Brain mechanisms of unconscious cognitive control

van Gaal, S.

Citation for published version (APA):
5. Unconscious activation of the prefrontal No-Go network

Abstract
Cognitive control processes involving prefrontal cortex allow humans to overrule and inhibit habitual responses in order to optimize performance in new and challenging situations, and traditional views hold that cognitive control is tightly linked with consciousness. We used functional magnetic resonance imaging to investigate to what extent unconscious “No-Go" stimuli are capable of reaching cortical areas involved in inhibitory control, particularly the inferior frontal cortex (IFC) and the pre-supplementary motor area (pre-SMA). Participants performed a Go/No-Go task that included conscious (weakly masked) No-Go trials, unconscious (strongly masked) No-Go trials as well as Go trials. Replicating typical neuroimaging findings, response inhibition on conscious No-Go stimuli was associated with a (mostly right-lateralized) frontoparietal “inhibition network”. Here we demonstrate, however, that also an unconscious No-Go stimulus can activate prefrontal control networks, most prominently the IFC and the pre-SMA. Moreover, if it does so, it brings about a substantial slow-down in the speed of responding; as if participants attempt to inhibit their response but just failed to withhold it completely. Interestingly, overall activation in this “unconscious inhibition network” correlated positively with the amount of slow-down triggered by unconscious No-Go stimuli. In addition, neural differences between conscious and unconscious control are revealed. These results expand our understanding of the limits and depths of unconscious information processing in the human brain, and demonstrate that prefrontal cognitive control functions are not exclusively influenced by conscious information.

Introduction
Recent experiments revealed a plethora of often astounding effects of unconscious stimuli on behavior, perception and cognition. To name a few, unconscious information has been shown to influence motivation (Pessiglione et al., 2007), reward value and decision-making (Pessiglione et al., 2008), emotional processing (Whalen et al., 1998), object recognition (Stoerig & Cowey, 1997), semantic processing (Dehaene et al., 2001) and action planning/execution (Binsted et al., 2007). These thought-provoking results have raised important questions about the limits of unconscious cognition and the evolutionary function of consciousness (Dehaene, 2008; Dehaene & Naccache, 2001).
Though unconscious information seems to have a wide range of effects on many psychological mechanisms, a debatable case is the existence of unconscious cognitive control (Dehaene et al., 2003; Eimer & Schlaghecken, 2003; Jack & Shallice, 2001; Lau & Passingham, 2007; Mayr, 2004; Umilta, 1988; van Gaal et al., 2008). Cognitive control processes are those processes that regulate and monitor ongoing actions to optimize goal-directed behavior, which become necessary when environmental demands change. The recruitment and implementation of cognitive control is strongly associated with the prefrontal cortex (PFC) (Fuster, 2000; Miller, 2000; Ridderinkhof et al., 2004). It is also the PFC that has been most strongly associated with conscious perception, whereas unconscious perception is usually not (Crick & Koch, 2003; Kouider & Dehaene, 2007; Rees et al., 2002). Therefore, it seems likely that consciousness and cognitive control are intimately related and this belief is sometimes so strong that authors naturally refer to the concept of “conscious cognitive control” as if “unconscious cognitive control” is inconceivable (Hommel, 2007). However recently, we (van Gaal et al., 2008; van Gaal et al., 2009) and others (Lau & Passingham, 2007) put this long-held assumption to a direct test and provided evidence for the existence of unconsciously triggered cognitive control.

To illustrate, in a recent electroencephalographic (EEG) study we demonstrated that an unconscious No-Go stimulus can trigger inhibitory control (van Gaal et al., 2008), commonly assumed to conscious control mechanisms (Eimer & Schlaghecken, 2003; Libet, 1999; Pisella et al., 2000). Source imaging suggested that unconsciously triggered inhibitory control was associated with activity in the lateral PFC. In the present work we tried to further illuminate the possible depth of processing of unconscious information using methods that allow more spatial precision in our neuroanatomical inferences, namely functional magnetic resonance imaging (fMRI). We devised a new Go/No-Go task in which conscious (weakly masked) and unconscious (strongly masked) No-Go signals were randomly mixed with Go signals. In this task, unconscious No-Go signals triggered a substantial slow-down in the speed of responding; as if participants attempted to inhibit their response but just failed to withhold it completely. This allowed us to investigate to what extent unconscious No-Go signals are capable of activating brain areas involved in inhibitory control, such as the inferior frontal cortex (IFC) and the pre-supplementary motor area (pre-SMA) (Aron, Behrens, Smith, Frank, & Poldrack, 2007; Chikazoe et al., 2009; Forstmann, van den Wildenberg, & Ridderinkhof, 2008; Leung & Cai, 2007; Mostofsky & Simmonds, 2008; Wager et al., 2005).
Methods and Materials

Participants
Twenty-nine undergraduate psychology students of the University of Amsterdam were recruited for a behavioral experiment in which the task was explained and practiced. Twenty-four of these participants were selected to participate in the fMRI experiment (for selection methods see below). All participants gave their written informed consent prior to participation, were right-handed as assessed by the Edinburgh inventory (Oldfield, 1971), had normal or corrected-to-normal vision and were naive to the purpose of the experiments. All procedures were executed in compliance with relevant laws and institutional guidelines and were approved by the local ethical committee.

The masked Go/No-Go task
The blood-oxygen level dependent (BOLD) signal was measured while participants performed a newly devised Go/No-Go task in which we randomly mixed conscious (weakly masked) and unconscious (strongly masked) No-Go trials as well as Go trials in a Go/No-Go block. In this task, participants were instructed to respond as fast as possible to a white annulus (the Go signal, visual angle of 0.80°), but withhold their response when a white square (the No-Go stimulus, visual angle of 0.47° x 0.47°) briefly preceded the annulus. However, when a diamond (i.e. the same square but tilted by 45°) preceded the annulus, they were instructed to respond as quickly as possible. In one condition, the annulus was ineffective in masking the preceding stimulus (square/diamond), since the square/diamond was presented relatively long (233 ms) and the annulus fairly brief (16.7 ms). Therefore the square/diamond was clearly visible. We will refer to this condition as the weakly masked condition. Crucially, in a second condition the square/diamond was presented very briefly (16.7 ms) and was followed after only a brief delay (33 ms) by the Go annulus (duration 200 ms). We will refer to these conditions as the strongly masked condition, because the annulus functioned as a metacontrast mask, which is known to strongly reduce stimulus visibility (Breitmeyer, 1984). The combination of these factors effectively rendered the participants incapable of perceiving the square/diamond, as evidenced by chance-level performance on a forced-choice discrimination task administered after the experiment (see Results). In the strongly masked condition, participants just perceived a white annulus and therefore treated these trials as Go trials.

Because the square functioned as a No-Go signal in the weakly masked conditions, this stimulus was consistently associated with response inhibition. On
the contrary, the diamond was not associated with response inhibition because participants were instructed to respond to trials containing a diamond. Because each of the four conditions (weakly/strongly masked * square/diamond) was presented in 25% of the occasions, overall, a conscious No-Go signal was presented in 25% of the trials (30 trials of each conditions; 120 in total per block). Because all conditions are presented equally frequently, we could investigate the processing of No-Go signals without confounding response inhibition with the processing of infrequent stimuli (see also Chikazoe et al., 2009) (see Figure 5.1 for stimulus and trial timing). The stimulus used as No-Go signal (square or diamond) was counterbalanced across subjects. Note that from fixation cross presentation, the duration until annulus offset of all conditions was equal (duration 750 ms).

Procedure
Before the actual fMRI experiment participants were invited to the lab to practice the task (1h). In a pilot study participants reported to see 'flicker' before the appearance of the annulus in the strongly masked conditions, as if the annulus quickly expanded when it appeared on the screen. Therefore, in the practice session before the actual fMRI experiment, participants were told that the white annulus quickly expanded (grew bigger) when it appeared on the screen. Most of the participants were able to follow the instructions and perform the task proficiently in the practice session already. However, in an informal interview during the practice session, five participants indicated to have difficulty to differentiate between the conditions. Therefore, based on this observation, these participants were not invited to participate in the upcoming fMRI experiment.

During the scanning session, participants performed one block of the task outside the scanner. Inside the scanner, participants performed four blocks of the Go/No-Go task. Immediately after the imaging session participants were informed about the fact that in the “annulus only” condition, actually, an annulus was always preceded by a diamond or a square. Next, to test their visibility of these stimuli, participants performed a two-alternative forced-choice discrimination task while still lying in the scanner (48 trials; 24 trials of each strongly masked condition). Stimulus and trial timing was exactly the same as in the masked Go/No-Go task. After the presentation of a trial, a pair of choices was presented left and right of fixation. Participants were asked to determine whether a square or a diamond was presented in the preceding trial. The two alternatives remained on the screen until the subject made a response, after which a new trial started. Before administering this task, participants were told that squares and diamonds were presented equally frequently
and were instructed to consider this in giving their response. They were also told that only accuracy was important in this task, not the speed of responding.

![Figure 5.1 Experimental design](image)

*Figure 5.1 Experimental design*

The duration of the square/diamond, the duration of the metacontrast Go-annulus and the stimulus onset asynchrony (SOA) between the square/diamond and the metacontrast Go-annulus was varied. To this end, the mask was unsuccessful in masking the preceding square/diamond on some occasions (weakly masked conditions), but rendered it invisible at others (strongly masked conditions, see Results). Thereby two factors were manipulated: Trial (Go or No-Go) and Visibility (weakly masked or strongly masked), which constitutes a 2 X 2 factorial design with the following four conditions: 1) a conscious No-Go condition, 2) a weakly masked Go condition, 3) an unconscious No-Go condition, and 4) a strongly masked Go condition.

*Behavioral data analyses*

For the RT analyses, responses between 100 ms and 1000 ms were included. For the RT analysis, a paired two-tailed t-test was performed on mean RT on responded strongly masked Go trials and responded unconscious No-Go trials. For the RT distributions, we calculated the number of responses in bins of 50 ms ranging from 100 to 700 ms. Inhibition rates were computed by taking all trials without a response before the start of the next trial. For the analysis of the inhibition rates, a paired two-tailed t-test was performed on the square root of the percentage of responding on strongly masked Go trials and unconscious No-Go trials. Discrimination performance was tested for significance for each individual
participant using a binominal test evaluated at a $p$-value of 0.05. Subsequently, on a group level, a one-sample t-test was performed on the $d$-scores (tested against 0).

**fMRI scanning and analysis**

Data was collected on a Philips 3T Intera scanner. The scanning session always started with a 3D T1 scan (T1 TFE, 250$^2$ mm FOV, 256$^2$ inplane resolution, 182 slices, 1.2 mm slice thickness, TR 9.6 sec, TE 4.6 ms, FA 8°, saggital orientation). Next, four runs (lasting ~8 minutes each) of functional data were collected (TR 2.29 sec, TE 30 ms, 220$^2$ mm FOV, 72$^2$ inplane resolution, 35 slices, 3.3 mm slice thickness, FA 90°, transversal orientation) covering the whole brain. Each run contained 120 trials (30 of each condition). Trial sequences were optimized using OptSeq (http://surfer.nmr.mgh.harvard.edu/optseq). We used a rapid presentation design with an average trial-time of 4 seconds (ranging from 2-16 seconds). Stimuli were presented on a back-projection screen, which was viewed via a mirror system attached to the MRI headcoil.

FEAT (FMRI Expert Analysis Tool) Version 4.0, part of FSL (FMRIB’s Software Library, www.fmrib.ox.ac.uk/fsl) was used to analyze the fMRI data. fMRI images were realigned to compensate for small head movements (Jenkinson, Bannister, Brady, & Smith, 2002). Functional data were aligned to the structural image of the subject and the data of each subject was transformed to the standard space of the Montreal Neurological Institute (MNI) using FLIRT. Next, the functional data were spatially smoothed using a 5 mm full-width-half-maximum Gaussian kernel and high-pass filtered in the temporal domain ($\sigma = 40 \text{s}$). Finally, the functional data were prewhitened using FSL (Woolrich, Ripley, Brady, & Smith, 2001).

The following conditions were separately modeled by convolution with a double-gamma response function in a General Linear Model (GLM): 1) inhibited conscious No-Go trials, 2) responded conscious No-Go trials, 3) responded weakly masked Go trials, 4) responded unconscious No-Go trials, and 5) responded strongly masked Go trials. The theoretically possible other trial types (e.g. non-responded weakly masked Go trials, or non-responded strongly masked Go trials or non-responded unconscious No-Go trials) were not encountered often enough in all subjects to warrant inclusion. Runs were pooled on a per subject basis using a fixed-effects model. Subsequently, a mixed-effects group analysis was conducted using FMRIB’s FLAME stages 1 and 2 in which relevant lower-level contrasts were combined. Two participants did not have any trials to model the condition in which conscious No-Go trials were responded to, in one out of the four runs. These two runs were excluded from all analyses.
First, we identified voxels involved in consciously triggered response inhibition by contrasting inhibited conscious No-Go trials with responded weakly masked Go trials. For this contrast we report cortical regions with a height threshold of $Z > 3.1$ ($p < 0.001$) and a cluster probability of $p < 0.05$, corrected for whole-brain multiple comparisons (using Gaussian random field theory (GRFT)) (Worsley, 2001). For a full list of conscious activations see Supplementary Table 5.1. To test which brain areas can be activated unconsciously, we looked for significant activations by contrasting responded unconscious No-Go trials with responded strongly masked Go trials (whole-brain analysis). As expected, the extent and strength of the unconscious No-Go activations was less strong and extensive than for conscious No-Go activations (see also Dehaene et al., 2001; Nakamura et al., 2007), therefore we selected cortical regions with voxels exceeding a $Z$ of 3.1 and clusters consisting of more than 20 contiguous significant voxels (four clusters, reported in Table 5.1).

Although the whole-brain cluster-corrected analysis of the consciously triggered No-Go activations did not reveal significant PCC activation (see Supplementary Table), the uncorrected ($Z > 3.1$) conscious activations revealed a rather specific cluster in the PCC (4, -22, 26; $z = 4.21$; see Figure 5.3) that was strikingly similar to the PCC activation revealed by the unconscious No-Go contrast (see Fig 5.4a) as well as in previous stop-signal and Go/No-Go studies (Aron et al., 2007; Aron & Poldrack, 2006; Boehler et al., 2008; Durston, Thomas, Worden, Yang, & Casey, 2002; Ray Li et al., 2006; Wager et al., 2005). However, this cluster was too small (92 voxels), with respect to the other clusters, to survive standardized whole-brain cluster correction. Since we show unconsciously activated clusters with more than 20 contiguous voxels with a $Z$-value larger than 3.1 in Figure 5.4a, for accurate comparison between the conscious and unconscious No-Go activations, the same was done in Figure 5.3 (conscious No-Go activations). It should be noted that all conscious No-Go activations displayed in Figure 5.3, except the PCC, survived whole-brain cluster correction (see Supplementary Table).

To visualize the temporal profile of the BOLD response in the right IFC for each of the four conditions we selected all overlapping right IFC voxels from the conscious contrast and the unconscious contrast. To increase the number of overlapping voxels and thereby the signal-to-noise ratio a slightly more liberal threshold ($Z > 2.3; p < 0.01$) was used to select overlapping voxels. A deconvolution analysis was performed on this right IFC cluster (195 voxels) to visualize the time-course of the BOLD response with slightly smoothed delta functions to model the individual components of the hemodynamic BOLD response (Dale & Buckner, 1997). For the deconvolution analysis, we up-sampled the temporal resolution of the BOLD-
signal to 1.14 seconds (TR/2) and modeled the BOLD response in the period of -3 to 16 seconds per run, per subject. These were subsequently averaged over runs and subjects (see Figure 5.4d). Two-tailed paired t-tests (evaluated at $\alpha = 0.05$) were performed to test for significant differences between the conditions. For all correlational analyses we performed nonparametric Spearman’s rank correlations evaluated at $\alpha = 0.05$ (two-tailed).

**Results**

*Probing the effectiveness of the masking procedure*

A prerequisite for studying the effects of unconscious No-Go signals on brain and behavior is the effectiveness of our making procedure. To assess whether participants were truly unaware of strongly masked squares and diamonds, a two-alternative forced-choice discrimination task was administrated after the Go/No-Go task, while participants were still lying in the scanner. The discrimination task was administered after the main experiment to rule out any effect of perceptual learning during the experiment. In this conservative measure of stimulus visibility, 20 out of 24 participants scored at chance-level (binominal test, $p > 0.05$), suggesting that these individuals were unable to perceive strongly masked squares/diamonds. Because we cannot ascertain that the other four participants were truly unaware of strongly masked signals during the Go/No-Go experiment, these participants were excluded from all further analyses. Also at the group-level, discrimination performance ($d'$) did not deviate from chance-level for the included 20 participants ($d' = 0.118, SD = 0.32, mean percentage correct = 52.3\%, SD = 6.15, t(19) = 1.667; p = 0.112, Figure 5.2a). After the presentation of the behavioral and neuroimaging results, several additional correlational analyses between behavioral/brain data and discrimination scores are reported that further indicate that participants were unable to perceive strongly masked squares/diamonds in the present experiment.

*Unconscious No-Go signals slow down responses*

Participants were able to perform this Go/No-Go task proficiently, as evidenced by typical inhibition rates of 69.9\% on conscious No-Go trials, while still responding quickly to weakly masked Go trials (367 ms). Whereas conscious No-Go trials triggered complete response inhibition on the majority of trials, participants did not inhibit their responses more often on unconscious No-Go trials (0.58\%) than on strongly masked Go trials (0.46\%). Crucially, however, RTs were significantly longer to unconscious No-Go trials than to strongly masked Go trials ($t(19) = 6.24; p < 0.001$, Figure 5.2b). Moreover, the entire response time distribution of unconscious
No-Go trials was shifted in time compared to the response time distribution of strongly masked Go trials (Figure 5.2c), which indicates that RT slowing induced by unconscious No-Go signals was not due to only a few trials. The combination of these results indicates that unconscious No-Go signals triggered inhibitory control processes, but not sufficiently to withhold the overt response. Although not successful as such, the attempt to inhibit may have resulted in a slower build-up of response activation, thus leading to slowing of the imminent response.

![Figure 5.2 Unconscious No-Go signals slow down responses](image)

**Figure 5.2 Unconscious No-Go signals slow down responses**

a) Participants were unable to discriminate between trials with a strongly masked square or diamond, as revealed by chance-level performance in a two-alternative forced-choice discrimination task administered after the main experiment. b) Although strongly masked (unconscious) No-Go signals could not be perceived consciously, they still triggered inhibitory control processes as revealed by a significant longer response times on these trials than on strongly masked Go trials. c) Response time distributions of unconscious No-Go trials and strongly masked Go trials. All plots indicate mean performance ± intersubjects standard deviations.

**Neural mechanisms of conscious and unconscious inhibitory control**

To examine the neural mechanisms underlying consciously triggered inhibitory control we contrasted inhibited conscious No-Go trials with responded weakly masked Go trials (Z > 3.1, whole-brain cluster-corrected). Consciously initiated response inhibition was associated with a typical (mostly right–lateralized) frontoparietal network associated with No-Go inhibition (for a full list of activations see Supplementary Table). This “conscious inhibition network” most prominently included the right and left inferior frontal cortex (IFC) bordering and extending into the anterior insula (AI), the pre-supplementary motor area (pre-SMA), the anterior cingulate cortex (ACC), the right superior frontal gyrus (SFG), the right dorsolateral prefrontal cortex (dLPPC), the right middle frontal gyrus (MFG), the posterior
cingulate cortex (PCC) and bilateral inferior and superior parietal cortices (see Figure 5.3). This network is consistent with previous results of (conscious) No-Go inhibition (Blasi et al., 2006; Chikazoe, Konishi, Asari, Jimura, & Miyashita, 2007; Garavan, Ross, Murphy, Roche, & Stein, 2002; Konishi et al., 1999; Rubia et al., 2003; Simmonds et al., 2008; Wager et al., 2005).

Figure 5.3 Neural activation associated with consciously triggered No-Go inhibition
The contrast between inhibited conscious No-Go trials versus responded weakly masked Go trials revealed activation in a (largely right-lateralized) frontoparietal inhibition network. For a full list of activated regions (Z > 3.1, whole-brain cluster-corrected), see Supplementary Table. To accurately compare conscious and unconscious activations, clusters with more than 20 voxels with a Z > 3.1 are displayed (see Methods).

To examine the activation related to the unconscious initiation of inhibitory control we contrasted responded unconscious No-Go trials with responded strongly masked Go trials (Z > 3.1, clusters containing more than 20 contiguous significant voxels). Unconsciously initiated response inhibition was associated with activation in the right and left IFC, the pre-SMA and the PCC (see Figure 5.4a and Table 5.1). All four clusters are typically observed during consciously initiated response inhibition, particularly the IFC and the pre-SMA are generally concerned as key brain regions involved in actively inhibiting responses in stop-signal and Go/No-Go tasks. Within this “unconscious inhibition network” the right IFC was most strongly activated by unconscious No-Go signals, as evidenced by the relatively high Z-value and large size of this cluster. If these areas co-operate during unconsciously triggered response inhibition then their activation level might be correlated. Indeed, across subjects
activation (unconscious No-Go > strongly masked Go) in the right IFC correlated with activation in both the left IFC (rho = 0.685, p = 0.001, see Figure 5.4b) and the pre-SMA (rho = 0.566, p = 0.009). No other significant correlations were observed between activation across any of these four brain regions (all rho’s < 0.33, all ps > 0.15).

To test whether this unconsciously initiated activation pattern is truly related to the initiation of inhibitory control, we further examined whether individual differences in activation levels in the unconscious inhibition network could explain why unconscious No-Go signals slow down responses for some participants more than for others. More specifically, the mean activation of all four brain regions was correlated with the amount of slow-down triggered by unconscious No-Go signals (mean RT unconscious No-Go trials > mean RT strongly masked Go trials) across all participants. Interestingly, overall activation in the unconscious inhibition network correlated positively with the amount of slow-down (rho = 0.452, p = 0.046, see Figure 5.4c). Thus the strength of activation in the unconscious inhibition network predicted the extent to which individuals slow down their responses to unconscious No-Go signals, which further supports that the observed activations are “functional” in the sense that they predict the impact of unconscious No-Go signals on subsequent behavior.

As a next step, we investigated whether this positive correlation between brain activation and behavioral performance was driven by activation in certain specific clusters within the unconscious inhibition network. Therefore, post-hoc correlational analyses between brain activation and RT slowing were performed on each of the four individual clusters. Significant positive correlations were observed in the right IFC (rho = 0.482, p = 0.031) and the left IFC (rho = 0.479, p = 0.033), but not in the pre-SMA (rho = 0.293, p = 0.209) or the PCC (rho = 0.270, p = 0.250). Thus, activation in the right IFC and left IFC predicted the extent to which individuals slowed down their responses after the presentation of an unconscious No-Go signal, suggesting a crucial role of these areas in processing unconscious No-Go signals and the subsequent implementation of response inhibition.

To assess the temporal extension of the frontal activations and to further assess whether brain activation could be utilized to discriminate between both strongly masked and weakly masked conditions, we performed a deconvolution analysis to extract the BOLD time-courses from the right IFC cluster. Figure 5.4d shows that the BOLD response to unconscious No-Go trials was significantly larger than the BOLD response to strongly masked Go trials for all data points from 3.52 s to 6.84 s (left panel). The difference between conscious No-Go trials and weakly
masked Go trials (right panel) was larger, started earlier (from 2.28 ms) and lasted longer (till 9.12 ms), but resembled the BOLD time courses observed on strongly masked conditions. Thus, the processing of conscious and unconscious No-Go signals differed in the temporal extension and strength of their activation.

**Figure 5.4 Neural activation associated with unconsciously triggered No-Go inhibition**

- **a)** The unconscious inhibition network revealed by the contrast between responded unconscious No-Go trials versus responded strongly masked Go trials ($Z > 3.1$, clusters with more than 20 contiguous significant voxels). Also activations at a more liberal threshold ($Z > 2.3$, in blue) are shown to observe the extension of the activations in each of the four unconsciously activated brain regions.
- **b)** Inter-regional across subjects’ Spearman’s rank correlations. Activation in the right IFC correlated with activation in the left IFC and activation in the pre-SMA.
- **c)** Across-subjects’ Spearman’s rank correlation between unconscious RT slowing and activation in the unconscious inhibition network (the mean activation of all four clusters).
- **d)** BOLD time-courses for all four conditions in the right IFC (see Methods). The left panel depicts the strongly masked...
conditions and the right panel depicts the weakly masked conditions. Bars are ± intersubjects standard errors of the mean (SEM). *p < 0.05 (two-tailed).

Table 5.1 Unconsciously triggered response inhibition

<table>
<thead>
<tr>
<th>No-Go &gt; Go</th>
<th>side</th>
<th>BA</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z-value at local maximum</th>
<th>Number of voxels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inferior frontal cortex</td>
<td>R</td>
<td>47</td>
<td>36</td>
<td>28</td>
<td>2</td>
<td>4.10</td>
<td>52</td>
</tr>
<tr>
<td>Inferior frontal cortex</td>
<td>L</td>
<td>47/48</td>
<td>-42</td>
<td>12</td>
<td>-4</td>
<td>3.71</td>
<td>34</td>
</tr>
<tr>
<td>Pre-supplementary motor area</td>
<td>L</td>
<td>6</td>
<td>-6</td>
<td>2</td>
<td>52</td>
<td>3.52</td>
<td>28</td>
</tr>
<tr>
<td>Posterior cingulate cortex</td>
<td>L</td>
<td>23</td>
<td>-6</td>
<td>-34</td>
<td>22</td>
<td>3.50</td>
<td>28</td>
</tr>
</tbody>
</table>

Note: Clusters of more than 20 contiguous voxels exceeding a Z of 3.1 (p < 0.001). Activation peaks in MNI coordinates.

Although all 20 included participants were unable to perceive strongly masked squares/diamonds consciously, as illustrated by chance performance on a two-alternative forced-choice discrimination task performed after the Go/No-Go experiment, we sought to further rule out the possibility that the reported behavioral or neuroimaging results can be explained by accidental visibility of masked stimuli. If incidental No-Go visibility would be responsible for the observed effects one would expect reliable positive correlations between discrimination scores and behavioral/neuroimaging correlates of inhibition. Discrimination performance (d') in the two-alternative forced-choice discrimination task did not correlate reliably with mean activation in the unconscious inhibition network (rho = -0.082; p = 0.730), or activation in any of the four clusters separately (largest rho = 0.26). Discrimination performance did also not correlate significantly with unconscious RT slowing (rho = 0.343; p = 0.139). Crucially, RT slowing effects were still significant when discrimination performance was extrapolated to zero visibility. The linear regression analysis revealed a significant intercept (21.9 ms; p < 0.001 for the regression of RT slowing against d'), which further confirms the conclusion that RT slowing was induced by No-Go signals that could not be perceived consciously (see Greenwald, Schuh, & Klinger, 1995; Hannula et al., 2005 for further discussion and justification of this method). The absence of consistent positive correlations between detection and brain/behavioral measures suggest that the reported effects are not related to (accidental) visibility of strongly masked stimuli.

Discussion

In a Go/No-Go paradigm, we masked No-Go signals to the point that they could no longer be detected to investigate the depth of processing of unconscious No-Go
signals in the human brain. Unconscious No-Go signals were observed to activate brain regions central to networks that have been associated with conscious response inhibition, namely the IFC, the pre-SMA and the PCC. Especially the right IFC and the pre-SMA are considered to be key brain regions responsible for response inhibition and subsequent action selection (Aron et al., 2007; Aron, Fletcher et al., 2003; Aron & Poldrack, 2006; Aron, Robbins, & Poldrack, 2004; Blasi et al., 2006; Chambers et al., 2006; Chikazoe et al., 2007; Forstmann et al., 2008; Konishi et al., 1999; Leung & Cai, 2007; Mostofsky & Simmonds, 2008; Simmonds et al., 2008; Wager et al., 2005) and interestingly, both areas are probably connected directly via white-matter tracts (Aron et al., 2007). Activation in the right IFC strongly correlated with activation in the pre-SMA and the left IFC, suggesting that these areas co-operate during unconsciously triggered response inhibition. These correlations cannot be explained by overall fluctuations in brain activation or general task-related effects, since activation in the PCC did not correlate with any of the other three brain regions. However, it might still be possible that the observed correlations reflect the influence of another, yet to be determined, brain region.

The observed activation in the unconscious inhibition network appears to be functional, in that this unconscious activation correlated with the amount of slowdown of manual responses elicited by unconscious No-Go signals; as if participants attempted to inhibit their response but just failed to withhold it completely. Interestingly, the correlation between brain activation and RT slowing triggered by unconscious No-Go signals was driven mainly by activation in the bilateral IFC, suggesting a crucial role of this area in actively implementing inhibitory control unconsciously. Although most attention is devoted to the right IFC in response inhibition, generally, both hemispheres are activated when participants inhibit their response in the Go/No-Go task as well as the stop-signal task, however, as also observed here, the right slightly more than the left (Aron et al., 2007; Aron & Poldrack, 2006; Blasi et al., 2006; Chikazoe et al., 2009; Chikazoe et al., 2007; Konishi et al., 1999; Leung & Cai, 2007; Wager et al., 2005; Xue, Aron, & Poldrack, 2008). Unconscious (and conscious) response inhibition was also associated with rather specific neural activation in the PCC. Whereas the functional significance of PCC activation is less clear, numerous studies have observed PCC activation during successful response inhibition (Aron et al., 2007; Aron & Poldrack, 2006; Boehler et al., 2008; Durston et al., 2002; Ray Li et al., 2006; Wager et al., 2005), which might not be directly related to motor inhibition, but could reflect subsidiary processes such as attentional selection that influence stopping success (Boehler et al., 2008).
These findings converge with and extend recent EEG results, obtained using a similar masked Go/No-Go paradigm, in important ways (van Gaal et al., 2008). In that EEG study, unconscious No-Go signals elicited a medium latency (~300 ms) frontal ERP component that correlated with the extent to which participants slowed down responses to those No-Go signals. Source imaging suggested this frontal ERP component to be localized in the lateral PFC. The present fMRI results specify these findings and pinpoint the IFC (and possibly the pre-SMA) as crucial components in linking unconscious No-Go signals to appropriate action; in this case, attempting to withhold the overt response.

Additionally, in the present fMRI experiment, participants slowed-down their responses much more than in the previous EEG study, which might be due to the fact that, in the present experiment, conditions were nicely balanced with respect to low-level stimulus properties, whereas this was less the case for the EEG experiment. This allowed us to estimate the time it takes for unconscious No-Go signals to influence inhibitory control. Interestingly, RT distributions of unconscious No-Go trials and strongly masked Go trials (Fig 5.2c) started to differentiate after ~300 ms, which suggests that it takes ~300 ms for unconscious No-Go signals to take effect. We verified that our sample did not consist of clearly separated fast and slow subgroups, such that the present pattern could not be attributed to artifacts resulting from averaging across such subgroups. These behavioral results converge nicely with the EEG results in which the first moment of significant deflection at frontal electrode sites was observed at 309 ms (van Gaal et al., 2008). In combination, the present fMRI results suggest that it takes ~300 ms for unconscious No-Go signals to trigger response inhibition, which is implemented by the IFC and pre-SMA.

The fate of unconscious prefrontal cognitive control

An outstanding question remains why we were able to provide evidence for prefrontal processing of unconscious information, whereas many other studies did not. It has been observed that the strength of unconscious activation decays rapidly during its way up in the cortical hierarchy (e.g. Dehaene et al., 2001; Grill-Spector, Kushnir, Hendler, & Malach, 2000), therefore the effects of masked stimuli at higher-level cortical areas have generally been observed to be small and not include the prefrontal cortex (for a review see Dehaene & Naccache, 2001; Kouider & Dehaene, 2007; but see Lau & Passingham, 2007; Thompson & Schall, 1999; van Gaal et al., 2008). We hypothesize that we were able to provide evidence for unconscious prefrontal cognitive control because our approach differs fundamentally from
previous masking studies, as it combines two factors that, to our knowledge, have been rarely combined before in neuroimaging studies. First, in the present experiment, the unconscious stimulus of interest is highly task-relevant and attended. This is important, since it has been shown that attended and task-relevant stimuli are processed faster and more deeply in the human brain than unattended and task-irrelevant information (for reviews see Kanwisher & Wojciulik, 2000; Lamme & Roelfsema, 2000; Ungerleider & Kastner, 2000). Interestingly, recent studies demonstrated that attention can also be oriented towards unconscious stimuli, which subsequently enhances the influence of these stimuli on behavior (Naccache et al., 2002; Sumner et al., 2006). Second, and perhaps more importantly, in our study the unconscious stimulus of interest was strongly associated with prefrontal cognitive control functions. Therefore, the instructed task-set of the participant required a deep level of information processing (incorporating prefrontal cortex) of the unconscious stimulus. Recently, we (van Gaal et al., 2008) and others (Nakamura et al., 2007; Nakamura et al., 2006) have shown that the instructed top-down task-set strongly determines the processing routes taken by masked stimuli. The combination of both factors allowed us to more directly tap into the possible scope and depth of unconscious information processing than previous studies using the masking priming task (or related paradigms).

Conscious and unconscious inhibitory control: not identical
Although we demonstrate that PFC mediated cognitive control mechanisms can be triggered unconsciously, our results also indicate differences between consciously and unconsciously triggered response inhibition. Behaviorally, the impact of unconscious No-Go signals appeared like a downscaled form of conscious No-Go signals (RT slowing instead of overt response inhibition). Similar patterns were observed in recent studies with the Go/No-Go tasks (van Gaal et al., 2008) and the stop task (van Gaal et al., 2009), in that unconscious control signals yielded considerable response slowing but less pronounced (albeit still significant) overt response inhibition. These findings are in line with results from many experiments that showed that visible stimuli exert stronger influences on behavior than do invisible stimuli (for a review see Dehaene & Naccache, 2001; Kouider & Dehaene, 2007) and that conscious information processing is more flexible (e.g. Jacoby, 1991) and durable (e.g. Greenwald et al., 1996; Kunde, 2003) than unconscious information processing. This dissociation in behavioral performance is nicely reflected in the neural signature associated with consciously vs. unconsciously triggered inhibitory control. In the present experiment, conscious No-Go signals elicited a typical large-
scale frontoparietal inhibition network, whereas unconscious No-Go signals triggered a more specific subset of (local) No-Go processors in prefrontal cortices. Additionally, the BOLD time-course analysis suggests that conscious No-Go signals trigger a relatively strong and long-lasting pattern of neural activations, whereas unconscious No-Go activations were more of a fleeting form.

These results are in accordance with the neural mechanisms proposed to underlie conscious and unconscious information processing (Baars, 2002; Dehaene et al., 2006; Lamme, 2006). In these influential models, it is supposed that when a stimulus triggers a feedforward sweep of activation, it is not experienced consciously. Only when recurrent interactions between higher and lower cortical areas are initiated, awareness of a stimulus arises. Many studies have shown that conscious stimuli evoke long-lasting, large-scale recurrent interactions between many distant brain areas (for reviews see Dehaene & Naccache, 2001; Kouider & Dehaene, 2007; Rees et al., 2002; Tononi & Koch, 2008); a neural state that has been referred to as ‘global ignition’ (Dehaene et al., 2006). This form of activation probably extends beyond the local and isolated information processors that were initially triggered by the stimulus (feedforward activity) and generally incorporates prefrontal, cingulate, parietal and temporal brain regions. On the contrary, masking a stimulus to the point that it can no longer be detected probably disrupts reentrant processes, but leaves feedforward activity relatively intact (Del Cul et al., 2007; Fahrenfort et al., 2007; Lamme et al., 2002).

With respect to the present data, it seems likely that conscious No-Go signals triggered a large-scale frontoparietal inhibition network, probably because these signals could durably reverberate in the neural system. Therefore, information might become available for a number of high-level brain areas (such as dorsolateral prefrontal cortices), which could lead to less automatic, more flexible and full-blown cognitive control operations (see also Dehaene, 2008; Dupoux et al., 2008), reflected in full-blown inhibition in the Go/No-Go task. Interestingly, the present data suggest that unconscious No-Go stimuli can travel along similar processing routes as conscious No-Go stimuli, even up to prefrontal cortex. However, if they do so, they do not activate a similarly strong, stable and extended activation pattern as conscious ones, but only cause a “trickle of activation” in specialized, but relatively isolated No-Go processors “that modulates decision time but does not determine the decision outcome” (Dehaene, 2008). Although we have yet to understand the exact functional and neural differences between conscious and unconscious cognitive control, these results stretch the alleged limits and depth of unconscious information processing in
the human brain and directly impact the current debate about the relationship between consciousness and cognitive control.
## Supplementary Table. Consciously triggered response inhibition

Inhibited conscious No-Go trials > responded weakly masked Go trials

<table>
<thead>
<tr>
<th>No-Go &gt; Go</th>
<th>Side</th>
<th>BA</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z-value at local maximum</th>
<th>Number of voxels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right Frontal network</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3784</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>R</td>
<td>47</td>
<td>32</td>
<td>22</td>
<td>-12</td>
<td>5.32</td>
<td></td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>R</td>
<td>47</td>
<td>44</td>
<td>22</td>
<td>-6</td>
<td>5.01</td>
<td></td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>R</td>
<td>9</td>
<td>36</td>
<td>54</td>
<td>24</td>
<td>4.93</td>
<td></td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>R</td>
<td>9</td>
<td>26</td>
<td>52</td>
<td>32</td>
<td>4.86</td>
<td></td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>R</td>
<td>9</td>
<td>42</td>
<td>48</td>
<td>26</td>
<td>5.08</td>
<td></td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>R</td>
<td>6</td>
<td>26</td>
<td>2</td>
<td>64</td>
<td>4.91</td>
<td></td>
</tr>
<tr>
<td><strong>Right parietal cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2166</td>
</tr>
<tr>
<td>Inferior parietal lobule</td>
<td>R</td>
<td>40</td>
<td>52</td>
<td>-52</td>
<td>42</td>
<td>5.75</td>
<td></td>
</tr>
<tr>
<td>Inferior parietal lobule</td>
<td>R</td>
<td>40</td>
<td>44</td>
<td>-58</td>
<td>52</td>
<td>5.45</td>
<td></td>
</tr>
<tr>
<td>Inferior parietal lobule</td>
<td>R</td>
<td>40</td>
<td>40</td>
<td>-48</td>
<td>44</td>
<td>4.66</td>
<td></td>
</tr>
<tr>
<td>Supramarginal gyrus</td>
<td>R</td>
<td>40</td>
<td>54</td>
<td>-46</td>
<td>40</td>
<td>5.49</td>
<td></td>
</tr>
<tr>
<td>Supramarginal gyrus</td>
<td>R</td>
<td>40</td>
<td>56</td>
<td>-46</td>
<td>28</td>
<td>5.23</td>
<td></td>
</tr>
<tr>
<td>Supramarginal gyrus</td>
<td>R</td>
<td>40</td>
<td>62</td>
<td>-52</td>
<td>28</td>
<td>5.18</td>
<td></td>
</tr>
<tr>
<td><strong>Left prefrontal cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>262</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>L</td>
<td>47</td>
<td>-30</td>
<td>26</td>
<td>-10</td>
<td>4.47</td>
<td></td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>L</td>
<td>47</td>
<td>-38</td>
<td>14</td>
<td>-20</td>
<td>4.06</td>
<td></td>
</tr>
<tr>
<td>Insula</td>
<td>L</td>
<td>13</td>
<td>-40</td>
<td>14</td>
<td>-12</td>
<td>4.24</td>
<td></td>
</tr>
<tr>
<td><strong>Left parietal cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>163</td>
</tr>
<tr>
<td>Supramarginal gyrus</td>
<td>L</td>
<td>40</td>
<td>-58</td>
<td>-50</td>
<td>28</td>
<td>4.10</td>
<td></td>
</tr>
<tr>
<td>Supramarginal gyrus</td>
<td>L</td>
<td>40</td>
<td>-66</td>
<td>-50</td>
<td>26</td>
<td>3.94</td>
<td></td>
</tr>
<tr>
<td>Supramarginal gyrus</td>
<td>L</td>
<td>40</td>
<td>-56</td>
<td>-48</td>
<td>38</td>
<td>3.84</td>
<td></td>
</tr>
<tr>
<td>Supramarginal gyrus</td>
<td>L</td>
<td>40</td>
<td>-56</td>
<td>-50</td>
<td>34</td>
<td>3.59</td>
<td></td>
</tr>
<tr>
<td>Inferior parietal lobule</td>
<td>L</td>
<td>40</td>
<td>-58</td>
<td>-56</td>
<td>46</td>
<td>3.62</td>
<td></td>
</tr>
<tr>
<td>Inferior parietal lobule</td>
<td>L</td>
<td>40</td>
<td>-56</td>
<td>-56</td>
<td>50</td>
<td>3.48</td>
<td></td>
</tr>
</tbody>
</table>

*Note: Z > 3.1 (p < 0.001), whole-brain cluster-corrected (p < 0.05). Activation peaks in MNI coordinates.*