Genetic & Environmental risk factors for Obsessive-Compulsive Symptoms: Do they affect the same brain?

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The use of fMRI to detect neural responses to cognitive tasks: is there confounding by task related changes in heart rate?

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D. van 't Ent, A. den Braber, E. Rotgans, E.J.C. de Geus, and J.C. de Munck. The use of fMRI to detect neural responses to cognitive tasks: is there confounding by task related changes in heart rate? (in revision, Psychophysiology).

Abstract

In agreement with the fact that functional Magnetic Resonance Imaging (fMRI) signals represent local Blood Oxygenation Level Dependent (BOLD) changes and variations in blood flow and blood volume, it has recently been demonstrated that fMRI signals during rest are strongly correlated with heart rate variations. Since heart rate/fMRI correlations show up in every part of the brain they may form an important confound in brain activation studies, particularly if heart rate is affected by the task. To assess the impact of task-related heart rate variation, we co-registered the electrocardiogram with fMRI in 91 subjects during a color-word Stroop task, administered using a block design, and a Tower of London (ToL) cognitive planning task, administered using an event-related design. We found that both Stroop interference and ToL planning were associated with significantly increased heart rate and confirmed significant main effects of heart rate regressors on the fMRI signals during both tasks. Nevertheless, statistical results from General Linear Model contrasts that test for increased fMRI signal during Stroop color-word interference and ToL planning were not significantly influenced by inclusion of heart rate regressors as nuisance variables. We conclude therefore that fMRI signal changes associated with fluctuations in heart rate do not impact strongly on higher-order fMRI task effects.

Introduction

The applicability of functional Magnetic Resonance Imaging (fMRI) as a neuroimaging tool rests on the assumption that fMRI signal changes include a Blood Oxygenation Level Dependent (BOLD) mechanism (Ogawa et al., 1992). This implies that regional brain activations result in local excess of oxy-hemoglobin supply, which leads to an increase in the homogeneity of magnetic susceptibility, a decrease in T2*, and hence increased fMRI signal (Buxton, 2009). Obviously, fMRI is only a very indirect measure of brain activity and, apart from the BOLD-effect, T2* is also influenced by other physiological factors such as respiratory and cardiac cycles which modulate blood oxygen levels and microvessel diameters (Birn et al., 2006; Glover et al., 2000; van Houdt et al., 2010; Windischberger et al., 2002; Bhattacharyya and Lowe, 2004; Katura et al., 2006; Tong et al., 2011). Indeed, it has recently been demonstrated for recordings during resting state conditions that fMRI signals over large parts of the brain are correlated with changes in heart rate (Chang et al., 2009; de Munck et al., 2008; Shmueli et al., 2007).

If there are no systematic differences in cardiac activity between different conditions of a task, one could argue that the effects of this physiological noise can always be compensated by recording a sufficient number of trials. However,

in paradigms where heart rate is modulated by the task, e.g., when there are different levels of task difficulty or emotional valence, fMRI signal changes correlated with cardiac activity pose a serious threat to the interpretation of the statistical parametric maps (SPMs). Statistically significant differences between task conditions may then not be caused by the BOLD-effect alone, but also by non-neuronal responses of the vascular bed to heart rate variations.

The goal of our study is to explore the extent to which fMRI signal changes between cognitive task conditions are influenced by between-condition differences in heart rate. To this end, we performed simultaneous electrocardiogram and BOLD fMRI recordings in a group of subjects during a color-word Stroop task and a Tower of London (ToL) cognitive planning task. The two tasks covered the two basic experimental setups for BOLD fMRI measurements; the Stroop task was administered in a blocked paradigm and the ToL in an event-related paradigm. The influence of non-neural contributions on fMRI task effects due to heart rate modulation was assessed by computing task related fMRI changes using a general linear model (GLM) that accounts for heart rate effects by adding heart rate regressors as confounders, and comparing the results with the fMRI signal changes from a GLM without inclusion of heart rate information, as is standard practice in BOLD fMRI research.

Methods

Subjects

From the Netherlands Twin Registry (Boomsma et al., 2002) we recruited a 'Test sample' of 46 subjects. All subjects (13M/33F: mean age 36.9 ± 8.9 yrs) were twins from monozygotic pairs, but by selecting only one of the members from each pair, shared family backgrounds were avoided. To investigate the stability of our findings, we repeated our analyses in the set of co-twins of the subjects in the test sample, which we will refer to as the 'Repetition sample'. However, for one of the co-twins (F: 35 yrs) no MRI data was available, leaving 45 subjects in the repetition set. None of the twins in both samples had a history of neurological illness as assessed from self-report surveys, and all twins provided written informed consent. The study was approved by the VU University medical centre Amsterdam ethical review board.

Tasks

The Stroop task of this study consisted of standard color-word stimuli as well as words with emotional content. For our purpose we investigated only the results pertaining to color-word interference, which had the largest effect on task performance and heart rate. During the Stroop color-word task, subjects had

to report the ink color of written color-words. Dutch translations of the words "red", "yellow", "blue" and "green" were used that could be written in any of these four colors. Word meaning and ink color could be either congruent (e.g., the word "green" written in green) or incongruent (e.g., the word "red" written in blue). The correct answer had to be indicated by pressing buttons: left middle finger for ink color yellow, left forefinger for green, right forefinger for red and right middle finger for blue. The task was administered in a block design with 18 blocks in total. Of these, 3 blocks contained congruent and 3 blocks contained incongruent color-word stimuli. The remaining blocks were filled with words that convey general emotional content (3 blocks, e.g., cancer, suffocate, etc), words with content related to obsessions/compulsions (3 blocks, e.g., guilty, dirty, etc), and neutral words with similar linguistic parameters (e.g., word length and frequency of occurrence) as the words with emotional content (3 blocks) and neutral words with similar linguistics as the words conveying obsession/compulsion related content (3 blocks). In each individual block of 35 seconds, 16 words were presented for 2 seconds and separated by small intervals of 200 milliseconds. The subjects were asked to respond to the stimuli as quickly and accurately as possible. Total task duration was 10.5 minutes.

Stimuli for the ToL task consisted of images of colored beads (red, blue, yellow), placed on three vertical rods of decreasing height **(figure 7.1)**. On each trial a start configuration and final target configuration were simultaneously depicted at the bottom and top of the screen, respectively. Subjects were requested to count the number of steps from the starting configuration to reach the target configuration. Five planning difficulty levels were included that corresponded with the minimal number of moves (1–5) actually needed to achieve the target. As a baseline condition, similar stimuli were presented but this time the subject only had to count the number of beads with specified colors. Each time, two possible answers (one correct and one incorrect) were presented at the bottom left and right of the screen, from which the correct one had to be indicated by pressing a corresponding left or right hand button. No feedback was provided during the task. The stimuli were presented in an event-related design with self-paced stimulus timing, i.e., a subsequent trial was presented on the screen with a delay of 32 ms after the response on a previous trial. For all subjects the stimulus presentation order was the same, but the total number of trials depended on the subject's reaction times. Total task duration was 17 minutes. Here we will focus on the comparison of 4-steps planning versus baseline, because it showed the largest modulation of heart rate. The heart rate effect for 5-steps planning was less pronounced and not statistically significant (Test sample: $p = 0.148$: Repetition sample: $p = 0.859$). This is likely because several of our subjects experienced this condition as very difficult and reported that they had given up on a number of trials. On average subjects completed 16 ± 3 trials

with 4-steps planning stimuli (~9% of the total number of trials) versus 62 ± 15 trials with baseline stimuli (~36%).

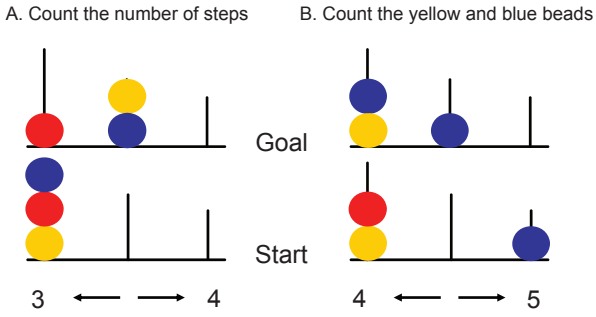


Figure 7.1. *Examples of Tower of London stimuli. (A) 4-steps planning condition; (B) baseline condition (adapted from van den Heuvel et al. (2005a)).*

For both the Stroop and ToL, stimuli were projected on a screen at the end of the MRI scanner table, viewed by the participants through a mirror. Two magnetic compatible response boxes were used to record the subject's performance. Before the experiment, the subjects practiced a number of trials on a computer outside the scanner and again inside the scanner, prior to the actual start of the session.

MRI and ECG

MR scans were made on a 3.0 T Intera MR system (Philips, Medical Systems, Best) with an 8-channel standard SENSE receiver head coil. Of each subject a three-dimensional T1-weighted gradient-echo sequence anatomical scan was made consisting of 182 coronal slices of 256×256 pixels; voxel size was 1.0×1.0×1.2 mm^3. For fMRI, an echo planar imaging (EPI) sequence (flip angle 80°; repetition time = 2300 ms; echo time = 30 ms, matrix, 96 × 96 pixels; field of view 220 × 220 mm) was used, covering the whole brain (40 axial slices; 2.29 mm × 2.29 mm in-plane resolution; 3.0 mm slice thickness; no gap between slices). For the Stroop task a total of 260 and for the ToL a total of 440 EPI volumes were scanned per subject in one single run. During fMRI scanning a four-lead electrocardiogram (ECG) was recorded using the ECG system provided with the MR scanner, and sampled at 500 Hz. The ECG was stored in an ASCII log file and time aligned to the fMRI scanning using information from the additionally stored MRI field gradient onsets.

Inter heart Beat Interval (IBI) regressors

Inter heart Beat Interval (IBI) regressors were constructed similarly as described by de Munck et al. (2008). In brief, first the R-peaks in the ECG were detected automatically and large changes (> 30%) in consecutive RR intervals were flagged

and if necessary manually corrected after visual inspection. Subsequently, the ECG time series were subdivided into epochs corresponding to each of the fMRI scans (a single volume was acquired in 2.3 s). Since the RR interval times are irregularly sampled over time they cannot be directly used as IBI regressor. To compute an IBI value per fMRI epoch, all RR intervals were averaged having at least one point of overlap with that epoch. Since the effect of time varying heart beats on the fMRI signal does not follow the standard hemodynamic response function used for neuroimaging, IBI-regressors were shifted in time over multiple time steps of the MRI volume repetition time (TR), to account for possible delayed responses of the fMRI-signal to heart beat variations. In this way, the effect of heart beat on fMRI is described with a general impulse response model, where the optimal response shape is a priori unknown and is extracted from the data. In this study we used 7 IBI-regressors corresponding to time shifts of [-2, -1, 0, 1, 2, 3, 4]*TR. The IBI time series were either considered as effects of interest, or as 7 additional nuisance regressors in the GLM for statistical testing, or ignored.

We also used the ECG data to test if heart rate changed with task difficulty. To compute a mean IBI value per stimulus type for the Stroop task in each individual we took the mean of all RR interval times that overlapped each individual stimulus block and then averaged these mean RR times separately across the 3 blocks with congruent and incongruent color-word stimuli, respectively. For the ToL we first computed, for each stimulus, the mean of all RR times that overlapped the interval between stimulus onset and the subject's response and then averaged the mean RR times per stimulus type. Similar to the computation of IBI values per fMRI epoch, all RR times were included having at least one point of overlap with the relevant epoch (i.e., stimulus block for the Stroop task and interval between stimulus onset and the subject's response for the ToL task).

Statistical analyses

MRI data were analyzed using Statistical Parametric Mapping version 5 (SPM5: Wellcome Department of Imaging Neuroscience, London, UK). Echo planar imaging scans were slice time corrected, realigned and normalized to the standard Montreal Neurological Institute (MNI) brain of SPM. Subsequently, data were re-sliced to 3 mm × 3 mm × 3 mm voxels and spatially smoothed using an 8 mm isotropic Gaussian kernel. After high-pass filtering with cut-off at 128 s (0.0078125 Hz), functional scans were analyzed in the context of the general linear model using delta functions convolved with a canonical hemodynamic response function. For the ToL task, event duration, computed as the time between stimulus and response onset, was included in the GLM to account for hemodynamic responses of varying lengths to each type of stimulus. Error trials and head-movement parameters were modeled as regressors of no interest. For the Stroop task, a 'color-word interference' main effect was computed in which brain

Table 7.1. Task performance and IBI changes

		Test sample			Repetition sample		
Task	Condition	Reaction time (ms)	Accuracy (%)	IBI (ms)	Reaction time (ms)	Accuracy (%)	IBI (ms)
Stroop	congruent	808.7 ± 145.5	95.2 ± 6.0	836.3 ± 137.1	792.5 ± 129.8	96.3 ± 5.0	820.2 ± 129.4
	incongruent	956.9 ± 168.4	82.6 ± 12.9	827.7 ± 132.9	967.7 ± 157.9	83.4 ± 10.9	815.3 ± 121.0
ToL	baseline	3566.1 ± 874.8	93.2 ± 4.3	825.1 ± 125.8	3636.6 ± 995.6	94.2 ± 2.6	814.5 ± 121.6
	planning	11580.0 ± 5229.7	75.1 ± 13.1	817.3 ± 124.8	11830.8 ± 4716.1	77.9 ± 13.5	809.2 ± 120.1

Mean reaction times (ms ± sd), mean reaction accuracies (% ± sd) and mean Inter heart Beat Intervals (IBIs: ms ± sd) on color-word congruent and incongruent trials for the Stroop task and on baseline and 4-steps planning trials for the ToL task. Results for the twins in the Test sample are listed in the columns on the left; results for the twins in the Repetition sample are listed in the columns on the right.

activation during trials with color-word incongruent stimuli was compared with brain activation during trials with color-word congruent stimuli. For the ToL task, we applied a 'planning versus baseline' contrast to compare brain activation during 4-steps planning with brain activation during baseline. The 1st-level results for each individual were computed twice; once with and once without taking into account all 7 IBI-regressors. The influence of including IBI-regressors in the GLM was assessed statistically by means of a paired t-test that compared the 1st level results of each subject with and without inclusion of the 7 IBI-regressors. Task main effects across subjects are reported after correction for multiple comparisons using a false discovery rate of 5% and a minimal cluster size of 10 voxels.

Results

Table 7.1 shows mean reaction times, mean reaction accuracies and mean Inter heart Beat Intervals (IBIs) on color-word congruent and incongruent trials for the Stroop task and on baseline and 4-steps planning trials for the ToL task in the Test sample (left columns) and Repetition sample (right). Differences in task performance and IBIs between the two trial types of both tasks are highlighted in **figure 7.2**. Both Stroop color-word interference **(figure 7.2: left)** and ToL planning **(figure 7.2: right)** were associated with significantly reduced reaction accuracies and significantly increased reaction times. Furthermore, reduced performance in both tasks was accompanied by shorter IBIs (= increased heart rate) which were all significant, except for Stroop performance in the Repetition sample. These task related IBI changes create the potential for a confounding effect on the computed fMRI responses.

Figures 7.3 and **7.4** show fMRI main effects of the 7 individual IBI regressors included in the GLM when interpreted as effects of interest. Results are shown separately for tests of positive statistical contrast (T-test contrast: +1) and negative statistical contrast (T-test contrast: -1). Since decreased IBIs indicate increased heart rate and vice versa, the positive contrast tests for an inverse relation between heart rate and fMRI, whereas the negative contrast tests for covariation between heart rate and fMRI. The correlation patterns across the 7 regressors are highly equivalent in the Test sample (top) and Repetition sample (bottom) and also appear similar for the Stroop task **(figure 7.3)** and ToL task **(figure 7.4)**, although the patterns are most robust for recordings during the ToL. For the unshifted regressor (0*TR) and the regressor with a positive time shift of 1*TR, co-variations between heart rate and fMRI (suprathreshold voxels for negative statistical contrast) are evident across the whole brain, with posterior dominance. In contrast for larger positive shifts of 2, 3 and 4*TR, heart rate and fMRI are inversely related (suprathreshold voxels for positive statistical contrast).

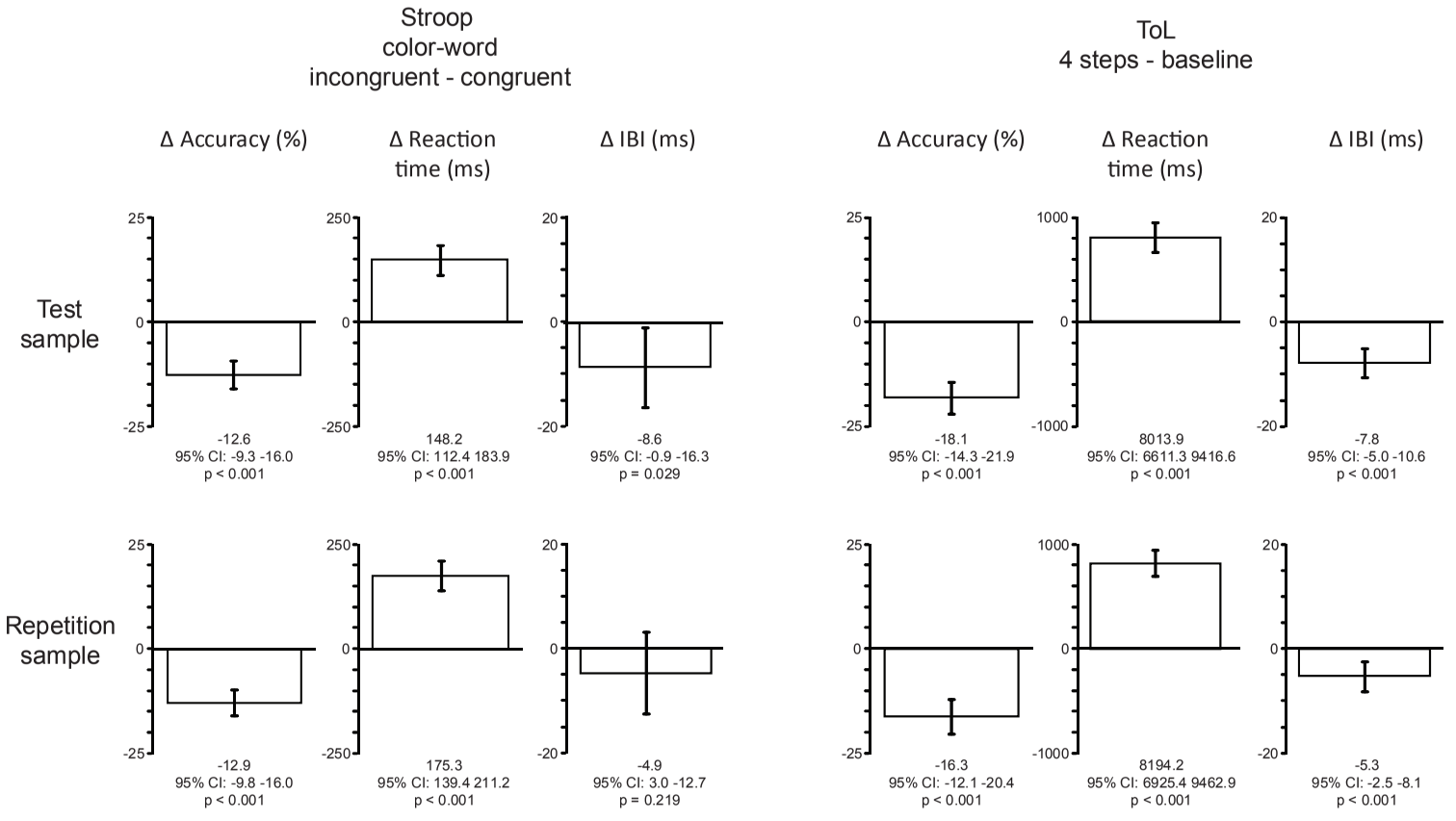


Figure 7.2. *Means of within subject differences in task performance (accuracy and reaction time) and Inter heart Beat Intervals (IBIs) on color-word incongruent compared to congruent trials for the Stroop task (left) and on 4-steps planning compared to baseline trials for the ToL task (right). Results of between condition paired t-test comparisons, for the Test sample (top) and Repetition sample (bottom), are indicated by 95% confidence intervals of the differences (CI: Error bars) and p-values.*

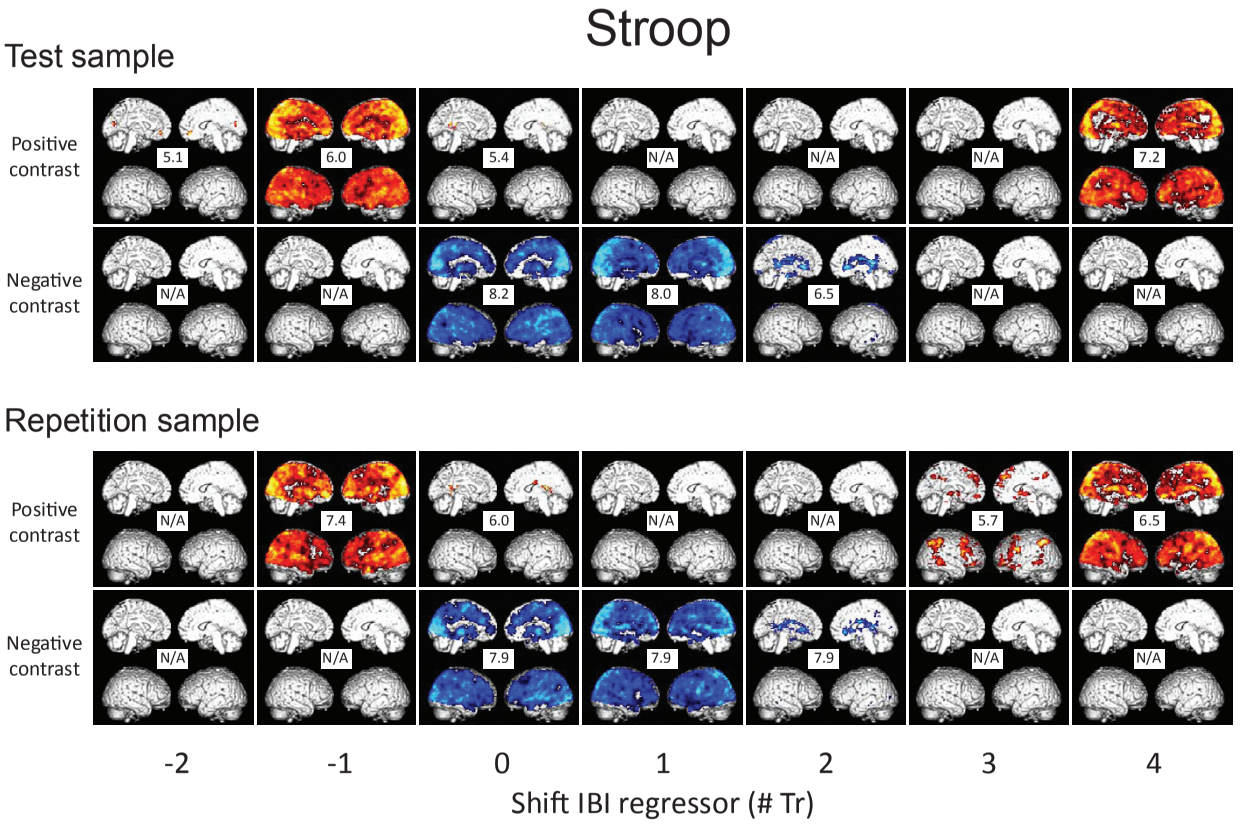


Figure 7.3. *Stroop task main effects of the 7 individual IBI regressors included in the GLM, corresponding to timeshifts relative to the recorded fMRI signals of [-2, -1, 0, 1, 2, 3, 4]*Repetition time (TR=2.3s), for the Test sample (top) and Repetition sample (bottom).*

Since decreased IBIs indicate increased heart rate and vice versa, the tests for positive contrast between IBI and fMRI changes (top rows) indicate voxels where heart rate and fMRI are inversely related, whereas the test for negative contrast (bottom rows) indicate voxels with covariation between heart rate and fMRI modulations. Numbers in each plot indicate statistical T-values for the voxel with highest test significance.

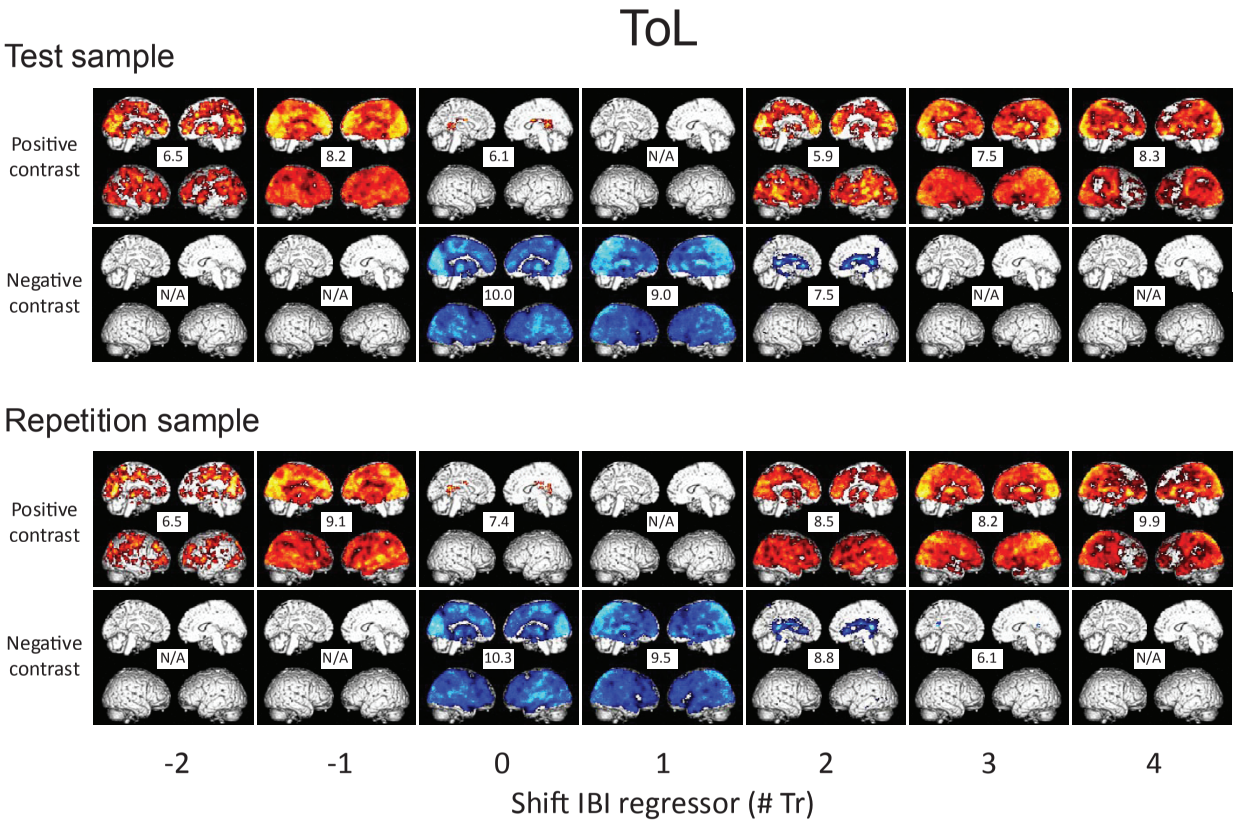


Figure 7.4. *ToL task main effects of the 7 individual IBI regressors included in the GLM, corresponding to timeshifts relative to the recorded fMRI signals of [-2, -1, 0, 1, 2, 3, 4]*Repetition time (TR=2.3s), for the Test sample (top) and Repetition sample (bottom). Numbers in each plot indicate statistical T-values for the voxel with highest test significance.*

Similar inverse heart rate versus fMRI associations are observed for shifts of the IBI regressor back in time, in particular for a shift of -1*TR.

Next, we examined the extent to which the higher heart rates (decreased IBIs) after Stroop color-word incongruent stimuli and after ToL planning stimuli confounded the fMRI main effects obtained using the color-word incongruent versus congruent contrast for the Stroop task and 4-steps planning versus baseline contrast for the ToL task. **Figure 7.5** shows glass brain projections of SPMs for Stroop color-word interference (left panels) and ToL planning (right) in the Test sample (top) and Repetition Sample (bottom). Lists of significant clusters for both tasks and samples are depicted in **tables 7.2** and **7.3**. In both samples, the SPMs computed without IBI-regressors as confounders were highly similar to the SPMs obtained with the 7 IBI-regressors as nuisance effects. Although generally SPM maximum T-scores and number of suprathreshold voxels were lower after including heart rate regressors. To statistically assess the influence of including IBI-data in the GLM, we applied paired t-tests that compared the 1st level results of each subject with and without accounting for the 7 IBI-regressors. Despite this highly sensitive test, we found no significant differences at our statistical threshold of $p<0.05$, FDR corrected. For further exploration, we lowered the threshold to a more lenient value of $p < 0.005$, uncorrected, with no cluster extend limit. The results for the Stroop and ToL task, depicted in **figures 7.6** and **7.7**, revealed a general tendency to find more clusters when testing for larger fMRI activations when using a GLM without heart beat regressors. However, as can be observed by comparing the glass brain projections as well as the MRI slice overlays which indicate the anatomical location with lowest statistical p-value, the results for both tasks and for the Test and Repetition samples did not reveal a consistent pattern of differences at specific brain locations. Furthermore, the effect sizes of the difference contrast at the most significant voxel, as indicated by the bar graph inserts on the slice overlays, were very small (< 0.2).

Finally, we investigated if the impact of heart rate related fMRI changes on computed brain activations was larger in individuals with strong versus weak task related heart rate variation. For this, we computed correlations between task related heart rate change in each subject and the difference in activated number of voxels (at $p < 0.005$, uncorrected) as well as maximum statistical T scores as derived from a GLM with versus without heart rate regressors. The correlation values for both samples, listed in **table 7.4**, indicated no significant associations. For the Stroop task, there were close to significant positive correlations for number of activated voxel and maximum T scores, indicating a tendency for a higher reduction in number of activated voxels and maximum T scores after inclusion of IBI regressors in individuals with a stronger task related heart rate increase, but this was true only in the Repetition sample. For the ToL task there was a close

to significant negative correlation for activated number of voxels, indicating a tendency for an increase in number of suprathreshold voxels after inclusion of IBI regressors in individuals with a stronger task related heart rate increase, but this was found only in the Test sample.

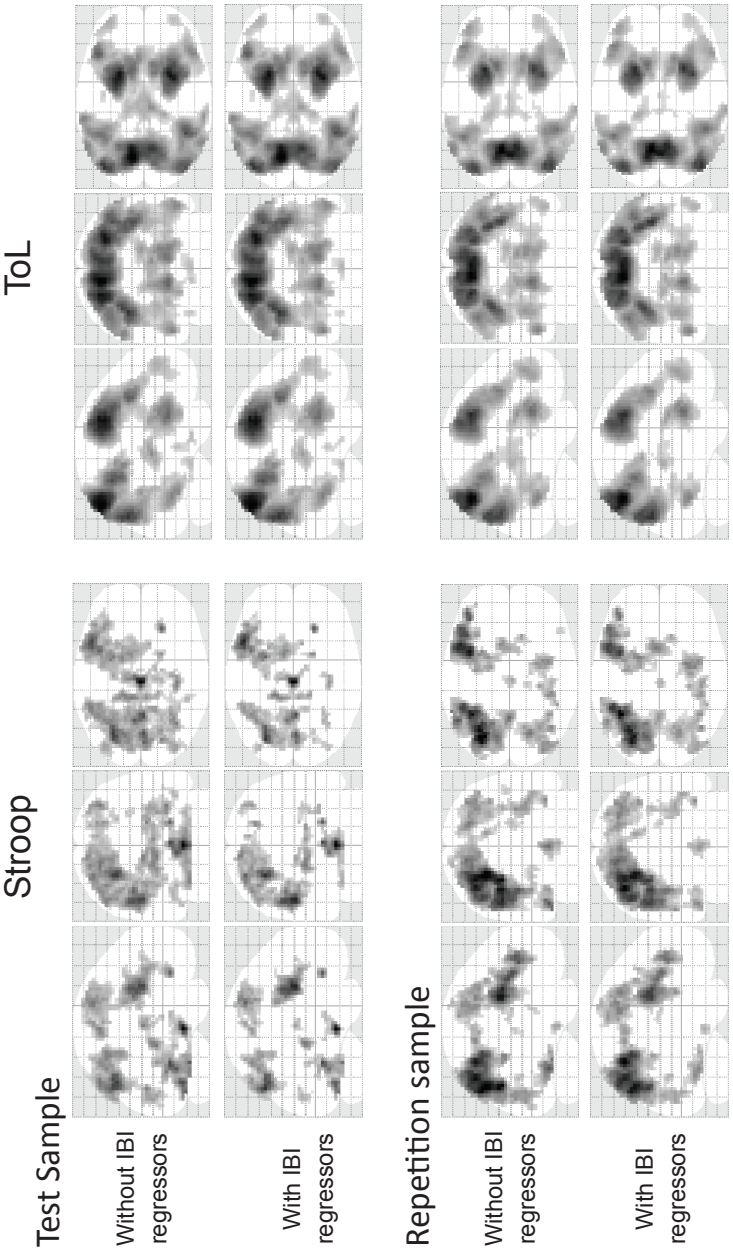


Figure 7.5. *Glass brain projections of SPMs for Stroop color-word interference (left) and Tol planning versus baseline (right) in the Test and Repetition samples. In each pane, top row projections indicate results without the IBI-regressors as confounders; bottom row projections indicate results obtained with the 7 IBI-regressors as nuisance effects.*

Table 7.2. Significant clusters from the SPMs for Stroop color-word interference

		Test sample										Repetition sample									
		Without IBI regressors					With IBI regressors					Without IBI regressors					With IBI regressors				
Anatomical location		MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels
		x	y	z			x	y	z			x	y	z			x	y	z		
L./R.	Brainstem	0	-21	-18	7.06	925	0	-21	-18	6.69	96	3	-18	-24	3.95	31	3	-21	-21	3.96	21
L./R.	Cerebellum	--	--	--	--	--	3	-51	-6	5.34	347	0	-54	-15	5.13	86	0	-54	-15	4.37	74
L.	Caudate/Glob. Pall.	-21	0	21	3.69	21	--	--	--	--	--	-18	-3	0	3.34	12	--	--	--	--	--
R.	Caudate	21	30	-6	5.03	39	21	30	-6	5.06	22	21	-3	18	3.47	14	21	-9	21	3.27	12
L.	Thalamus	--	--	--	--	--	-12	-18	18	3.98	25	--	--	--	--	--	--	--	--	--	--
R.	Thalamus	--	--	--	--	--	--	--	--	--	--	18	-21	15	3.33	13	--	--	--	--	--
L.	Parahippocampus	-9	-36	6	4.71	453	-18	-33	9	4.66	103	--	--	--	--	--	--	--	--	--	--
R.	Parahippocampus	--	--	--	--	--	30	-57	-3	3.83	34	--	--	--	--	--	--	--	--	--	--
L.	Occipital/Parietal	-27	-69	42	5.76	928	-27	-75	45	5.78	532	-27	-75	42	7.47	1365	-36	-51	51	7.63	2089
		--	--	--	--	--	-33	-81	12	3.72	21	-27	-93	0	3.56	18	--	--	--	--	--
R.	Occipital	21	-75	15	4.57	101	21	-75	15	3.65	24	33	-84	15	4.54	162	48	-78	0	4.14	132
R.	Parietal	21	-66	60	4.14	133	21	-66	60	3.45	10	21	-69	57	4.90	413	36	-75	27	3.72	22
		24	-60	42	3.56	19	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
		30	-72	33	3.28	14	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
L.	Temporal	-42	-45	-9	3.65	41	--	--	--	--	--	-51	-57	-15	3.65	41	-51	-57	-15	4.69	128
R.	Temporal	45	-36	-12	3.54	20	--	--	--	--	--	42	-63	-12	3.54	20	42	-63	-12	4.61	15
R.	Precentral	36	-21	57	4.60	88	36	-21	57	4.20	40	42	-30	51	4.57	264	42	-30	51	4.37	88
L.	Frontal	-48	18	18	5.62	1188	-48	18	18	5.97	418	-39	9	27	6.90	785	-39	9	27	6.25	690
		--	--	--	--	--	-27	3	63	4.98	376	-30	6	51	4.65	404	-3	12	54	4.86	487
R.	Frontal	21	6	54	4.13	39	21	6	54	4.33	16	48	27	24	3.25	13	--	--	--	--	--

List of significant clusters from the SPMs for Stroop color-word interference in the Test sample (left) and Repetition sample (right). Left columns indicate results without the IBI-regressors as confounders; right columns indicate the results obtained with the 7 IBI-regressors as nuisance effects. MNI coordinates and T-score are listed for the voxels with largest effect size in each cluster.

Table 7.3. Significant clusters for Tol 4-steps planning versus baseline

		Test sample										Repetition sample									
		Without IBI regressors					With IBI regressors					Without IBI regressors					With IBI regressors				
Anatomical location		MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels	MNI coordinates			T score	# voxels
		x	y	z			x	y	z			x	y	z			x	y	z		
L./R.	Par./Occ./Temp./	-9	-72	57	13.70	4686	-12	-72	60	13.64	4563	-6	-63	54	14.82	5552	-6	-63	54	14.13	3778
	Thalamus/Caudate	-15	9	-3	8.27	1466	-15	9	-3	8.01	1335	--	--	--	--	--	-12	15	0	8.37	855
		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	-21	-33	18	5.18	80
		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	18	-57	21	5.14	81
		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	54	-57	-12	4.91	81
L./R.	Frontal/ Cingulate	27	6	54	12.61	2897	27	6	54	12.64	2843	-24	12	54	12.26	2562	-24	12	54	11.44	1172
		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	24	12	51	11.28	1263
L.	Frontal	-33	57	9	4.83	142	-33	57	9	4.70	138	-39	51	6	6.17	198	-39	51	6	5.96	173
L.	Precuneus	-21	-57	21	3.66	24	-21	-57	21	3.50	15	--	--	--	--	--	--	--	--	--	--
L.	Temporal	-39	-12	-24	3.72	14	-39	-12	-24	3.17	11	--	--	--	--	--	--	--	--	--	--
	Brainstem	0	-33	-24	4.08	59	3	-33	-24	4.52	48	--	--	--	--	--	--	--	--	--	--
		-15	-18	-12	4.00	24	-15	-18	-12	3.59	13	--	--	--	--	--	--	--	--	--	--

List of significant clusters from the SPMs for Tol planning versus baseline in the Test sample (left) and Repetition sample (right). Left columns indicate results without the IBI-regressors as confounders; right columns indicate the results obtained with the 7 IBI-regressors as nuisance effects. MNI coordinates and T-score are listed for the voxels with largest effect size in each cluster.

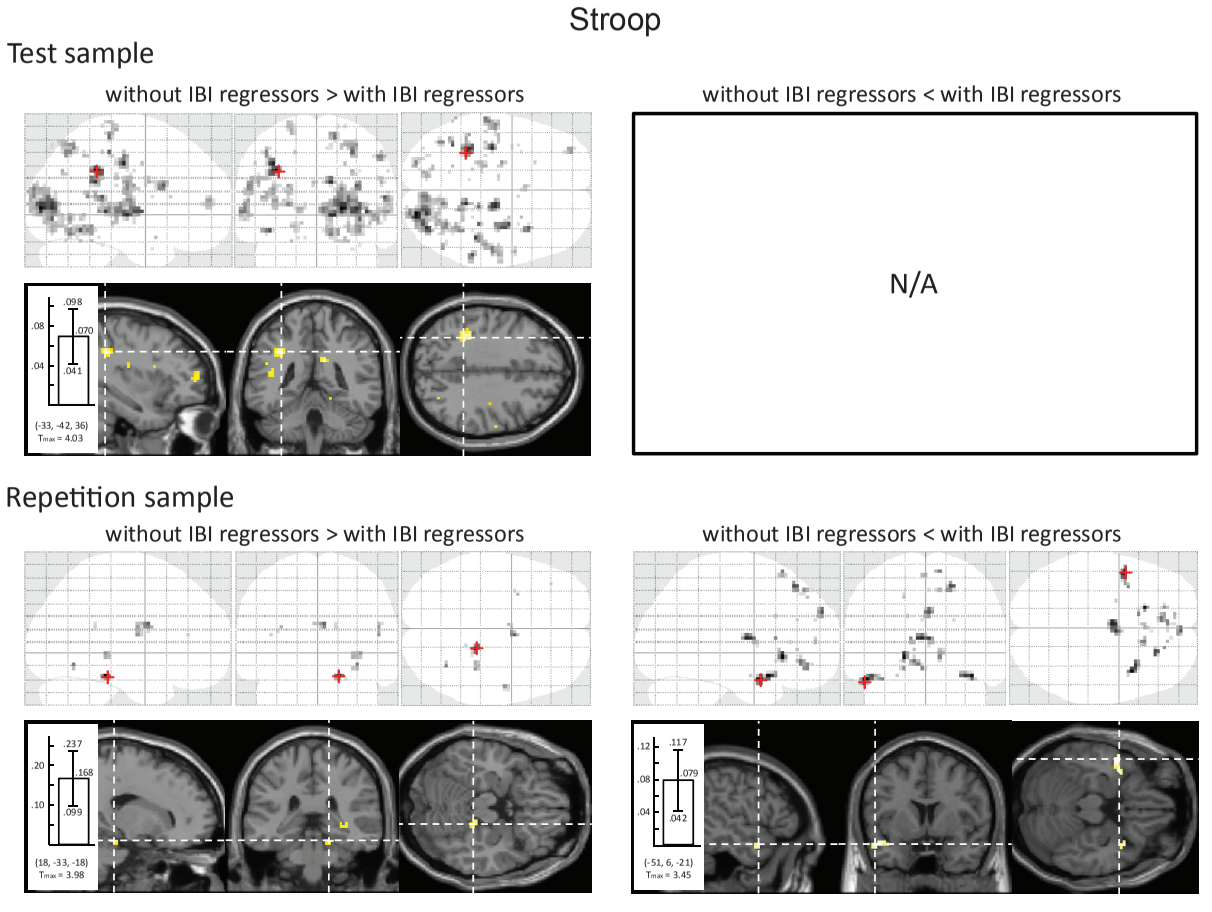
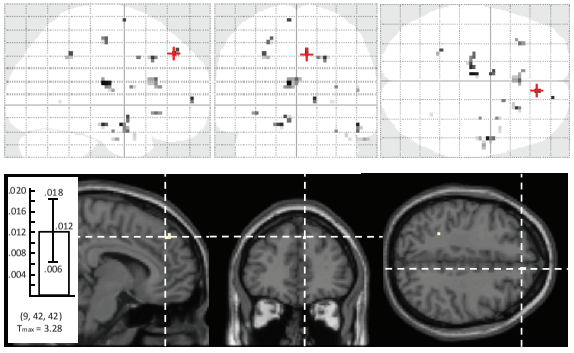


Figure 7.6. *Results of paired t-tests, for the Test sample (top) and Repetition sample (bottom), that compare brain activations during Stroop color-word interference in each individual as estimated with versus without accounting for the 7 IBI-regressors in the GLM. On the left, tests for regions with higher activation when IBI-regressors were not included; on the right, tests for regions with higher activation when IBI-regressors were included. Glass brain projections in each panel indicate the overall distribution of the observed clusters. MRI slice overlays show the voxel with largest statistical difference, with bar graph inserts on each sagittal slice depicting the contrast estimates and 90% confidence interval at this location (numbers within brackets below each bar indicate the MNI coordinates of the voxel and Tmax; the maximum statistical T-value).*

ToL

Test sample

without IBI regressors > with IBI regressors

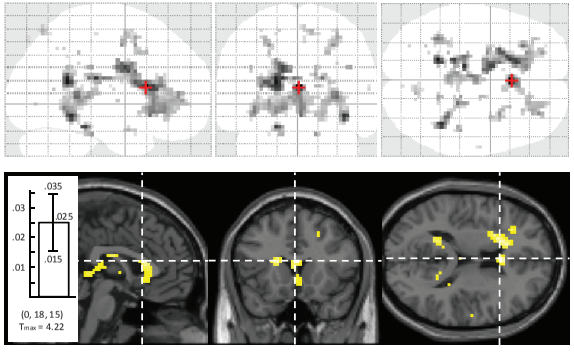


without IBI regressors < with IBI regressors

N/A

Repetition sample

without IBI regressors > with IBI regressors



without IBI regressors < with IBI regressors

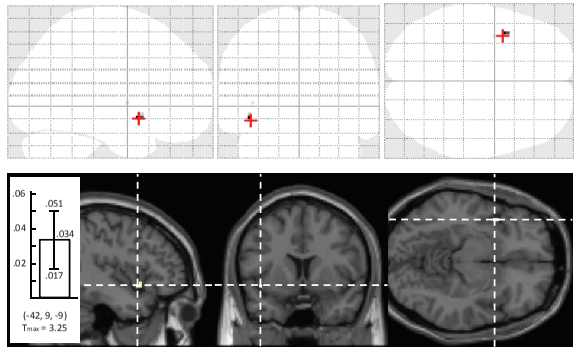


Figure 7.7. *Results of paired t-tests, for the Test sample (top) and Repetition sample (bottom), that compare brain activations for Tol planning versus baseline in each individual as estimated with versus without accounting for the 7 IBI-regressors in the GLM.*

Table 7.4. Correlations of 1st level results with task related IBI changes

	Test sample						Repetition sample					
task	# activated voxels			Maximum T score			# activated voxels			Maximum T score		
	N	*p*	Pearson r	N	*p*	Pearson r	N	*p*	Pearson r	N	*p*	Pearson r
Stroop	46	0.665	0.066	46	0.563	-0.088	45	0.083	0.261	45	0.077	0.266
ToI	46	0.067	-0.273	46	0.305	-0.155	45	0.485	0.114	45	0.238	0.180

Correlations between the task related heart rate change in each subject and the difference in number of suprathreshold voxels (# activated voxels: at p < 0.005, uncorrected) and maximum statistical T scores (Maximum T score) as derived from a GLM with versus without heart rate regressors. Left columns: results for the Test sample; right columns: results for the Repetition sample. N: number of subjects; Pearson r: Pearson's bivariate correlation coefficient; p: statistical significance of correlation. Significant positive correlations would indicate that inclusion of heart rate regressors results in a relatively larger reduction in number of activated voxels/maximum T scores for subjects with stronger task related heart rate increases and vice versa. Significant negative correlations would indicate a weaker reduction (or an increase) in number of activated voxels/maximum T scores after inclusion of heart rate regressors in subjects with stronger increases in heart rate and vice versa.

Discussion

In this study we found that heart rate was influenced by task difficulty. This is in line with previous evidence for substantial momentary variation in heart rate both at rest and during conditions of mild cognitive load, assumed to reflect a complex set of hormonal, thermoregulatory, hemodynamic and respiratory effects on neural control over the heart (Berntson et al., 1997). The increase in heart rate to ToL planning stimuli was quantitatively similar to the heart rate change noted after color-word incongruent Stroop stimuli, despite the fact that the ToL task was administered in an event-related design, rather than a blocked design as used for the Stroop. It must be emphasized however that the inter-stimulus time windows in the present ToL task were determined by the planning reaction times of the subjects which amounted to about 12 seconds for the 4-steps condition. These relatively long trial spacings provide a substantial amount of time to allow for significant heart rate changes.

The spontaneous variations in heart rate were highly correlated with the fMRI modulations during our cognitive paradigms. Similar correlations across large parts of the brain, but most robust over posterior regions, have been noted for functional recordings during rest (Chang et al., 2009; de Munck et al., 2008; Shmueli et al., 2007). Furthermore, the pattern of correlations across the 7 heart rate regressors in this study is highly consistent with the response function between heart rate and fMRI modulations during the resting state condition as reported by de Munck and colleagues [(2008), cf. fig. 4]. For the IBI regressor aligned in time with the fMRI data (0*TR) and a regressor delayed by 1*TR, positive associations

between heart rate and fMRI changes were observed, while for larger delays of 2, 3 and 4*TR, there were inverse associations. This essentially indicates that heart rate increases are initially followed, between 0 and 4.6 seconds, by enhanced fMRI signal, and thereafter, between 4.6 and 9.2 seconds, by reduced fMRI signal (and vice versa). Similar inverse relations were observed for the IBI regressors advanced in time by -2*TR, and in particular -1*TR. This would mean that fMRI signal increases (decreases) are generally followed up to 4.6 second later by decreased (increased) heart rate.

It should be kept in mind that the associations between heart rate and fMRI modulations observed in this study do not necessarily imply causal relations. For example, the possibility that shifting the IBI regressor backward or forward in time just results in bringing the low frequency component of the IBI changes either in or out of phase with respect to the low frequency component of the fMRI modulations may play a role. These low frequency fluctuations in IBI and fMRI signals may for example be caused by respiratory effects that are ignored in our study. However, the fact that the correlation pattern across the 7 heart beat regressors was similar during both tasks of this study, in particular for the unshifted regressor and the regressor delayed by 1*TR, and that IBI-fMRI response functions comparable to the present ones have been observed in the resting state study by de Munck et al. (2008), indicates that the observed heart rate-fMRI relations, at least partly, represent systematic effects.

Irrespective of the exact biological background, the presence of associations between heart rate and fMRI changes together with our finding that heart rate was influenced by task demands creates a theoretical potential for confounding of computed fMRI group effects. In the paradigms and control subjects we explored, however, the effect was small and not statistically significant. For both Stroop color-word interference and ToL planning, comparison of fMRI main effects after ignoring IBI information in the GLM with the main effects obtained after using IBI time series as nuisance regressors revealed the same general neurocircuitry engaged. In this context, it should be noted that the reduction of inter beat times after incongruent compared to congruent color-word stimuli for the Stroop and 4-steps planning versus baseline for the ToL were both in the order of about 8 milliseconds. Although statistically significant, these changes are only marginal relative to the standard deviations of IBI times in this study which were in the order of 130 ms. Overall, SPMs from the GLM with regressors did show a tendency towards small reductions in voxel T-scores and number of suprathreshold voxels compared to the SPMs obtained without heart beat regressors. This effect of heart rate regressors on the SPMs, although not statistically significant, does imply that their inclusion in the GLM can potentially influence final conclusions, in particular in studies dealing with weak-to-detect fMRI signal changes.

Our final conclusion is that there are substantial correlations between heart rate and fMRI signal changes across large parts of the brain during performance of cognitive tasks. However, even if heart rate is significantly modulated by task demands, the fMRI signals associated with heart rate variations only marginally impact on higher-order fMRI task effects. This conclusion is based on heart rate and fMRI data recorded during a commonly used cognitive task administered in a block design as well as a standard cognitive task presented in an event-related design, with relatively wide trial spacing. However, we expect similar results for cognitive test paradigms with closer trial spacing, in particular since smaller trial-to-trial onset times allow less time for significant heart rate changes. The absolute values of the observed IBI changes in this study were relatively small (around 8 ms). During tasks with higher emotional valence or in specific patient samples, heart rate changes and their effect on fMRI signals may be more pronounced. In particular for these cases we still recommend to evaluate the effect of including IBI data as nuisance regressors, also considering the small effort of deriving the regressors from the ECG.