Fear processing is differentially affected by lateralized stimulation of carotid baroreceptors

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Abbreviations:

fMRI- Functional Magnetic Resonance Imaging

HRV- Heart Rate Variability

CS- Carotid Stimulation

RMSSD- Square root of the mean of the squares of differences between adjacent N-N intervals

VAS- Visual Analogue Scale

ITI- Inter-Trial Interval

BOLD- Blood Oxygenation Level Dependent

ROI- Region of Interest

FWE- Family Wise Error

ABSTRACT

Information processing, particularly of salient emotional stimuli, is influenced by cardiovascular afferent signals. Carotid baroreceptors signal the state of cardiovascular arousal to the brain, controlling blood pressure and heart rate via the baroreflex. Animal studies suggest a lateralization of this effect: Experimental stimulation of the right carotid sinus has a greater impact on heart rate when compared to left-sided stimulation. We tested, in humans, whether the processing of emotional information from faces was differentially affected by right versus left carotid afferents. To achieve so, we used an automated neck suction device to stimulate the carotid mechanoreceptors in the carotid sinus (parasympathetic pathway) synchronously with functional magnetic resonance imaging (fMRI) acquisition whilst participants were engaged in an emotional rating task of fearful and neutral faces. We showed that both right and left carotid stimulation influenced brain activity within opercular regions, although a stronger activation was observed within left insula during right stimulation compared to left stimulation. As regards the processing of fearful faces, right, but not left carotid stimulation attenuated the perceived intensity of fear, and (albeit to a lesser extent) enhanced intensity ratings of neutral faces. Mirroring the behavioural effects, there was a significant expression-by-stimulation interaction for right carotid stimulation only, when bilateral amygdala responses were attenuated to fear faces and amplified to neutral faces. Individual differences in basal heart rate variability (HRV) predicted the extent to which right carotid stimulation attenuated amygdala responses during fear processing. Our study provides unique evidence for lateralized viscerosensory effects on brain systems supporting emotional processing.

Keywords: autonomic, baroreceptor, carotid stimulation, emotion, fear, visceral, neuroimaging

INTRODUCTION

Brain and body interact to support perceptions, thoughts and feelings (Critchley, Eccles and Garfinkel, 2013; Critchley et al., 2002; 2004). The processing of threat is coupled to physiological arousal via the autonomic nervous system, which facilitates evasive reactions, and enhances feelings of fear. Cardiovascular arousal is signaled to the brain by the activation of arterial baroreceptors, which respond to increased intraluminal pressure at systole. Vagus and glossopharyngeal nerves carry these signals to the brainstem where they trigger the baroreflex, lowering blood pressure by (parasympathetic) slowing of the next heartbeats and inhibiting (sympathetic) vasoconstriction within muscle vascular beds (reviewed Fadel et al., 2003). Emotional challenges inhibit the baroreflex, allowing heart rate and blood pressure to rise together (Gianaros et al., 2009). This decreases heart rate variability (HRV), an index of cardiac parasympathetic effects (Thayer and Lane, 2000; Thayer et al., 2009), and a proposed signature of wellbeing and capacity for emotional regulation (Thayer and Lane, 2000; Thayer and Brosschot, 2005).

Thus, phasic baroreceptor firing informs the brainstem about the strength and rate of heartbeats. This information is also relayed up the neuraxis to insular cortex (Critchley et al, 2004) and amygdala (Cechetto and Calaresu, 1984, 1985). Natural fluctuations in baroreceptor activity can modulate memory encoding (Garfinkel et al., 2013), pain processing (Edwards et al., 2008; Gray et al., 2009) and appraisal of emotional stimuli (e.g. Gray et al., 2012; Garfinkel et al., 2014). Artificial stimulation of baroreceptors can be achieved using external neck suction (Cooper and Hainsworth, 2009) which increases transmural pressure within the carotid sinus, and enhances parasympathetic cardiovascular drive. Carotid stimulation (CS) can be applied to study the influence of baroreceptor afferent activity on psychological processes and underlying brain function.

Recently, CS was shown to decrease the perceived intensity of fearful faces and enhances the intensity of neutral faces via changes in amygdala activation (Makovac et al., 2015a). These findings endorse the role of the amygdala in the integration of viscerosensory information with fear processing (Phelps and LeDoux, 2005; Garfinkel et al., 2014; Makovac et al., 2015a) and extend previous evidence showing CS modulates amygdala and insula activity at rest, when engaged in a cognitive task, and during emotional challenges (Basile et al., 2013a; 2013b).

Animal studies suggest that perturbation of the right carotid sinus induces greater effects on heart rate when compared to left-sided stimulation (Worthen et al., 1972; Greene et al., 1986). In humans, there is some evidence for the superiority of right over left CS (Tafil-Klawe, Raschke, and Hildebrandt, 1989; Furlan et al., 2003). Heart rate slowing is observed to be greater during right, compared to left CS (Tafil-Klawe et al., 1989; Furlan et al., 2003), although other studies find no difference between the two sides (Williamson and Raven, 1993). In hypertensive individuals, therapeutic use of *baroreflex activation therapy* suggests that right-sided baroreflex activation elicits a more profound long term effect on blood pressure than bilateral or left-sided (de Leeuw et al., 2015). Such lateralization is proposed to have a basis in asymmetric cardiac innervation and baroreceptor inputs to brain (Hagemann et al. 1975; Tafil-Klawe et al., 1989).

So far, there has been no assessment in humans of whether unilateral CS elicits differential psychological or neural responses. Motivated by observed bilateral CS effects on fear processing (Makovac et al., 2015a), we tested the hypothesis that unilateral right and left CS will have a different impact on cardiac activity, brain activations and behavioural responses during the appraisal of fearful and neutral faces. Specifically, we predict differential amygdala engagement, reflected in a greater impact of right CS on the subjective appraisal of fear. At

both neural and behavioural levels, we expect to observe a significant emotion-by-CS interaction with right CS only, where amygdala's activity will decrease during fearful appraisal and increase during neutral appraisal. In accordance with this, we predict a decrease in fearful ratings and increase in neutral ratings during right CS, mirroring the results with our previous study using bilateral CS (Makovac et al., 2015a). Overall, we sought to gain greater insight into forebrain asymmetry in emotion (Davidson and Fox, 1982; Grimshaw and Carmel, 2014) and its putative basis in peripheral asymmetry within the autonomic nervous system (Craig et al., 2005; Craig 2014).

MATERIALS AND METHODS

Participants

Twenty right-handed volunteers (11 females/ 9 males; mean age = 24.15 years; SD = 3.32; range, 20-31) with no neurologic, psychiatric disorders and other major clinical conditions, underwent detailed autonomic examination to characterize autonomic function and exclude autonomic dysfunction before participating in a neuroimaging study. The research was approved by the Santa Lucia Foundation ethical committee, with written informed consent was obtained from all participants.

Heart rate variability (HRV) evaluation

The ECG data were collected in two different occasions: 1) Pre-scanning (collected the day before the MRI scanning); these data were used to evaluate each participant's autonomic activity and to perform HRV analysis; 2) During the MRI scanning, to monitor the cardiac response to the CS.

The HRV analysis was performed on ECG data collected during an autonomic evaluation carried out prior to the experiment, to characterize the autonomic (parasympathetic)

(re)activity of each participant. HRV analysis of normal (R-R) interbeat intervals was used to index autonomic activity (Malik and Camm, 1995).

R-R interval was calculated as the temporal difference between two consecutive R-wave peaks on ECG. QRS complexes were detected using a derivative + threshold algorithm. Parabolic interpolation was used to refine the R-wave fiducial points (Malik et al., 1996). All the detected QRS were visually searched for artefacts, and, if any, manually corrected.

To evaluate possible autonomic dysfunctions in our participants, the Valsalva maneuver was performed consecutively three times, interleaved with a two minute rest, and was followed by ten minutes orthostatic (45^{o} tilt) and supine electrocardiography (ECG) recordings.

In order to investigate the association between the central neural response to CS and each individual autonomic activity, correlational analyses were carried out using the square root of the mean of the squares of differences between adjacent R-R intervals (RMSSD), obtained from the supine position. This is a reliable index of vagally-mediated HRV that reflects changes in the parasympathetic arm of the autonomic nervous system (Task Force, 1996), stable over short recording intervals (Nussinovitch et al., 2012).

Carotid stimulation delivery

Using a laboratory-built device for fMRI compatibility, neck suction was delivered through two individual cuffs within a neck collar, following a well-established procedure (see Basile et al., 2013b; Makovac et al, 2015a). The pressure was set by controlling, using a computer interface, the aspiration level of a vacuum source (placed in the MRI control room). The actual pressure within each cuff of the neck collar was continuously and independently monitored. Specific placement of neck suction cuffs was tailored for each participant, using carotid angiograms acquired earlier to localise the points of carotid artery bifurcation. CS was

delivered in pulses of variable duration, ranging from 7.2-8.2 s. In order to ensure the effective influence of this autonomic nervous system perturbation on emotional processing of faces, delivery of the CS was time-locked to the onset of the face stimulus and offset of the VAS (Figure 1B). CS periods were randomly presented on the right or left side. The unilateral stimulation consisted of an efficacious unilateral (left or right) stimulation (-60 mm Hg pressure; RIGHT-ON, LEFT-ON) and a simultaneous contralateral non-efficacious stimulation (-20 mm Hg pressure). The use of a contralateral non-efficacious stimulation (perceptively non-distinguishable from the efficacious one) allowed us to rule out the possibility of a unilateral attentional bias. The sham condition consisted of a bilateral non-efficacious simultaneous stimulation (-20 mm Hg pressure, BILATERAL-OFF). Carotid stimulation pressures for efficacious and non-efficacious CS were established from literature data and previous studies assessing the effect of CS on the average increase of the heart periods in normal subjects (Calcagnini et al., 2010), and has been already validated by our previously published studies (Basile et al., 2013b; Makovac et al., 2015a). Active pulses were always followed by an ITI of a variable duration, during which CS was not delivered and participants were not engaged in any active task. This reduced the likelihood of baroreceptor response accommodation. ECG, pulseoximetry, and respiration were recorded during fMRI (Biopac Systems Ins, CA). R-R interbeat intervals were extracted from the ECG. No motion artefacts were detected when the CS was applied using the two-cuff device. Physiological monitoring and CS delivery did not induce an increase in radio frequency noise.

Paradigms and procedure

During the functional magnetic resonance imaging (fMRI), datasets were acquired over two 20 min runs of an experimental task to avoid fatigue-related confounds. The experiment used a randomized event-related design: Within each run, 45 fearful and 45 neutral

faces, taken from the Ekman set [10 identities were selected from the dataset; each face was repeated 4 times on average; Ekman and Friesen, 1974], were randomly presented to give a total number of 90 fearful and 90 neutral faces over two runs. Within each run, 15 faces were presented under left CS, 15 under right CS and 15 under sham (non-efficacious) CS. Trials lasted on average 8.7 s each (range 9.2-10.2 s), followed by a variable inter-trial interval (ITI) (adopted to prevent a learning effect), lasting on average 4 s (range 3.05-4.95 s) in 80% of the trials, and 9 s (range 8.05-9.95 s) in 20% of the trials (see Figure 1).

For each trial, first, a fixation cross appeared for 1 sec, followed by brief presentation of a face stimulus (200 ms). A post-stimulus fixation cross was then presented for 500 ms, followed by a blank screen with a 'ready' message (3-4 sec). A visual analogue scale (VAS) was then presented for 3.5 s for the participant to rate emotional intensity of the face on a scale ranging from 0 – no emotional intensity- to 100 –extreme emotional intensity – (see Figure 1A). Responses on the VAS were made by button press, where a continuous press caused the cursor to move continuously, and tapping provided unit-by-unit movement for precision (Garfinkel, et al., 2014; Makovac, et al., 2015s). The cursor began at the mid-point (50).

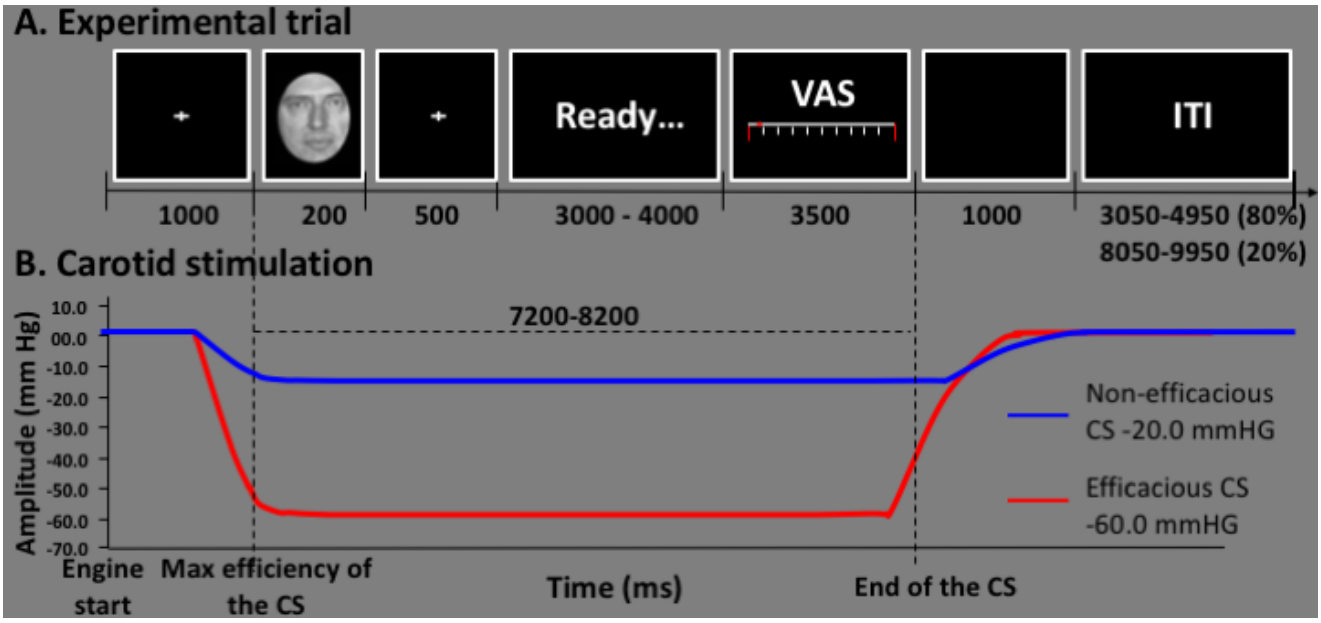

Figure 1. Emotional rating paradigm. **A)** Each trial started with a fixation cross, followed by the presentation of the visual expression (neutral or fearful, 200 ms), and a second fixation cross, a variable "ready" interval, and a VAS task in which the subject had to label faces' emotional intensity by button pressing on a scale ranging from 0 – no emotional intensity- to 100 –extreme emotional intensity. A variable ITI was introduced at the end of the trial. **B)** The unilateral efficacious (ON) and bilateral non-efficacious (BILATERAL-OFF) stimulation was randomly delivered to each participant. The neck suction engine started 500 ms after the onset on the pre-face fixation cross, and reached the set value (-20 or -60 mmHG) at the onset of the face. Accordingly, each stimulation offsets at the end of the VAS rating event**.**

fMRI acquisition and preprocessing

Neuroimaging data was acquired using a head-only 3.0T MR scanner (Siemens Magnetom Allegra, Siemens Medical Solutions, Erlangen, Germany). Functional brain images optimised for blood oxygenation level dependent (BOLD) contrast were collected using an echo-planar (EPI) T2*-weighted sequence (TR=2.08s, 32 axial slices, slice thickness=2.5mm, gap=1.3mm; voxel size 3 x 3 x 3 mm). Data were processed using MATLAB 7.0 (MathWork, Natick, MA) and SPM8 (Statistical Parametrical Mapping, http://www.fil.ion.ucl.ac.uk). In both fMRI runs, the first four volumes were removed to allow for T1 equilibration effects.

EPI images were realigned to the first image and normalized to a standard EPI template. Normalized functional scans were smoothed with a Gaussian kernel of 8-mm (full-width half maximum).

ECG and behavioural data analysis

A repeated measure 3x2 ANOVA was conducted to test the effect of CS on R-R cardiac intervals, with CS side (RIGHT-ON, LEFT-ON, BILATERAL-OFF) and time (pre_CS, post_CS) as main factors.

As regards behavioural data, a 3x2 ANOVA was conducted to test the effect of CS on VAS rating phase. CS side (RIGHT-ON, LEFT-ON, BILATERAL-OFF) x emotion (fear, neutral) was entered as within-participant variables. The stimulation-by-emotion interaction effect on both R-R interval and ratings was then explored using post-hoc t-tests Bonferroni-corrected for multiple comparisons.

fMRI data analysis

First-level analyses estimating contrasts of interest for each participant were followed by second-level analyses for statistical inference at the group level (Friston et al., 2002). The first-level multiple regression model included six conditions corresponding to the VAS task, reflecting a combination of emotion (fear, neutral) and CS (RIGHT-ON, LEFT-ON, BILATERAL-OFF), which were modelled as a boxcar and convolved with a canonical haemodynamic response function. The six VAS conditions were time-locked at the onset of the VAS rating event with a duration of 3500 ms. All predictors were convolved with the SPM8 hemodynamic response function, and realignment parameters were included as covariates of no interest.

At the group level, two different analyses were carried out. In a first analysis, the six

conditions resulting from the emotion x CS combination of the VAS event were modelled within a 3 x 2 within-participant ANOVA, to test for the main effect of the stimulation (RIGHT-ON/LEFT-ON < BILATERAL-OFF; RIGHT-ON/LEFT-ON > BILATERAL-OFF) across emotional conditions, and for the interactions between the two factors [(fear ON< BILATERAL-OFF) - (neutral ON< BILATERAL-OFF)] for left and right CS separately. This was deemed necessary since we have only one sham condition, which was common to both lateralized CS conditions; therefore, a separate comparison between each unilateral CS stimulation condition (LEFT-ON, RIGHT-ON) and the sham condition was needed to elucidate whether the CS x emotion interaction was present during only right stimulation.

Statistical threshold was set to $p < 0.05$, FWE-corrected at cluster level (cluster size defined using uncorrected voxel-level threshold $p < 0.005$) at a whole-brain level. Given our strong predictions on the involvement of the amygdala and insula, anatomical masks were constructed for the region-of-interest (ROI) analyses, using the anatomical toolbox in SPM (Tzourio-Mazoyer et al., 2002) for bilateral insula and bilateral amygdala. For completeness, we reported also significant results outside the regions of the amygdala and insula, which might be additionally informative of the influence of the unilateral CS on emotional appraisal.

Finally, we tested whether the areas showing an effect of CS over emotional appraisal were associated with behavioural, HRV and ECG measures. We considered a sphere (10 mm radius) centred on peak voxel within the ROI analyses described above. The association between contrast estimates from our ROIs and behavioural, cardiac and autonomic measures were evaluated using Pearson correlations.

RESULTS

Effect of carotid stimulation on cardiac activity

After careful inspection of ECG data acquired during the scanning, one participant has been excluded from the analysis due to significant artefacts. The final analysis of the effect of CS on cardiac activity has been performed on 19 participants.

We observed a significant CS side (RIGHT-ON, LEFT-ON, BILATERAL-OFF) x time (pre_CS, post_CS) interaction [$F(1, 18) = 6.94$, $p<0.01$, partial eta squared = 0.45], driven by a significant increase in R-R interval from pre to post CS for right CS (pre vs post RIGHT-ON = 0.879 (0.092) vs. 0.882 (0.092), $t(17)= 2.61$, $p=0.02$, partial eta squared = 0.16), whereas no difference for the pre vs. post CS contrast were observed for the left CS (pre vs post LEFT-ON= 0.884 (0.095) vs 0.886 (0.095); $t(17)= 1.76$, $p= 0.11$, partial eta squared = 0.08) and for the bilateral non-efficacious CS (pre vs post= 0.877 (0.092) vs 0.878 (0.092); $t <1$). We did not observed a main effect of the CS side ($F(1,18) = 2.8$, $p= 0.11$, partial eta squared = 0.18) or time ($F(1,18)= 1.8$, $p=0.19$, partial eta squared = 0.14). The R-R values for each participant (for the pre and post CS, RIGHT-ON, LEFT-ON and BILATERAL-OFF conditions) are reported in Table 1.

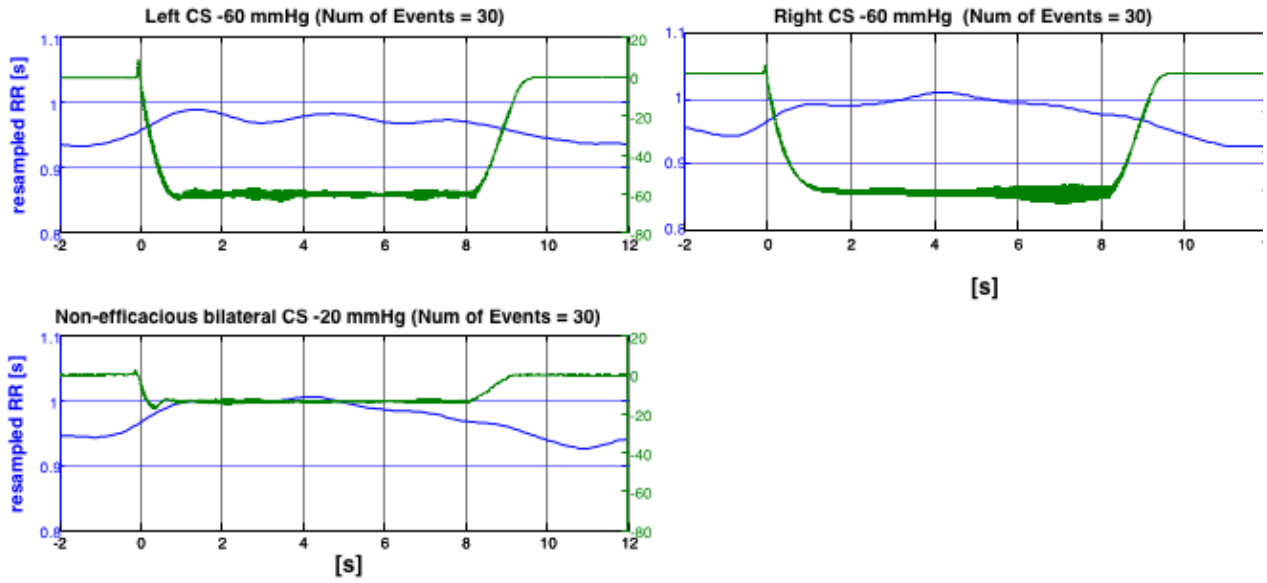

Figure 2. Hearts-period stimulus response curve (shown in blue) and CS pressure (shown in green) for efficacious (- 60 mm Hg) and non-efficacious (-20 mm Hg) stimuli, in a representative participant. The ECG was collected during the scanning, to evaluate the cardiac response to the CS. In this example, stimuli response curves were collected for one volunteer. Each curve is obtained as an average over 30 unilateral CS pulses at 60 mm Hg for each side (combined with a contralateral non-efficacious stimulation at 20 mm Hg) and 30 CS bilateral pulses at 20 mm Hg. The left axis refers to the R-R interval values, while the right axis reports the suction pressure applied to the neck.[1]

[1] The ECG traces represent the average of all the R-R intervals as measured during the efficacious (green line) and non-efficacious (blue line) trials in one representative individual. The increased width of the green line indicates an increased variability of the R-R interval during the maximum efficacious stimulation.

Table 1. Individual inter-beat intervals -R-R (s) – before (pre) and after (post) the carotid stimulation

	RIGHT-ON		LEFT-ON		BILATERAL-OFF	
	Pre-CS R-R (ms)	Post-CS R-R (ms)	Pre-CS R-R (ms)	Post-CS R-R (ms)	Pre-CS R-R (ms)	Post-CS R-R (ms)
Subj *#1*	1.005	1006	1019	1017	996	999
Subj *#2*	792	798	791	798	797	801
Subj *#3*	724	726	724	727	716	715
Subj *#4*	723	724	718	719	724	718
Subj *#5*	965	969	964	965	968	970
Subj *#6*	801	800	802	801	811	810
Subj *#7*	1009	1001	1014	1011	1009	994
Subj *#8*	964	968	949	953	982	985
Subj *#9*	882	888	885	890	884	890
Subj *#10*	784	784	789	789	786	788
Subj *#11*	887	888	897	902	874	874
Subj *#12*	911	909	902	898	906	903
Subj *#13*	970	982	995	1012	967	977
Subj *#14*	928	922	945	933	916	909
Subj *#15*	927	933	926	926	936	935
Subj *#16*	860	868	869	873	853	862
Subj *#17*	757	762	761	766	757	759
Subj *#18*	876	882	886	891	860	863
Subj *#19*	947	955	965	976	930	929

Behavioural results

We observed a significant main effect of emotion, reflecting greater intensity ratings for fear faces relative to neutral faces [$F(1, 19) = 110$, $p<0.001$, partial eta squared= 0.85], and no main effect of CS [$F(2, 38) = 1.07$, $p= 0.3$, partial eta squared= 0.13] on overall emotional intensity ratings.

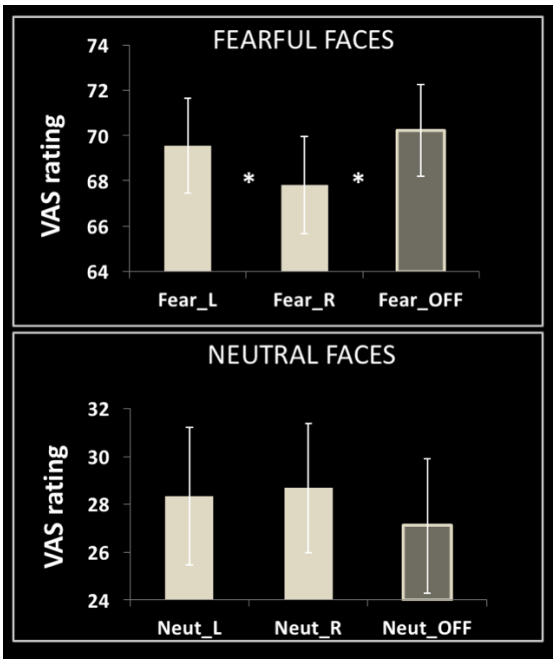

Figure 3. Effect of right and left CS during intensity ratings of fearful (upper panel) and neutral (lower panel) face stimuli. Right-sided CS produced a negative shift (lower rating) for fearful faces and a weak positive shift (higher ratings) for neutral faces. No significant effect of the left CS was observed when compared with the sham (OFF) condition.

Importantly, we observed a significant CS-by-emotion interaction [$F(3, 38) = 6.63$, $p< 0.001$, partial eta squared= 0.47], driven by lower rating of fearful faces [fearful RIGHT-ON versus fearful BILATERAL-OFF, 67.83 (9.33) vs. 70.22 (8.83), $t(19)= 3.54$, $p< 0.001$, partial eta squared = 0.25] and higher rating of neutral faces approaching statistical significance [neutral RIGHT-ON vs. neutral BILATERAL-OFF, 28.68 (11.76) versus 27.10 (12.30), $t(19)= 2.08$, $p= 0.051$, partial eta squared = 0.11], both during right CS (Figure 3). Importantly, when directly comparing ratings during left and right stimulation, a significant difference was observed in the fearful condition (fearful RIGHT-ON versus LEFT-ON, 67.83 (9.33) vs. 69.54 (9.12), $t(19)= 2.97$, $p< 0.01$, partial eta squared = 0.19) whereas no significant difference was observed for neutral ratings (neutral RIGHT-ON vs. neutral LEFT-ON, 28.68 (11.76) versus 28.33 (12.51), $t(19)= 1.25$, $p= 0.23$, partial eta squared = 0.04). No difference

in fearful and neutral rating during left CS was observed (fearful LEFT-ON vs. fearful BILATERAL-OFF $t(19)=1.40$, $p=0.18$, partial eta squared = 0.05 and neutral LEFT-ON vs. neutral BILATERAL-OFF $t(19)= 1.25$, $p=0.23$, partial eta squared = 0.04).

Neuroimaging results

Main effect of efficacious carotid stimulation: We tested, using a whole-brain neuroimaging analysis, the effect of CS during the appraisal of facial emotion (i.e. covering the VAS rating period), accommodating a previously observed delay in effects of maximum baroreceptor stimulation on brain activity (as also in the published study, Makovac et al., 2015a). For completeness, exploratory analysis has been conducted to test the effect of CS on perception of facial expressions (see Supplementary material).

The main effect of efficacious CS vs. non-efficacious (sham) stimulation was tested separately for right and left CS. For right CS, a distributed relative increase in brain activity was evident in bilateral insula, bilateral parietal operculum, Heschl's gyrus, planum polare, precentral gyrus (see Figure 4 and Table 2 for the complete list of brain areas). During left efficacious CS, a relative increase (in comparison to the BILATERAL-OFF condition) in brain activity was observed contralateral to the stimulation, in the right parietal operculum cortex, right insula and right superior temporal gyrus. When directly comparing efficacious right vs. left CS (RIGHT-ON> LEFT-ON contrast), a relative enhanced activation was maximally localized to left insula (Figure 4, Table 2).

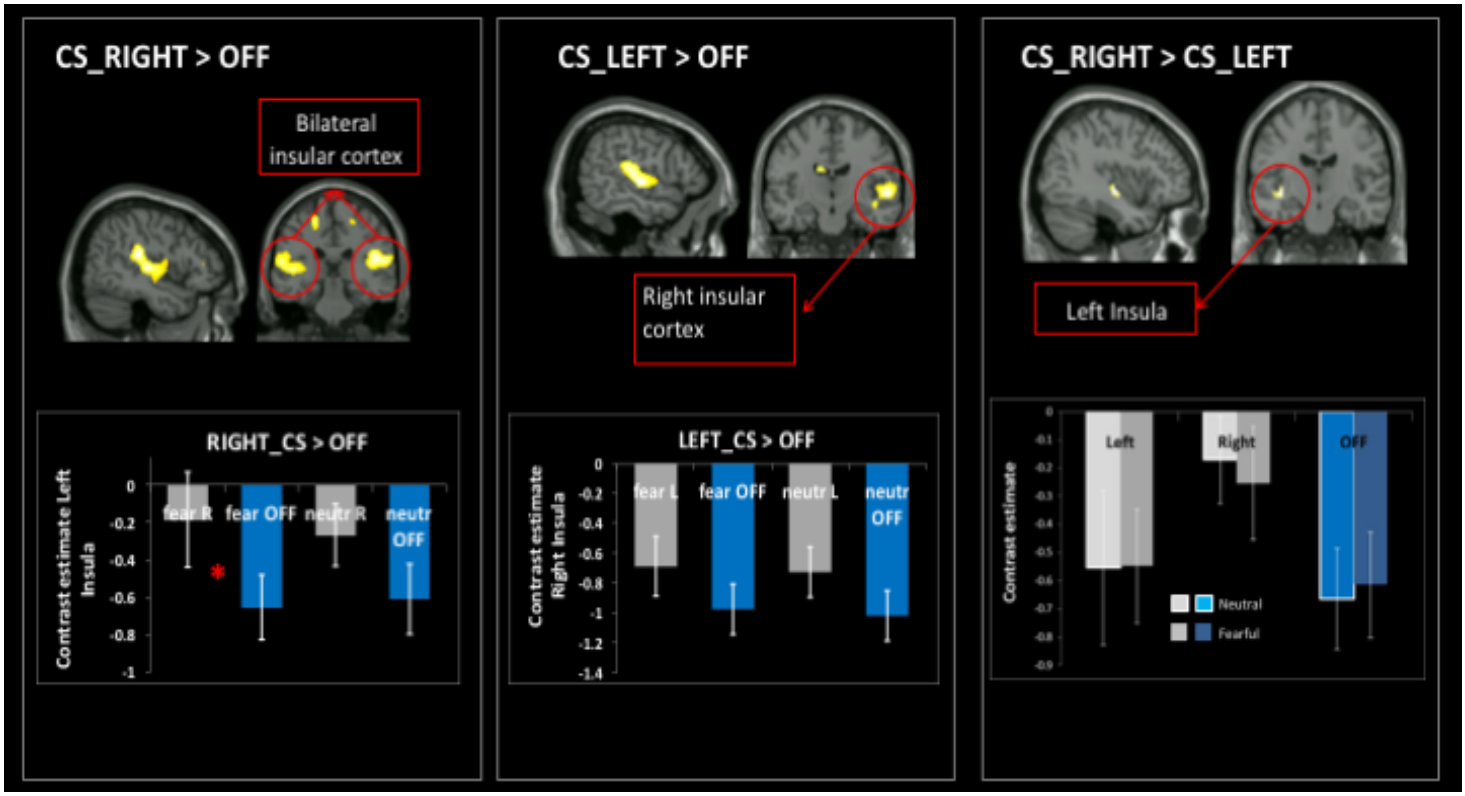

Figure 4. Main effect of CS (averaged across emotional conditions). Brain activity was increased in bilateral parietal operculum/planum temporale/insular cortex during right CS (left panel). The same regions were activated during left CS, but only for the controlateral side of the brain (right panel). When directly comparing right vs. left CS, a stronger activation was evident within left insula during right CS.

Table 2. Brain activation underlying main effect of left and right CS

Brain region		Cluster		Voxel	
	Side	*k*	*p FWE*	*Z*	*MNI xyz*
LEFT-ON > BILATERAL-OFF					
Insula	R	25	0.023 ° #	3.33	*44 -16 -10*
Superior temporal gyrus	R	1156	0.011*	4.14	*58 -14 0*
Parietal operculum	R			4.09	*48 -28 14*
RIGHT-ON > BILATERAL-OFF					
Insula	R	74	0.006 ° #	3.74	*44 -12 -12*
	L	147	0.006 ° #	3.80	*-40 -16 9*
Parietal operculum	R	1787	0.001*	4.25	*48 -30 20*
Planum polare	R			3.74	*44 -12-12*
Central opercular cortex	R			3.56	*56 -2 8*
Tenporal pole/insular cortex	R			2.77	*38 8 -20*
Planum polare/insular cortex	L	1369	0.000*	3.96	*-44 -8 -12*
Parietal operculum	L			3.84	*-60 -26 16*
Planum polare	L			3.78	*-46 -26 6*
Precentral gyrus	L			3.48	*-50 0 12*
RIGHT-ON > LEFT-ON					
Insula	L	45	0.054 ° #	3.14	*-36 -16 -4*
Emotion-by-CS interaction during Right CS					
Amygdala	L	20	0.031 ° #	3.30	*-22 -8 -6*
	R	24	0.017 ° #	3.63	*26 0 -32*
RIGHT-ON < BILATERAL- OFF during fearful appraisal					
Amygdala	L	9	0.052 ° #	3.20	*-32 -4 -30*

FWE peak level
* FWE whole brain cluster level
° FWE after ROI small-volume correction

Interaction between carotid stimulation and emotional rating condition

We next tested for interaction between CS (RIGHT/LEFT-ON, BILATERAL-OFF) and emotion ([fear ON > BILATERAL-OFF] - [neutral ON > BILATERAL-OFF]) to investigate specific effect of CS on emotional appraisal (i.e. covering the VAS period). For RIGHT-ON CS only, **t**he activity within bilateral amygdala showed a significant CS–by-emotion interaction (T = 3.39, 20 voxel and T = 3.63, 24 voxel, both $p< 0.05$ FWE-corrected for small-volume for the left and right amygdala respectively, Figure 5A), where the activity within the amygdala decreased during the fearful RIGHT-ON condition in compared to the fear BILATERAL-OFF condition, and increase in the neutral RIGHT-ON condition in comparison to the neutral BILATERAL-OFF.

No significant CS–by-emotion interaction was evident during LEFT-ON CS.

Correlations with behavioural, ECG and HRV measures

With the correlational analysis, we explored whether a) the cardiac response to the CS (shift in R-R interval) was directly associated with the neural activation following the CS; b) the neural activation following the CS (in our region of interest i.e. the amygdala) was directly associated with the behavioural measure (rating of fearful and neutral faces) and whether c) the neural activation following the CS was depending on each individual baseline autonomic activity (as measured by the RMSSD index, acquired in a pre-scanning session in supine position).

Overall, a positive correlation was evident between activity within left amygdala during the appraisal of fearful faces (T = 3.20, 9 voxel) and changes in the rating during the appraisal of fearful faces [fearful RIGHT-ON – BILATERAL-OFF] during right CS ($r= 0.63$, $p< 0.02$, Figure 5B1), showing the correspondence between RIGHT-ON CS –induced attenuation of left amygdala reactivity and reduced subjective impact of the fearful faces. No signif-

icant correlations were observed for ratings during LEFT-ON CS. Further, individuals with higher HRV (i.e. greater cardiac parasympathetic tone) showed greater attenuation by right CS of amygdala response associated with fear processing. This was evident as a negative correlation between the basal RMSSD HRV and the degree of activation of the left amygdala when RIGHT-ON CS was administered during the appraisal of fearful faces ($r = -0.62$, $p < 0.01$) (Figure 5B2). No correlation between HRV and brain activation during LEFT-ON CS was observed.

Finally, we did not observe a significant association between the cardiac response to the right CS (ie. the shift in R-R interval following right CS) and the activation of the left amygdala during right CS.

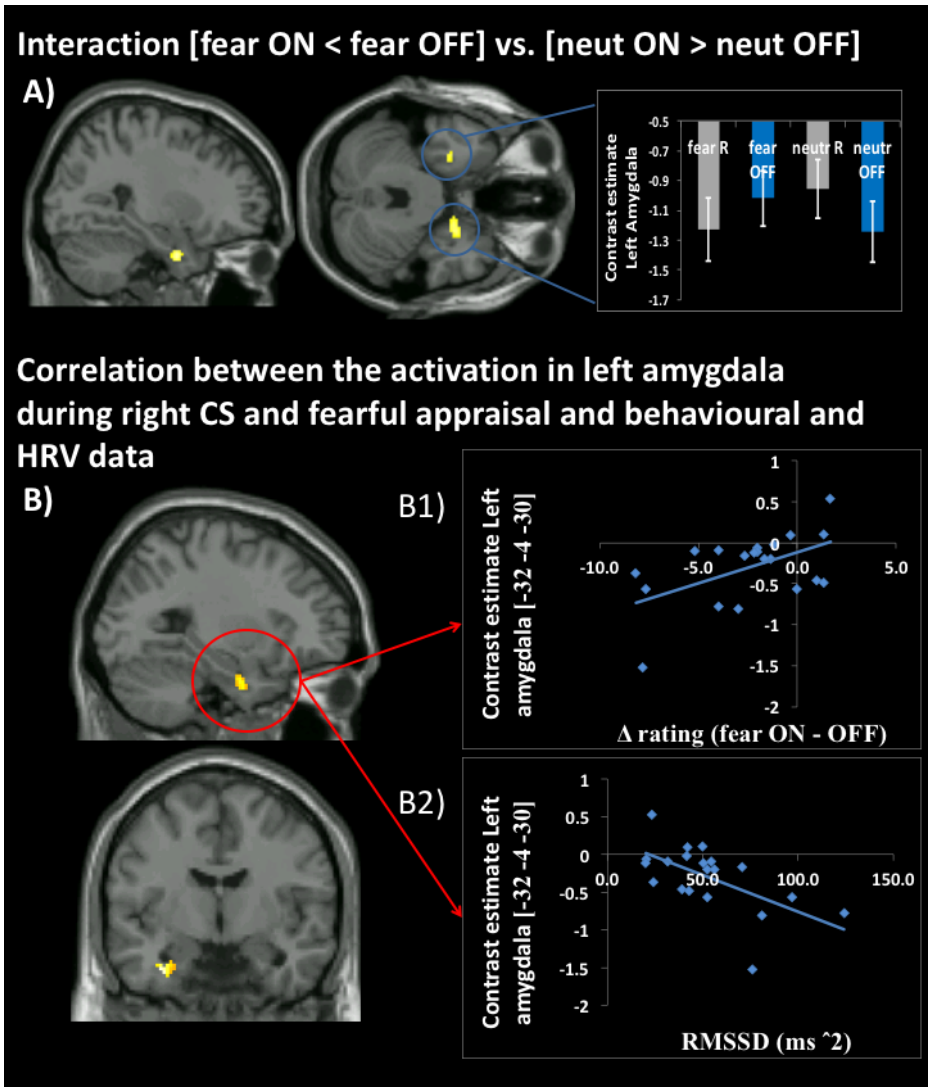

Figure 5. A). An effect of the CS x emotion interaction emerged in the activity of bilateral amygdala, driven both by a decrease in amygdala activity engendered by right CS when judging fearful faces, and a relative increase in amygdala activity when judging neutral faces. **B1**) Positive correlation between the change in fearful rating and the activation of left amygdala by RIGHT-ON CS. **B2)** Negative correlation between baseline RMSSD and the activation of left amygdala by RIGHT-ON CS during appraisal of fearful faces.

DISCUSSION

The present study examined whether there was evidence for a lateralization of viscero-sensory afferent influences on emotional processing. We tested for differential effects of right and left arterial baroreceptor signals on the appraisal of fearful and neutral stimuli and on the underlying neural substrates. Motivated by earlier observations (Makovac et al.,2015a), we used a neck suction device to stimulate baroreceptors within the carotid sinus separately for each side. We demonstrated lateralization of effects on both emotional judgement and on regional brain activity. Moreover, following detailed autonomic characterization of each individual, we found an association between the basal parasympathetic tone and the degree to which CS influenced emotional appraisal and underlying neural responses.

There was a general lateralization of CS effects: independently of stimulus type, CS increased neural activity (in comparison to the sham condition) in parietal operculum cortex, superior temporal gyrus and insula, yet this was more pronounced during right CS, which bilaterally modulated activation of these brain regions. In contrast, left CS produced a weaker activation only of contralateral insular cortex. The involvement of the insula in cardiovascular responses is well-documented in the literature (Oppenheimer & Cechetto, 2016). The insula represents an interface between cognitive, homeostatic, and affective systems, and is regarded as being the link between externally-driven processing and interoception (Craig 2009). An association has been described between the activity in the right anterior insula, perception of one's own bodily state, and emotional elaboration (Critchley et al., 2004) suggesting that insula is a key brain region involved in this process where signals from the autonomic nervous system shape emotional experience. Interestingly, some studies have already suggested a lateralization of cardioregulatory activity of the insula, where the stimulation that the right anterior insula resulted in activation of the cardiovascular sympathetic activity,

whereas the stimulation of the left anterior insula was associated with cardiac parasympathetic regulation (Oppenheimer et al., 1992).

Further, an increase in the activity of the left amygdala was evident only during right CS. Our results extend previous reports of right CS superiority on the afferent control of baroreflex (expressed in heart rate slowing), observed both in animal models (Worthen et al., 1972; Greene et al., 1986) and in humans (Tafil-Klawe, Raschke, and Hildebrandt, 1989; Furlan et al., 2003). In addition to replicating the major influence of right arterial baroreceptors on heart rate, this is the first study that shows the stronger influence of right arterial baroreceptor influences on emotional appraisal and forebrain activity. Interestingly, while other studies (including the one from our group) have observed a decrease in neural activity following bilateral CS (Makovac et al., 2015a; Dworkin et al., 1994), here we observe increase in brain activation following unilateral CS. Effects of bilateral and unilateral stimulation on heart rate are already reported, where the sum of responses to unilateral stimulation is greater than the responses to bilateral stimulation, suggesting an engagement of inhibitory mechanisms by bilateral stimulation (Williamson and Raven, 1993). Speculatively, this might be one mechanism responsible for different influences on brain activity in our study. Moreover, these fundamental differences between unilateral and bilateral afferents might also explain why we failed to observe an association between the cardiac response to the right CS and the neural activations during the same stimulation, contrary to what suggested by other studies with bilateral CS (Reyes del Paso et al., 2014). A direct comparison between unilateral and bilateral stimulation might be misleading here, given the lack of a bilateral stimulation condition within the present experimental design. This methodological choice was dictated by time-constrains and represents the main limitation of the present study. Moreover, even if an inhibitory effect on higher brain areas after bilateral baroreceptor stimulation is widely acknowledged, this has not always been replicated and results to the contrary have also been

reported (Brody et al., 1997; Edwards et al., 2003; Elbert et al., 1988). However, these observations highlight the need for future investigations to disentangle potentially very interesting mechanisms underlying the different effects of bilateral and unilateral carotid stimulation on brain activity.

Although the main effect of CS resulted in a tendency for enhanced neural activation, the activity within the amygdala shows a different response to CS depending on the emotional valence of the stimuli, i.e. a deactivation during fearful appraisal and activation during neutral appraisal. It is important to note that while the spatial extend of the amygdala activation in our study might appear small, this result replicates our previous findings with bilateral CS and is in accordance with our initial hypothesis. Moreover, other published studies reported that fearful faces not only activate the amygdala itself, but also other surrounding areas (Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002; Johnstone et al., 2005; Knight, Nguyen, & Bandettini, 2005), which might explain the limited spatial extent of our results. Despite this evidence, our results should be interpreted with this limitation in mind, and role of the amygdala as a hub in the mediation between right carotid afferents and emotional processing should be further addressed in future studies.

The interaction between CS and emotional condition was also reflected in our behavioural data, where the rating of fearful faces significantly decreased while the rating of neutral faces marginally increased during right CS.

It is noteworthy that we observed a main effect of left CS on brain activity (within left insula), yet this was not reflected on our behavioral data, where no effect of left CS on emotional rating was evident. This discrepancy suggests that afferent input from left CS does not contribute directly to emotional appraisal, but might still support other cognitive and visceral functions mediated by the insula which were not measured in our study. These could include

interoceptive awareness (Critchley et al., 2004), multimodal sensory processing (Bushara et al., 2003) or body self-consciousness (Tsakiris et al., 2007). The influence of left carotid afferent on functions different from emotional rating will be addressed in future investigations.

As regards the effects of right CS, even the relatively small differences in the ratings suggest that systematic changes are both physiologically meaningful and functionally significant. They are also in line with the observed differential effects on ratings of emotional (including fear) stimuli of cardiac afferent signaling within the heart cycle (Garfinkel et al., 2014; Gray et al., 2012). Here, the modulation of the subjective intensity of brief stimuli by cardiac timing (systole: concurrent with baroreceptor firing versus diastole during baroreceptor quiescence) was of a comparable magnitude. Moreover, this previous work also demonstrates that these seemingly small physiological differences were sufficient to heighten the detection of periliminal fear stimuli time-locked to cardiac systole using the emotional attentional blink paradigm (Garfinkel et al., 2014), thereby demonstrating functional impact in the domain of attention. Nevertheless, fear faces are generally potent stimuli, hence future experiments that exert more experimental control over this potency (e.g. through morphing to systematically vary fear intensity of the stimuli) will provide fine-grained insight into how the effects of CS on emotional stimuli are influenced by the degree of initial intrinsic intensity.

Our data add to evidence countering the assumption that arterial baroreceptors activation results in a general inhibition of sensory processing and cortical excitability, since we show an emotion-specific modulation, (Lacey and Lacey, 1970; Gahery and Vigier, 1974; Koriath and Lindholm, 1986). We also replicate the findings regarding fear processing of our previous study (Makovac et al., 2015a), which parallel the observations concerning the influence of cardiac timing on emotional processing (Garfinkel et al., 2014). The present study illustrates the dominance of right-sided baroreceptors in driving these effects. Importantly, this differential effect of right CS on emotional ratings develops upon our previous study

with bilateral CS, by allowing us to rule out alternative causal mechanisms that might relate to a more general asymmetry in sensory pathways from the body and the mechanisms that drive an orienting reflex (and a consequent cardiac deceleration; Bradley, 2009). These would result in a general inhibitory effect independently of the emotional condition and of the side of CS.

We observed a direct association between the modulation of the activity within left amygdala by right CS and the modulation of emotional appraisal, apparent in the linear correlation between CS-evoked reduction in left amygdala response and lower behavioural intensity ratings of fearful faces. This same association was not evident for neutral ratings, reinforcing evidence for a fundamental role of the amygdala in fear perception and its elaboration by cardiovascular afferents (Makovac et al., 2015a; Garfinkel et al., 2014).

Our results are provide valuable insights concerning the emotion lateralization within forebrain regions (e.g. Davidson and Fox, 1982; Grimshaw and Carmel, 2014). Established models associate the right hemisphere with emotional arousal. A more recent and compelling neurobiological account that suggests functional hemispheric asymmetry originates in lateralized representations of homeostatic activity, which ultimately reflect asymmetries in the peripheral autonomic nervous system (Craig et al., 2005). While peripheral anatomical and functional asymmetry in autonomic efferents and homeostatic afferents is broadly recognised for specific organs, the impact on central processes (both homoeostatic and cognitive/affective) has received little attention. This is the perhaps the first study that attempts to elucidate this point in humans. The control of autonomic activity at the level of the cerebral cortex is also asymmetric (Oppenheimer et al., 1992; Cechetto and Shoemaker, 2009). There is segregation of responsibility wherein which sympathetic activity is mainly controlled by the right hemisphere and parasympathetic activity by the left hemisphere (Wittling et al., 1998). Accordingly, stimulation of insular cortex can elicit strikingly asymmetric effects on

cardiac activity that parallel the afferent asymmetries (Oppenheimer et al., 1992). Stimulation of left insula produces parasympathetic effects (bradycardia and blood pressure depression), whereas right insula produces sympathetic effects (tachycardia and pressor response). The dominant involvement of the left hemisphere in parasympathetic control is also suggested by our own results: We found that the left amygdala activity was associated with the influence of basal HRV (RMSSD) on right CS induced changes in fear appraisal. Thus, an individual's parasympathetic vagal tone determined the degree to which left amygdala responses were attenuated by right CS, predicting a diminished emotional impact of fearful faces. A similar left amygdala finding was observed in our previous study with bilateral stimulation (Makovac et al., 2015a).

The major limitation of the present study is the small sample size, which increases the chance that that estimate of the magnitude of a significant effect is exaggerated (Button et al.,2013), and increase the likelihood of type II errors (Lieberman & Cunningham, 2009). Moreover, comparison with pleasant faces (which would help us to distinguish further between effects of emotional valence and arousal) was not possible due to time constraints. This should be addressed in future investigations.

Limitations notwithstanding, our results may be particularly relevant for the understanding of pathologies such as anxiety disorders, in which an enhanced perception of fear and a withdrawal of parasympathetic influences of the heart are well-known hallmarks of this pathology (Lang et al., 2000; Ottaviani et al., 2015; Makovac et al., 2015b). In fact, a thorough understanding of the two-way communication between the brain and the cardiac system during fear processing is an important milestone for the optimization of existing therapies and the development of new efficacious treatments for this very common expression of psychopathology. Parasympathetic withdrawal is a key feature of stress responses (Porges, 1992). Moreover there are promising findings from studies of the potential therapeutic benefits of

the vagus nerve stimulation (VNS) in treatment-resistant anxiety disorders, with evidence of acute and long-term improvement in some patients (George et al., 2008; Furmaga et al., 2011). VNS is known to increase activity in regions involved in anxiety modulation or perception including thalamus, amygdala, insula, and brainstem (Chae et al., 2003; Lomarev et al., 2002, Critchley et al., 2007). Moreover, VNS paired with exposure to conditioned cues enhanced the extinction of conditioned fear (Peña et al., 2013). Future investigations should explore whether this right lateralized CS methodology may be beneficial to patients with anxiety disorders and further elucidate the mechanism underlying the elaboration of fear through afferent influences from the heart.

In conclusion, this is the first study to evaluate the impact of unilateral carotid stimulation on brain activity and the consequent appraisal of fearful faces. Our study brings important insights in the interaction between body and brain, by highlighting the differential involvement of right and left carotid afferents in brain functions, where only right (but not left) carotid feedbacks are involved in emotional processing.

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