Investigation of the Autonomic Nervous System Control of Cardiovascular Variables using fMRI and Carotid Stimulation

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Abstract

Noninvasive neuroimaging using fMRI has the potential to identify the brain regions involved in the processing of autonomic signals. An event-related design was employed to randomly administer 50 efficacious (-60 mmHg) and 30 non-efficacious (-10 mmHg) neck-suction stimuli, with a duration of 8 s each. Six-hundred echo-planar imaging volumes (TR=2.08 ms) with BOLD contrast, covering the whole brain, were collected in each experiment (total duration 20 min). A stimulus-response curve was obtained by averaging the uniformly resampled RR intervals following each stimulation. Fifteen informed volunteers underwent fMRI at 3T during neck suction stimulation. Nine subjects responded to the stimulation, whereas in six subjects the heart period did not show a significant increase during the suction. Efficacious versus non-efficacious stimuli induced a significantly cluster-level increased activation ($p<0.005$), in the right superior temporal lobe (p-corrected<0.004) and within limbic circuits, involving left amygdala and putamen (p-corrected<0.01).

1. Introduction

Cardiovascular parameters such as heart rate, blood pressure and peripheral vascular resistances are continuously regulated by the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). Afferent signals from a complex network of peripheral sensors (arterial aortic and carotid baroreceptors, cardiac and lung receptors, to name a few) are processed at central level. Several factors make the study of the central processing and control of ANS in humans difficult. Non invasive neuroimaging using fMRI may provide re-levant information to identify the brain regions involved, but technical and methodological issues need still to be addressed: appropriate perturbations have to be applied to the ANS and regressors to be used for fMRI modeling have to be identified and validated. The use of behavioral tasks and of regressors derived from the heart rate variability (HRV) measures has been proposed by Napadow et al [1]. We propose the use of non-invasive carotid baroreceptor stimulation during f-MRI imaging to study the central processing and control of ANS in humans.

2. Methods and material

2.2. Neck suction technique

The carotid baroreceptors are located at the medial–adventitial border of blood vessels in the carotid sinus bifurcation. These mechanoreceptors function as the sensors in a negative feedback control system that regulates the beat-to-beat changes in arterial pressure modulating the autonomic neural outflow. Stretching of carotid baroreceptors causes an increase in afferent neuronal firing which results in a reflex-mediated increase in parasympathetic nerve activity and decrease in sympathetic nerve activity [2].

The application of neck suction (NS) causes an increase in carotid sinus transmural pressure which in turn stretches the carotid baroreceptor, mimicking the delivery of an hypertensive stimulus. The advantages of NS techniques include: accurate control of timing, intensity and duration of the stimulus; non-invasiveness; assessment of the perturbation on the ANS in terms of stimulus- response curve. Various neck chamber designs have been proposed so far; the main difference is between collars that enclose the entire neck (or at least the anterior two-thirds of the neck [3]) and small individual chambers. As far as the shape, duration and intensity of the NS are concerned, two approaches have been proposed and applied: trials of square impulses and continuous sinusoidal stimulation[2][4]. Square impulses were used in this study because they better fit the event-related design used in fMRI investigations.

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2.3. MRI compliant neck suction device

The neck suction device is similar to those already used, with modifications needed to comply with the MRI environment. The pressure is set by controlling the aspiration level of a vacuum source. The control signal is generated by the analog out of an acquisition card (NI6212, National Instrument). This reference signal was fed in an analog comparator together with the pressure signal at the output of the pump, to obtain a feedback control signal to drive a suction pump motor. Since the suction pump can only generate sub-atmospheric pressure, an air leakage was added to the line. The pump and its controlling unit were placed in the MRI control room. A 5-m silicon tube connected the pump with the neck chambers. The collar neck chamber was realized using PVC sheets and silicon rubber, while the small individual chambers were obtained modifying facial masks used for ventilation. The size of the masks was chosen according to the size of the neck of each volunteer.

2.4. Stimulus Response Curve

RR interval series were obtained from the ECG trace, using a simple threshold algorithm. RR intervals from each MRI scanning were arranged as a Discrete Event Series (DES). DES was interpolated and uniformly resampled at 10 Hz, using cubic splines (resampled RR). Stimulus-response curves were obtained by averaging the individual RR response to each stimulation: resampled RR segments corresponding to each neck pulse were aligned to the time of 5 mmHg pressure drop, and averaged. We categorized as 'responders' to the stimulation those subjects who had a significant increase of the RR intervals during the application of the suction pulse, respect to the baseline. For each subject, the statistical significance of the RR response to the stimulation was assessed using the Student t-Test for paired data (RR at baseline vs RR during stimulation).

2.5. Signal acquisition and fMRI study protocol

Acquisition and analog conditioning of ECG, pulseoximetry and respiration were obtained using an MRI-compliant system (BIOPAC Systems Ins, CA, USA).

Pressure signals in the left and right chambers were measured and conditioned inside the MRI chamber. The pressure transducers and the conditioning amplifiers were housed in a shielded box, approximately 2 meters apart from the RF coil. ECG, respiration, Pulseoximetry and pressures in the left and right chambers were acquired using a DaqCard NI USB 6212, located in the MRI control room. Signal acquisition and pulse generation were synchronized to the imaging acquisition, using a RS-232 trigger from the scanner.

To avoid synchronization of the respiratory efforts, neck suction pulses (NS) were applied randomly in time. Each pulse lasted 8 s, with a random inter-pulse delay ranging from 3.0 to 5.2 s. Two suction pressures were used: -60 mmHg (efficacious stimulation) and -10 mmHg (non-efficacious stimulation). The pressures were applied in a random order. Fifteen healthy volunteers [all men; mean (SD) age=23.0 (3.4) years] underwent fMRI at 3T. Six-hundred echo-planar imaging volumes (TR=2.08 ms) with BOLD contrast, covering the whole brain, were collected in each experiment (total duration= 20 min). An event-related design was employed, which randomly administered 50 efficacious and 30 non-efficacious stimuli. Within subjects ANOVA was used to assess the differences in brain activation for the two experimental conditions. f-MRI data were analysed using the Statistical Parametric Mapping for neuroimaging data (SPM5).

3. Results

The use of a PVC collar enclosing the two-thirds of the neck was found to create significant motion artifacts during fMRI scanning. In addition the volunteers referred discomfort during the application of the 60 mmHg suction, mainly due to mechanical stimulation to upper airways and trachea. The further use of this kind of chamber was thus excluded. No significant discomfort was reported when suction was applied using the two-chamber device.

Fifteen subjects were studied. Table 1 shows the average RR period 2 s before (baseline), and during (stim) the application of the neck suction pulse, in the 15 subjects studied. Nine subjects had a significant RR increase during the efficacious stimulation ($p<0.005$). One subject had also a significant increase of RR interval with the non-efficacious stimulation.

Stimulus-response curves for two volunteers are displayed in Fig. 1 and Fig. 2. The time 0 corresponds to the drop of -5 mmHg and it is the reference point for the averaging. The left axis refers to the resampled RR values, while the right axis reports the pressure level applied to the neck. Fig. 1 shows that in this subject the application of -60 mmHg pulses evoked a marked response in the heart period, whereas the stimulation at -10 mmHg did not. Fig. 2 shows the stimulus-response curve of one subject in which no changes in RR intervals due to the application of suction pulses were observed.

Efficacious versus non-efficacious stimuli induced a significantly cluster-level increased activation ($p<0.005$), in the right superior temporal lobe (BA41) (p-corrected<0.004) and within limbic circuits, involving left amygdala and putamen (p-corrected<0.01) (see Fig. 3).

Additional activations were observed in the left lateral occipital cortex (p-unc<0.03), and in the right putamen (p-unc<0.01). Non-efficacious stimuli did not produce any cluster-level significant activation compared to the efficacious stimuli, although, p-uncorrected significant activations were observed in the cingulate gyrus (p-unc<0.003) and in some right frontal regions (p-unc<0.01).

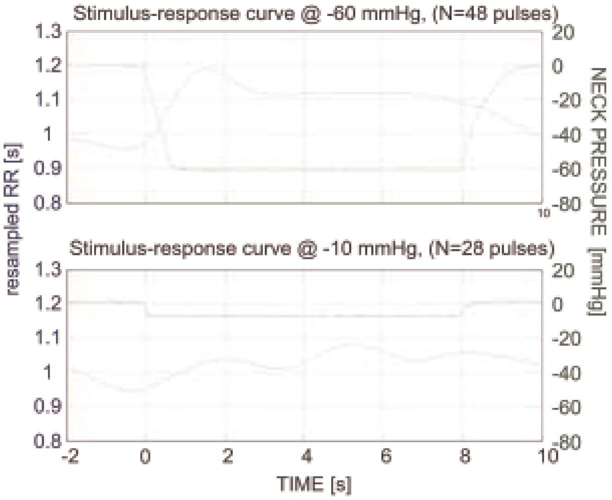


Fig. 1. Stimulus-response curves of a volunteer which responded to the application of the neck suction pulses (-60 mmHg). Note the absence of RR response for the non-efficacious (-10 mmHg) stimulation.

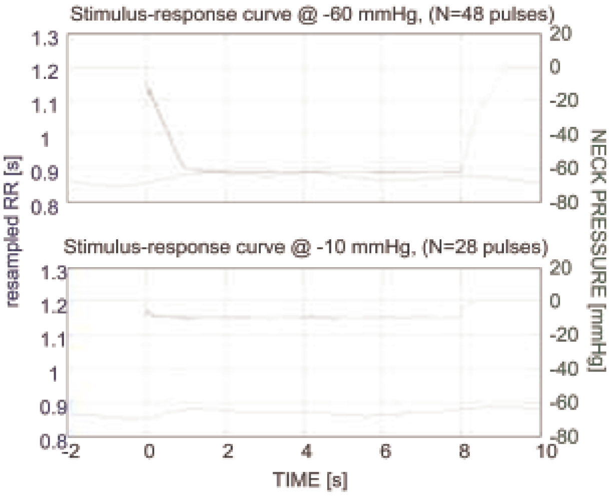


Fig. 2. Stimulus-response curves of a volunteer which did not respond to the application of the neck suction pulses. The RR curves for -60 mmHg and -10 mmHg stimulations are comparable, and no response to the suction is observable.

When the sample was splitted according to the cardiac response to application of the neck pulses, "responder" subjects showed a significantly increased activation in the efficacious stimulation, compared against "not-responders". As shown in Fig.4, significant cluster-level increased activation (p<0.005) was observed in the right inferior and superior parietal lobule (p-corrected<0.02), and bilaterally in the inferior and middle frontal gyrus (p-corrected<0.05).

4. Discussion and conclusions

Investigation of ANS central network using fMRI poses peculiar constraints both in the realization of a neck suction device and in design the of a stimulus-response protocol.

The main concern with the neck chamber technique is that only a portion of the pressure is transmitted to the carotid baroreceptors, or, in other words, that the change in pressure measured in the chamber may not represent the change in the transmural pressure. In addition, due to the anatomical variability in the location of carotid baroreceptors, some patients may not be stimulated at all [2]. Klawe et al [5] compared the effect of single chamber vs dual chambers neck suction on airway resistance in humans. 50% of the negative pressure in the single chamber was transmitted to the esophagus, whereas the pressure changes in the single capsules were not reflected at esophageal level. They concluded that neck suction, applied using a single chamber, probably excites mechanoreceptors in the trachea, the larynx and the esophagus as well the carotid baroreceptors. A more selective method of stimulation is thus achievable using two-chambers device. In addition, in our experiment, a single chamber device induced motion artifacts in MRI imaging and discomfort to the subjects, while the two-chambers device did not show these inconveniences.

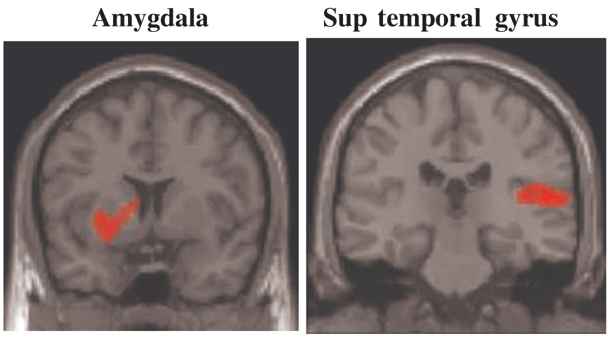
The duration of the stimulation pulses should be chosen long enough to allow the detection of the heart period response and to be consistent with the fMRI volume scanning requirements, but shorter enough to avoid adaptation of baroreceptors or counteraction from aortic and cardiopulmonary baroreceptors. Fadel et al [2] used trial of 5s pulses and showed carotid cardiac stimulus-response curve obtained by averaging 4 pulses of -60 mmHg. We used 8 s pulses of -60 mmHg, to take into account the longer transient of our MRI-compliant neck suction system to set the pressure level. Stimulus-response curves were obtained by coherent averaging of the resampled RR response to each pulse. Nine out of fifteen subjects were categorized as "responders", i.e. had a significant increase of RR period due to application of the neck pulses.

Non-efficacious stimulations (-10 mmHg) did not induce significant changes in the RR intervals. The results obtained comparing efficacious vs non-efficacious stimulation showed that baroreceptor stimulation induces expected peripheral responses together with modulation of activity in several brain regions.

Table 1. Cardiac response to efficacious stimulation.

CARDIAC RESPONSE (-60 mmHg)				
Sbj	**Baseline RR [s]**	**Stim RR [s]**	**ΔRR %**	**p(*)**
#1	0.85	0.88	3.67	0.000
#2	0.75	0.75	1.72	0.781
#3	0.80	0.84	5.84	0.000
#4	0.92	0.96	4.85	0.000
#5	0.88	0.95	8.78	0.000
#6	0.70	0.70	0.59	0.426
#7	0.90	0.89	-0.77	0.169
#8	0.85	0.88	2.91	0.003
#9	1.02	1.06	4.86	0.005
#10	1.22	1.29	7.53	0.090
#11	0.53	0.53	0.47	0.783
#12	0.98	1.19	20.90	0.000
#13	0.89	0.94	6.92	0.003
#14	0.97	1.12	15.86	0.000
#15	0.98	1.00	2.72	0.231

(*) t-Student test for paired data.

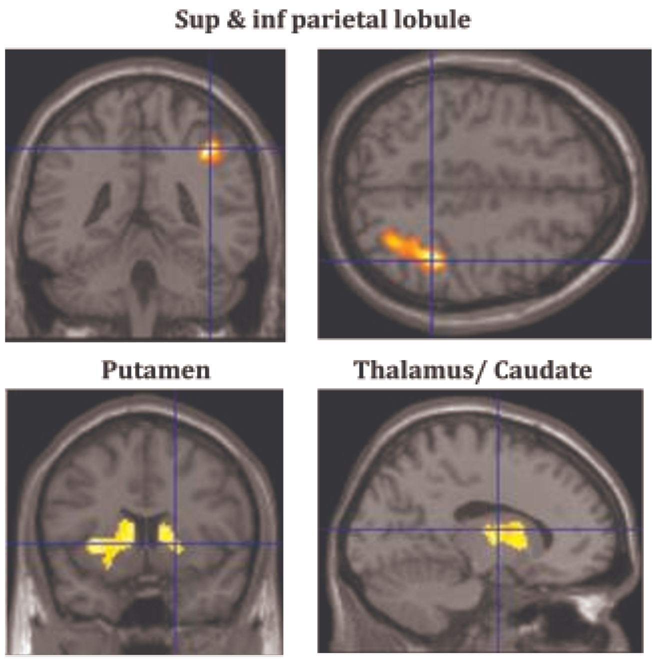


Amygdala **Sup temporal gyrus**

$P_{corrected}<0.005$ Min. cluster size=20voxels

Fig. 3. cluster-level increased activation due to efficacious stimulation, in the whole population.

Some regions were previously associated with autonomic functions (limbic areas, like the amygdala), while other regions are involved in higher level functions (putamen). Specific comparison between efficacious stimulation in “responders”, versus “not-responders”, showed additional activations in more prefrontal regions of the brain and in the left parietal lobule, both involved in attentional processes. Conversely, “not-responders” did not show any significant brain activation during the efficacious stimulation.



Sup & inf parietal lobule

Putamen **Thalamus/ Caudate**

$P_{corrected}<0.005$ Min. cluster size=20voxels

Fig. 4. Cluster-level increased activation due to efficacious stimulation, in the “responders” population, compared to not-responders.

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