cardio-visual inputs with spatial and temporal conflicts (between the seen and physical body) induced a mental state characterized by bilocation and body reduplication, thus resembling heautoscopy in neurological patients. Such illusory effects of owning more than one body are not usually observed during the classic FBI based on visuo-tactile stimulation (unless participants are visually presented with two virtual bodies) (Heydrich and Blanke, 2013). We argue that the manipulation of interoceptive signals induces different patterns of BSC alterations including bilocation and body reduplication that are absent in paradigms using purely exteroceptive signal manipulations. Future research will have to address this hypothesis more systematically.

Other clinical evidence, supporting an association between insular cortex and BSC, comes from patients reporting a delusional misattribution of a body part and abnormal self-identification (somatoparaphrenia). These patients often suffer right brain damage, centering on the insula, although in more posterior and medial insular regions as compared to the present results (Levine et al., 1991, Cereda et al., 2002, Vallar and Ronchi, 2009, Karnath and Baier, 2010). Neuroimaging results in healthy subjects have also involved the insula in the sense of limb ownership that is related to self-identification. Tsakiris et al. found that activity in the right insula and the right operculum correlated with the strength of illusory ownership over fake hand induced by visuo-tactile stimulation (as measured using proprioceptive drift of the perceived location of the participants' hand towards the rubber hand) (Tsakiris et al., 2007). Finally, Ionta et al. have shown that the insula is part of a cortical network of multisensory regions processing BSC, including the temporoparietal cortex, the supplementary motor area and the ventral premotor cortex (Ionta et al., 2014).

Therefore, clinical evidence and neuroimaging findings, both suggest a prominent involvement of the opercular-insular cortex in neurologically or experimentally driven alterations of BSC.

Recent theoretical accounts posit a substantial role for interoceptive processing for self-awareness and consciousness (Craig, 2002, Damasio, 2003, Seth, 2013). It has been proposed, in particular, that the anterior insular is of key importance in processing and integrating multiple inputs from within the body and that such integration plays a pivotal role in self and consciousness (Craig, 2002, Craig, 2011). Recently, it was shown that neural responses to interoceptive signals (i.e. heartbeat) can predict the conscious detection of a visual grating (Park et al., 2014), suggesting a prominent role of brain mechanisms related to the processing of afferent cardiac information for subjective conscious experience, including visual awareness and self-consciousness (Park et al., 2014, Park and Tallon-Baudry, 2014, Park et al., 2016, Salomon et al., 2016). The present data extends this proposal by showing that not only awareness for interoceptive signals (the heartbeat) but also changes in self-consciousness (experimentally induced by interoceptive-exteroceptive, cardio-visual, stimulation) co-activate bilateral Rolandic operculum and the contiguous insula, underlining its pivotal role in self-consciousness and awareness.

Previous work reported lateralization differences in the involvement of the insular cortices in BSC as well as self-awareness, highlighting either right or left insula contributions. Here, we found a body and stimulation specific effect and a stimulation, but not body-specific effect in the right Rolandic operculum, and a body and stimulation specific effect in the left Rolandic operculum. In heautoscopy patients the lesion was mainly located in the left insula (Heydrich and Blanke, 2013) whereas the right insula was involved in the case of experimentally induced changes in hand ownership during the visuo-tactile rubber hand illusion (Tsakiris et al., 2007) and a disorder of BSC related to the contralesional limb as in somatoparaphrenia (Vallar and Ronchi, 2009, Karnath and Baier, 2010). Finally, Ronchi et al. associated

experimentally induced changes in BSC, including bilocation and body reduplication, with a lesion of the right insula (Ronchi et al., 2015). Moreover, bilateral operculum and adjacent insula have been involved in interoception as well as interoceptive awareness. The present findings and the previous work on BSC, however, suggest that although each operculum-insula cortex processes multisensory bodily cues relevant for BSC, the contributions of left versus right insula for BSC may differ.

Finally, when using cardio-visual stimulation in the present study, we did not find a strong modulation of activity in regions of the temporo-parietal junction (the angular gyrus, the posterior superior temporal gyrus and the supramarginal gyrus) a region previously involved in changes in BSC as induced by the FBI (Ionta et al., 2011, Ionta et al., 2014, Guterstam et al., 2015, Ronchi et al., 2015). In the present study, we found a trend (i.e. cluster did not survive Bonferroni correction) of body and stimulation specific effect for cardio-visual stimulation in the left supramarginal gyrus, overlapping with those described by (Ionta et al., 2011). Thus, it seems that cardio-visual stimulation also partially taps into regions, previously linked to BSC changes induced by visuo-tactile stimulation. More work is necessary to understand the differences that may exist between the involvement of right and left opercular-insular cortex in interoception, interoceptive awareness, and BSC and also on the different brain structures recruited during cardio-visual stimulation versus visuo-tactile stimulation.

We used the MBD task to investigate implicit changes in self-location, as performed in several previous FBI studies using visuo-tactile stimulations (Ionta et al., 2011, Pfeiffer et al., 2013). We argue that in the present study we may not have found the predicted stimulation X visual stimulus interaction with a significant drift in self-location towards the virtual body (in the synchronous body condition) for the following reason. As in two previous studies (Heydrich and Blanke, 2013, Ronchi et al., 2015), we found that cardio-visual stimulation was associated with the feelings of body reduplication and of bilocation (Q5 and Q6). The

subjective experience of being located at two distinct places may not be reflected by a forward drift in self-location at all as the drift may require a subjective experience (illusory or not) to be at a single unitary location.

Author contributions

O.B. and R.M. designed the study. M.L.B., J.B.-R., and B.H. implemented the experimental paradigm. M.L.B. performed all the measurements inside and outside the MRI. R.M. supervised the fMRI sessions. M.L.B. analyzed the behavioral and fMRI data and wrote the paper. R.S., A.S., O.B. critiqued the interpretation of results and revised the manuscript. All the authors proofread the final text of the paper for submission to EJN.

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Data Availability

The behavioral data, the relevant fMRI contrasts and beta values can be freely accessed at the following link.

Abbreviations

BA = Asynchronous Body

BPM = Beats Per Minute

BS = Synchronous Body

BSC = Bodily Self-Consciousness

CV = Cardio-Visual

ECG = Electrocardiography

FBI = Full Body Illusion

HAT = Hear awareness task

MBD = Mental Ball Drop

OS = Synchronous Object

OA = Asynchronous Object

PPG = Photoplethysmography

ROI = Region of Interest

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Figure legends:

Figure 1 - Cardio-visual stimuli: The temporal delay between the PPG peak and the R-wave was estimated on *Day 1* (right). Every time a PPG peak was detected, a marker (gray vertical line) was sent through a parallel port to the Biosemi Active II system and recorded together with the ECG tracks. Post-processing analysis was performed to calculate the pulse transit time (x = time distance between ECG-R wave to the PPG peak). During the fMRI session on *Day 2* (right), the synchronous visualization was provided by displaying the white outlined contour at Peak'(t) of the PPG signal dt-corrected (dt = Peak(t) – Period + x; Period = difference between two PPG peaks). Such synchrony ensured that the outlined contour matched the R peak of the QRS complex of the next heartbeat. The visual flash lasted 500 ms and followed the positive section of a sinusoidal function (i.e. progressive illumination from total transparency (0%) to full opacity (100%) and back to transparent (0%)).

Figure 2 - 2x2 factorial cardio-visual stimulation design: Each FBI-CV run consisted of 16 trials randomly ordered and reflecting the 2x2 factorial design with stimulation (Synchronous (S) and Asynchronous (A)) and visual stimulus (Body (B) and No-body (O)) as the main factors. In the synchronous Body/No-body condition, the white outlined contour was displayed at the peak of the PPG signal corrected for the mean pulse transit time (i.e. the average lag between the peak of the ECG-R wave and the marker of the peak of the PPG signal). In the asynchronous Body/No-body condition the algorithm introduced a random delay between the PPG signal corrected for the mean pulse transit time and the predicted ECG; thus the white outlined contour was displayed at a different time than the predicted ECG.

Figure 3 - Subjective questionnaire ratings: The boxes show the distribution of questions score in the four experimental conditions (e.g. Synchrony Body (SB), Asynchrony Body (AS), Synchrony Object (SO) and Asynchrony Object (SO) where a significant modulation depending on the pattern of CV-stimulation was found (see Table 2 for the mean scores and standard errors of all the questions and the results of the two-way repeated measures ANOVA). The boxes are based on the minimum value, first quartile (25% of the data fall below it), mean (green dot), third quartile (75% of the data fall below it) and maximum value.

Figure 4 - Group-level whole-brain analysis during Heart Awareness Task: Activity reflecting the heartbeat awareness (red) (Heart>Color) and the control task (blue) (Color>Heart), T-contrast, FWE=0.05. Cluster labeling was implemented using the Automated Anatomic Labeling (AAL) in MRIcro.

Figure 5 - ROI analysis: Six brain regions showing increased activity in the heart condition as compared to the control condition were used to define *a priori* functional areas of interest (i.e. bilateral Rolandic operculum, the bilateral supramarginal gyrus, the right frontal inferior operculum, and the left temporal superior gyrus). Statistical difference Bonferroni corrected in the mean beta values were measured only in two regions, the right and the left Rolandic operculum (see Table 4-b). The boxes reflect differentially activation of the bilateral Rolandic operculum, including the minimum value, first quartile (25% of the data is below it), mean (green dot), third quartile (75% of the data is above it) and maximum value.

Figure 6 - Group-level whole-brain analysis during cardio-visual stimulation: Activity reflecting a main effect of stimulation (a) and main effect of visual stimulus (b), T contrast, FWE=0.05. Activity reflecting the interaction did not survive FWE correction. To control for multiple testing, a topological FDR on the cluster level was used at q = .05 corrected, based on an initial feature-defining voxel threshold set to p = .001 uncorrected (c) (see Table 4 for the results of the two-way repeated measures ANOVA). Cluster labeling was implemented with the Automated Anatomic Labeling (AAL) in MRIcro.

Tables:

Table 1: Bodily self-consciousness questionnaire

Q1: I felt as if the virtual body/object was my body
Q2: It felt as if my real body was drifting towards the virtual body
Q3: It seemed as if the flashing was my heartbeat
Q4: I felt pressure on the sensor put on my finger
Q5: It seemed as if I was in two places at the same time
Q6: It seemed as if I might have more than one body
Q7: It felt as if my heart was in the virtual body/object
Q8: It seemed as if I had two hearts
Q9: It seemed as if I was feeling my heartbeat where I saw the flashing

Bodily self-consciousness questionnaire: Changes in bodily self-consciousness were measuring using subjective ratings to a questionnaire including nine items assessing self-identification with the virtual body (Q1), self-location (Q2, Q5, Q6), heart awareness (Q3, Q9) and heart perception (Q7, Q8).

Table 2: Questionnaire ratings and Mental Ball Drop

a)

Questions	*SB*	*AB*	*SO*	*AO*	*Stimulation*	*Visual Stimulus*	*Interaction*
	mean ± stderr					* p<0.05	
Q1	4.00 ± 0.58	3.06 ± 0.56	2.06 ± 0.37	2.37 ± 0.43	0.064	* 0.021	0.081
Q2	2.87 ± 0.52	2.50 ± 0.45	1.93 ± 0.35	2.31 ± 0.48	0.824	0.165	0.396
Q3	5.00 ± 0.53	4.93 ± 0.50	5.06 ± 0.46	4.43 ± 0.50	0.584	0.782	0.589
Q4	3.37 ± 0.60	3.87 ± 0.59	4.12 ± 0.61	4.25 ± 0.58	0.255	0.053	0.666
Q5	2.87 ± 0.53	2.25 ± 0.45	1.75 ±0.39	1.81 ± 0.30	0.386	* 0.028	0.272
Q6	2.75 ± 0.54	2.06 ± 0.43	1.50 ± 0.23	2.00 ± 0.42	0.681	* 0.038	* 0.044
Q7	3.81 ± 0.55	2.31 ± 0.46	3.43± 0.55	3.00 ± 0.55	* 0.013	0.602	0.245
Q8	2.37 ± 0.46	1.50 ± 0.23	1.68 ± 0.33	2.68 ± 0.53	0.807	0.604	* 0.005
Q9	4.43 ± 0.57	4.62 ± 0.56	4.68 ± 0.55	4.68 ± 0.54	0.685	0.542	0.703

b)

MBD	*SB*	*AB*	*SO*	*AO*	*Stimulation*	*Visual Stimulus*	*Interaction*
	mean ± stderr (ms)				* p<0.05		
	1205.7 ± 155.3	1197.7 ± 153.4	1196.7 ± 142.1	1223.2 ± 162.2	0.74	0.77	0.52

Questionnaire ratings and Mental Ball Drop: Each questionnaire item was rated on a 7-point Likert scale, where 1 indicated complete disagreement and 7 indicated complete agreement. The tables show (a) the means and standard errors of nine questions in all of the four experimental conditions and (b) the response time in the mental ball drop task. A two-way ANOVA with stimulation (Synchronous(S) and Asynchronous (A)) and visual stimulus (Body (B) and No-body (O)) as within factors revealed main effects and interaction for some of the nine questions ($p<0.05$) and longer response time in the Body as compared to the No-body condition ($p=0.04$).

Table 3: Brain activity during Heart Awareness Task

a)	*Heart>Color*	*Side*	*MNI coordinates X Y Z*			*Cluster size*	*T value*
	Rolandic Operculum	L	-57	6	8	310	6.05
	Supramarginal Gyrus	R	52	-27	28	89	5.9
	Supramarginal Gyrus	L	-51	-39	30	72	5.82
	Rolandic Operculum	R	60	3	14	43	5.56
	Superior Temporal Gyrus	L	-48	-44	14	9	5.41
	Frontal Inferior Operculum	R	56	14	8	8	5.12

b)	*Color>Heart*	*Side*	*MNI coordinates X Y Z*			*Cluster size*	*T value*
	Middle Occipital Gyrus	R	27	-70	26	186	6
	Inferior Occipital Gyrus	L	-39	-69	-6	21	5.82
	Superior Parietal Lobule	R	26	-58	50	15	5.55
	Middle Occipital Gyrus	L	-32	-80	15	48	5.5
	Calcarine Fissure	L	3	-86	3	26	5.27
	Superior Parietal Lobule	L	-21	-56	48	4	5.24
	Cuneus	L	-6	-86	24	10	5.2
	Fusiform	R	28	-70	-4	2	5.09
	Inferior Parietal Lobule	R	26	-51	50	2	5.02

Brain activity during Heart Awareness Task: (a) Enhanced activity during heart awareness task (Heart>Color, T contrast, FWE=0.05) and (b) decreased activity during the perceptual control task (Color>Heart, T contrast, FWE=0.05). Local maxima labeling was implemented using the Automated Anatomic Labeling (AAL) in MRIcro.

Table 4: ROI Analysis

a)

Two-way ANOVA	*SB*	*AB*	*SO*	*AO*	*Stimulation*	*Visual Stimulus*	*Interaction*
	Mean Beta Values				*p value<0.05		
Left Rolandic Operculum	-0.32	0.57	0.49	-0.69	0.51	0.49	0.0002*
Right Supramarginal Gyrus	0.59	0.86	0.90	0.04	0.49	0.45	0.2318
Left Supramarginal Gyrus	-0.36	0.78	0.62	-0.26	0.58	0.91	0.0087*
Right Rolandic Operculum	-0.29	1.13	0.44	0.19	0.05	0.72	0.0000*
Left Superior Temporal Gyrus	-0.21	0.65	0.84	-0.60	0.49	0.78	0.0021*
Right Frontal Inferior Operculum	0.31	-0.64	-0.34	-0.87	0.06	0.25	0.5429

b)

Post-hoc Paired T-tests	*SB-AB*	*SO-AO*
	*** Bonferroni corrected (p<0.05/nr of ROIs)*	
Left Rolandic Operculum	$t_{(1,15)}$=-2.73 p=0.01	$t_{(1,15)}$=4.23 p=7.21e-04**
Left Supramarginal Gyrus	$t_{(1,15)}$=-2.41 p=0.02	$t_{(1,15)}$=2.77 p=0.01
Right Rolandic Operculum	$t_{(1,15)}$=-4.46 p=4.54e-04**	$t_{(1,15)}$=0.80 p=0.43
Left Superior Temporal Gyrus	$t_{(1,15)}$=-1.47 p=0.15	$t_{(1,15)}$=3.18 p=0.006

ROI Analysis: (a) A two-way ANOVA revealed four regions as being differently modulated by the FBI-CV experimental conditions. (b) Only two brain areas, namely the right and the left Rolandic operculum survived Bonferroni correction (p<0.05/6) (b).

Table 5: Brain activity during cardio-visual stimulation

a)

Stimulation	*Side*	*MNI coordinates X Y Z*			*Cluster size*	*T value*	*Mean Beta Values*			
							SB	*AB*	*SO*	*AO*
Middle Cingulate Cortex	L	-3	-21	40	4	5.68	0.17	0.30	0.47	-0.13
Thalamus	R	18	-28	-2	2	5.62	0.14	0.22	0.08	0.12

b)

Visual Stimulus	*Side*	*MNI coordinates X Y Z*			*Cluster size*	*T value*	*Mean Beta Values*			
							SB	*AB*	*SO*	*AO*
Middle Temporal Gyrus	R	51	-72	6	1184	11.74	2.55	2.88	2.19	2.71
Inferior Occipital Gyrus	L	-39	-81	-3	1204	11.29	2.34	1.78	1.66	1.19
Middle Occipital Gyrus	R	24	-88	10	63	6.57	3.04	2.98	3.72	4.09

c)

Interaction	*Side*	*MNI coordinates X Y Z*			*Cluster size*	*T value*	*Mean Beta Values*			
							SB	*AB*	*SO*	*AO*
Anterior cingulate cortex	R	15	48	20	299	3.9	0.38	-0.06	0.08	0.14

Brain activity during cardio-visual stimulation: The tables show the brain regions revealing a significant (a) main effect of stimulation (T contrast, FWE=0.05), (b) main effect of visual stimulus (T contrast, FWE=0.05) and (c) interaction (T contrast, $q<0.05$ corrected at the cluster level using topological FDR). Local maxima labeling was implemented using the Automated Anatomic Labeling (AAL) in MRIcro.