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Research Report

Event-related near-infrared spectroscopy detects conflict in the motor cortex in a Stroop task

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ABSTRACT

The Stroop effect is one of the most popular models of conflict processing in neuroscience and psychology. The response conflict theory of the Stroop effect explains decreased performance in the incongruent condition of Stroop tasks by assuming that the task-relevant and the task-irrelevant stimulus features elicit conflicting response tendencies. However, to date, there is not much explicit neural evidence supporting this theory. Here we used functional near-infrared imaging (fNIRS) to examine whether conflict at the level of the motor cortex can be detected in the incongruent relative to the congruent condition of a Stroop task. Response conflict was determined by comparing the activity of the hemisphere ipsilateral to the response hand in the congruent and incongruent conditions. First, results provided explicit hemodynamic evidence supporting the response conflict theory of the Stroop effect: there was greater motor cortex activation in the hemisphere ipsilateral to the response hand in the incongruent than in the congruent condition during the initial stage of the hemodynamic response. Second, as fNIRS is still a relatively novel technology, it is methodologically significant that our data shows that fNIRS is able to detect a brief and transient increase in hemodynamic activity localized to the motor cortex, which in this study is related to subthreshold motor response activation.

1. Introduction

The Stroop (1935) effect is one of the most frequently used models of conflict processing in neuroscience and psychology. In Stroop tasks a stimulus with at least two dimensions is presented. Participants are instructed to give a behavioral response on the basis of the task-relevant stimulus dimension and neglect the other dimensions. In the congruent condition the task-relevant and task-irrelevant stimulus dimensions prompt the same behavioral response. In contrast, in the incongruent condition of Stroop tasks, in each presentation of a trial the task-relevant stimulus dimension prompts a certain behavioral response, while the task-irrelevant stimulus dimension prompts a different behavioral response. These opposing behavioral tendencies may then result in response conflict in ultimately correctly responded trials. Here we used functional near-infrared spectroscopy (fNIRS) to collect evidence for the presence of response conflict in the Stroop task, thereby also evaluating the ability of fNIRS to detect inappropriate subthreshold response tendencies in cognitive tasks.

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One prominent theory of the Stroop effect is the response conflict/competition or horse race theory[1] : it assumes that in each trial task-relevant and task-irrelevant stimulus dimensions are processed in parallel and they also activate the response tendencies assigned to them in a parallel manner (Morton and Chambers, 1973; Posner and Snyder, 1975). The response conflict theory of the Stroop effect can be best interpreted in the framework of the continuous flow model of information processing (Eriksen and Schultz, 1979). Unlike serial information processing frameworks (e.g. Sternberg, 1969), the continuous flow model assumes that processing stages are not rigidly separated and therefore response processes can be influenced as soon as enough perceptual information is available to prepare a certain response tendency. Several classical (Gratton et al., 1988; Coles et al., 1985; Eriksen et al., 1985; Eriksen and Schultz, 1979; Eriksen and Eriksen, 1974) and more recent (Szűcs et al., 2009a, 2009b; Bryce et al., 2011) psycho-physiological studies have provided support for the continuous flow model. Hence, it is a reasonable hypothesis that in the congruent condition of Stroop tasks both the task-relevant and task-irrelevant dimensions activate the same response, whereas in the incongruent condition these dimensions activate opposing response tendencies (Morton and Chambers, 1973; Posner and Snyder, 1975). In the overwhelming majority of incongruent trials, the incorrect response tendency gets suppressed before it could be elicited and ultimately these trials will be responded correctly. However, the Stroop conflict will still be expressed by longer reaction times (RTs) in the incongruent than in the congruent condition.

Importantly, behavioral methods cannot provide explicit/positive evidence for response conflict in Stroop tasks because they cannot observe subthreshold incorrect response activation assumed to happen before an overt response is actually given. In this regard, neuroimaging techniques might provide fruitful insight but, contrary to the popularity of the Stroop task as a model of conflict processing, only a few neuroimaging studies provided explicit evidence for response conflict in these tasks.

The first such study used fast optical imaging and sampled brain activity at 25 Hz temporal resolution in a manual spatial Stroop task (DeSoto et al., 2001). The study measured lateralized activity over motor cortices in order to characterize motor response preparation. The physiological activity of the brain was measured by detecting the phase-delay of the optical signal (Gratton and Limkeman, 1983). The data demonstrated increased phase-delay over the motor cortex ipsilateral to response hand in incongruent but not in the congruent trials, and increased phase-delay over the motor cortex contralateral to response hand in both congruent and incongruent trials. The results were interpreted to show that the motor cortex associated with the correct response hand was activated in both the congruent/incongruent conditions, while the motor cortex associated with the incorrect response was activated only in the incongruent condition. The data was considered as evidence demonstrating selective incorrect response preparation in the incongruent condition, supporting the response conflict theory of the Stroop effect, although it should be noted that some recent methodological studies (Steinbrink et al., 2005; Radhakrishnan et al., 2009) have questioned the reliability of the fast optical signal. Therefore, a replication of the above findings is necessary.

Surprisingly, there is a similar sparseness of results also in functional magnetic resonance imaging (fMRI) investigations. Whereas most fMRI investigations of the Stroop conflict aimed at isolating brain areas related to conflict resolution (e.g. Melcher and Gruber, 2009; Egner and Hirsh, 2005; van Veen et al., 2001) and/or tried to dissociate areas related to stimulus vs. response conflict (e.g. Liston et al., 2006; Egner et al., 2007; van Veen and Carter, 2005), to date only one fMRI study of the Stroop effect provided explicit evidence for response competition at the level of the motor cortex (Cohen-Kadosh et al., 2007). It was found that in the incongruent condition the motor cortex ipsilateral to the response hand became activated to a greater extent than in the congruent condition.

Response conflict theory has been investigated also with electro-encephalography (EEG): several event-related potential (ERP) studies attempted to use an ERP derivative, the Lateralized Readiness Potential (LRP), to detect incorrect response activation in Stroop tasks (Cohen-Kadosh et al., 2007; Szűcs et al., 2007, 2009a, 2009b; Szűcs and Soltész, 2007, 2008; Bryce et al., 2011). The LRP is computed from electrodes placed over the motor cortex and it is considered as a measure of motor cortex activation related to response hand preparation (Gratton et al., 1988; De Jong et al., 1988). However, among all the studies which have tested whether LRP amplitude actually deviated significantly from baseline, only two of them found that the LRP signaled incorrect response activation in the incongruent condition (Szűcs et al., 2009a; Bryce et al., 2011); furthermore, the significant effects found in those two studies had a duration of only about 100 ms. The inability to detect more robust effects with the LRP is probably due to the volume conduction of electro-encephalography signals (Szucs and Soltesz, 2010), given that the LRP is inevitably influenced by electrical activity of both motor cortices (i.e., incorrect response preparation effects may be canceled out by larger, temporally coincident correct response preparation effects).

In order to circumvent this problem, we have carried out an electro-myographic (EMG) investigation of response conflict in a manual Stroop task by measuring muscle activity from the muscles of response fingers (Szűcs et al., 2009b). We have found that there was subthreshold muscle activity in fingers associated with the incorrect response. This subthreshold activity was specific to the incongruent condition. This data provides evidence for the response conflict theory of the Stroop effect (see also similar findings in a spatial response compatibility task in Burle et al., 2005). However, one uncertainty about EMG data is that it is not clear how it relates to central (brain) level response organization.

Hence, although some studies provide evidence about the response conflict theory, previous results need to be confirmed by using a central level measure of overt and subthreshold response activation which is not limited by electrical volume conduction like the LRP. Furthermore, taken together with the temporally transient nature of response

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[1] It is to note that here we only examine motor response conflict/competition in a Stroop task and we do not investigate the predictions of the conflict monitoring theory regarding anatomical locations, for example anterior cingulate function.

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conflict effects in EEG studies (Szűcs et al. , 2009a, 2009b; Bryce et al., 2011), the sparseness of fMRI evidence (Cohen-Kadosh et al., 2007) suggests that relatively short-lived response conflict effects may not usually be observed by fMRI. Hence, there is a need for extending findings with a brain-level hemodynamic method that combines some particular advantages of EEG and fMRI. In this regard, functional near-infrared spectroscopy (fNIRS) should be considered as the neuroimaging technique which might provide the more complete description regarding the cortical dynamics involved in the Stroop effect: for instance, it is not subjected to volume conduction as EEG and it has higher temporal resolution than fMRI (10 Hz in our study).

Thus, here we have used fNIRS technology to address the question of whether response conflict can be detected at the level of the motor cortex hemodynamic activity in an event-related Stroop task. Similar to fMRI, fNIRS measures local, functionally related, hemodynamic changes in the brain (e.g. Chance et al., 1993; Villringer et al., 1993). Unlike fMRI, however, which records signals based on the paramagnetic properties of deoxygenated hemoglobin (HbR), fNIRS measures the local hemodynamic activity by detecting the changes in the optical properties of cortical surface. This method can estimate variations in the concentration of both HbR and oxygenated hemoglobin (HbO). The sum of HbO and HbR concentrations provides an estimate of total blood volume (HbT). Furthermore, fNIRS can sample the changes in hemoglobin concentration at a higher temporal resolution than those typically used in fMRI, thus it could potentially provide a richer picture of the dynamics of cortical activations during the execution of cognitive tasks (see Cutini et al., 2012). Indeed, although the hemodynamic response remains sluggish in comparison with the neuronal response, the higher sampling frequency of fNIRS with respect to fMRI allows us to evaluate more precisely the temporal structure of the hemodynamic activity (e.g. Cutini et al., 2011a;'Minati et al., 2009). For instance, Minati et al. (2009) have shown that the hemodynamic response peak latency of the visual cortex was modulated by the emotional valence of the stimuli.

The presence of several fNIRS studies that have successfully investigated the neural correlates of the Stroop effect (see Discussion) confirms that this technique is perfectly suited to investigate this effect. Nevertheless, none of those studies compared motor cortex activity in ipsi- and contralateral motor cortices (relative to response hands) in congruent/incongruent conditions of Stroop tasks.

In the present fNIRS investigation of the Stroop effect, we have applied an animal-size decision Stroop task because, by using the LRP, we have previously demonstrated that this task elicits clear incorrect response activation (Szűcs et al., 2009a; Bryce et al., 2011). In the present study, the same event-related experimental design was adopted except for the inter-stimulus interval, which has been increased to 12 s to take into account the slowness of the hemodynamic response (Schroeter et al., 2002, 2004).

Our analysis was focused on fNIRS channels positioned over the left and right motor cortices. Based on the previous fast optical imaging, fMRI and EEG/EMG studies (DeSoto et al., 2001; Cohen-Kadosh et al., 2007; Szűcs et al., 2009a, 2009b; Bryce et al., 2011), we hypothesized that in the motor cortex contralateral to the response hand fNIRS would detect equal or higher signal amplitude in the congruent than in the incongruent condition. In contrast, we expected that in the motor cortex ipsilateral to the response hand fNIRS will detect stronger signal amplitude of hemodynamic response in the incongruent than in the congruent condition. Several outcomes from this experiment could be valuable. First, the results could provide evidence (proof of principle) that fNIRS has the sensitivity to detect potentially occurring subthreshold motor cortex activation in correctly responded incongruent trials in Stroop tasks. Second, such a finding would provide a strong and direct evidence for the motor response conflict theory of the Stroop effect. Third, the examination of the temporal characteristics of the hemodynamic response might provide major insights about the neural substrates of the Stroop effect. This added value could prompt the use of fNIRS in similar circumstances by the cognitive neuroscience field; in the present case, the results obtained by examining the cortical activity and the temporal dynamics with fNIRS could provide major implications for better understanding the cognitive processes involved in response conflict theory.

2. Results

2.1. *Behavioral data*

Accuracy was the same in both the congruent and incongruent conditions (congruent vs. incongruent: 95.8% vs. 94.8%). Mean RT was 90 ms longer in the incongruent (1356 ms) than in the congruent (1265 ms) condition ($t(12) = -5.67$; $p < 0.0001$). It is to note that these RTs are longer than in our previous studies (Szűcs et al., 2009a; Bryce et al., 2011). The longer RTs in the current study can probably be explained by the much longer ISI in the fNIRS (12 s) than in the EEG experiments (2.5 s). Participants probably adapted a much more relaxed way of task execution with the longer ISIs.

2.2. *ΔHbO*

ΔHbO data from the motor cortex is shown in the middle panel of Fig. 1B. Point-by-point Congruency × Hemisphere ANOVAs confirmed the presence of Congruency × Hemisphere interactions from 2.5 s to the end of the epoch ($p < 0.05$). These interactions are marked by boxes in Fig. 1B. There were two distinct patterns of the interaction. The first pattern appeared during the initial period of the peak of the ΔHbO response and it is shown in the left panel of Fig. 2B. The initial interaction period lasted from 2.5 s to 4.5 s (interaction: $F(1,11) = 7.56$; $p = 0.0188$). Post hoc contrasts showed that the difference between the congruent/incongruent conditions was significant in the ipsilateral ($p = 0.0398$; incongruent > congruent) but not in the contralateral hemisphere. The second interaction pattern appeared during the later period of the peak of the ΔHbO response and is shown in the right panel of Fig. 2B. The second interaction period lasted from 5.0 s to 8.0 s (interaction: $F(1,11) = 8.38$; $p = 0.0145$). Post hoc contrasts revealed that now the difference between the congruent/incongruent conditions ceased to be significant

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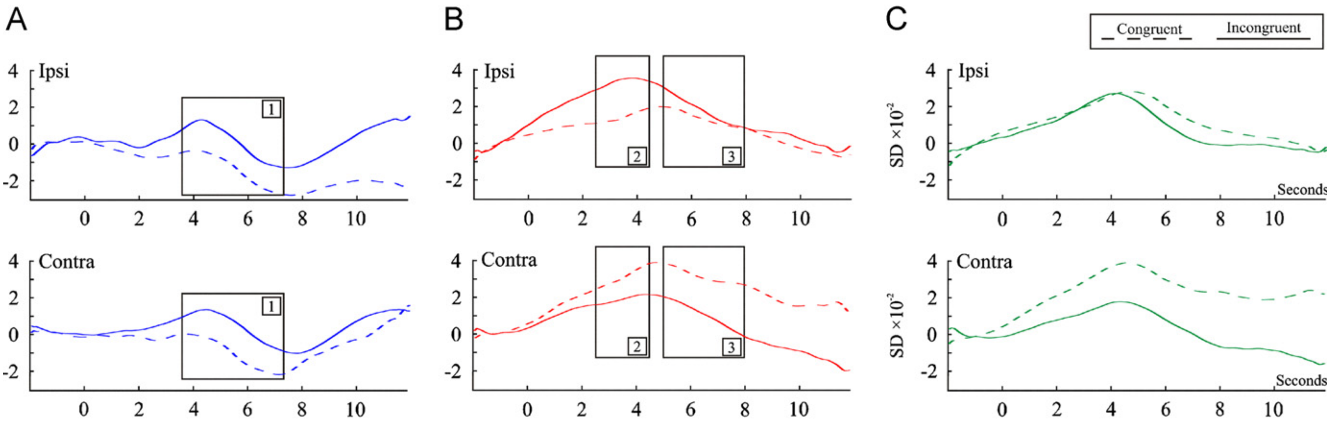

Fig. 1 – Time course of the fNIRS data. HbO (red), HbR (blue) and HbT (green) time course is shown in panels A–C, respectively (curves represent the congruent and incongruent conditions). Boxes mark the time intervals with significant Congruency effects in panel A (Box 1: 4–7.5 s) and with significant Congruency × Hemisphere interactions in panel B (Box 2: 2.5–4.5 s and Box 3: 5–8 s). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in the ipsilateral hemisphere. Rather, the difference between the congruent/incongruent conditions was now significant in the contralateral hemisphere ($p=0.0155$; congruent > incongruent).

At channels posterior to motor cortex channels there was a main effect of Hemisphere between 3.6 to 5.0 s ($p<0.05$) because ΔHbO amplitude was larger over the contralateral than the ipsilateral hemipshere. No effect was found for the channels anterior to motor cortex channels.

2.3. *ΔHbR*

ΔHbR data from the motor cortex is shown in Fig. 1A. Point-by-point Congruency × Hemisphere ANOVAs did not show any interaction, but only a Congruency main effect between 4 s and 7.5 s ($F(1,11)=13.44$; $p=0.0037$). The effect was due to a small increase in ΔHbR in incongruent trials with respect to congruent trials, rather than a classical decrease in ΔHbR. This Congruency effect is marked by boxes in Fig. 1A and is visible in Fig. 2A and B. There were no ΔHbR effects in data measured at channels anterior and posterior to motor cortex channels.

2.4. *ΔHbT*

ΔHbT data from the motor cortex is shown in Fig. 1C. There were no significant results in ΔHbT at motor cortex channels. There were no ΔHbT effects in data measured at channels anterior to motor cortex channels. At channels posterior to motor cortex channels there was a main effect of Hemisphere between 3.6 and 4.9 s ($p<0.05$) because ΔHbT amplitude was larger over the contralateral than the ipsilateral hemisphere.

3. Discussion

We have used event-related fNIRS to detect subthreshold incorrect response activation in the motor cortex in a Stroop task. Besides seeking evidence for the response conflict theory of the Stroop effect our secondary objective was to test the sensitivity of fNIRS to detect motor cortex activity related to the subthreshold incorrect response activation, and possibly exploit its superior temporal resolution with respect to fMRI.

Several fNIRS studies have compared brain activity in different conditions of the Stroop task, indicating that fNIRS is a sound instrument to investigate such paradigm, but none of those studies compared motor cortex activity in ipsi- and contra-lateral motor cortices to provide evidence of the response conflict theory. Schroeter et al. (2002, 2003) placed sensors over electrode positions (defined by the international 10–20 electrode system) F7/F8, F3/F4, Fc3/Fc4; C3/C4, P3/P4 and O1/O2, while Schroeter et al. (2004) used positions F7/F8, F3/F4, Fc3/Fc4. These studies used an event-related design in a manual task with an inter-stimulus interval (ISI) of 12 s and reported a larger increase in the concentration of HbT and HbO as well as a larger decrease in the concentration of HbR in the incongruent than in congruent trials in the bilateral dorsolateral prefrontal cortex (DLPFC) in adults (Schroeter et al., 2002, 2003) and in the left DLPFC in children (Schroeter et al., 2004). Ehlis et al. (2005) used 22 sensors covering the left hemispshere in 10 adults in a block design in a verbal task. It was found that the concentration of HbO and HbT increased more in the incongruent than in the congruent condition in left inferior frontal brain areas. León-Carrion et al. (2008) recorded data in a verbal task from 16 channels over the forehead (targeting the DLPFC along the line defined by electrode positions Fp1/Fp2) and analyzed 25 participants' data. Similar to Schroeter et al. (2002, 2004) it was found that subjects with faster RTs showed a higher concentration of HbO. In summary, the above fNIRS results are in line with fMRI data reporting congruency effects in Stroop tasks with similar localization (van Veen and Carter, 2002; Leung et al., 2000; Zysset et al., 2001), but do not provide any evidence about the response conflict theory.

Our hypothesis was that response conflict would be expressed by higher signal amplitude in the hemisphere ipsilateral to the response hand for the incongruent condition when compared to

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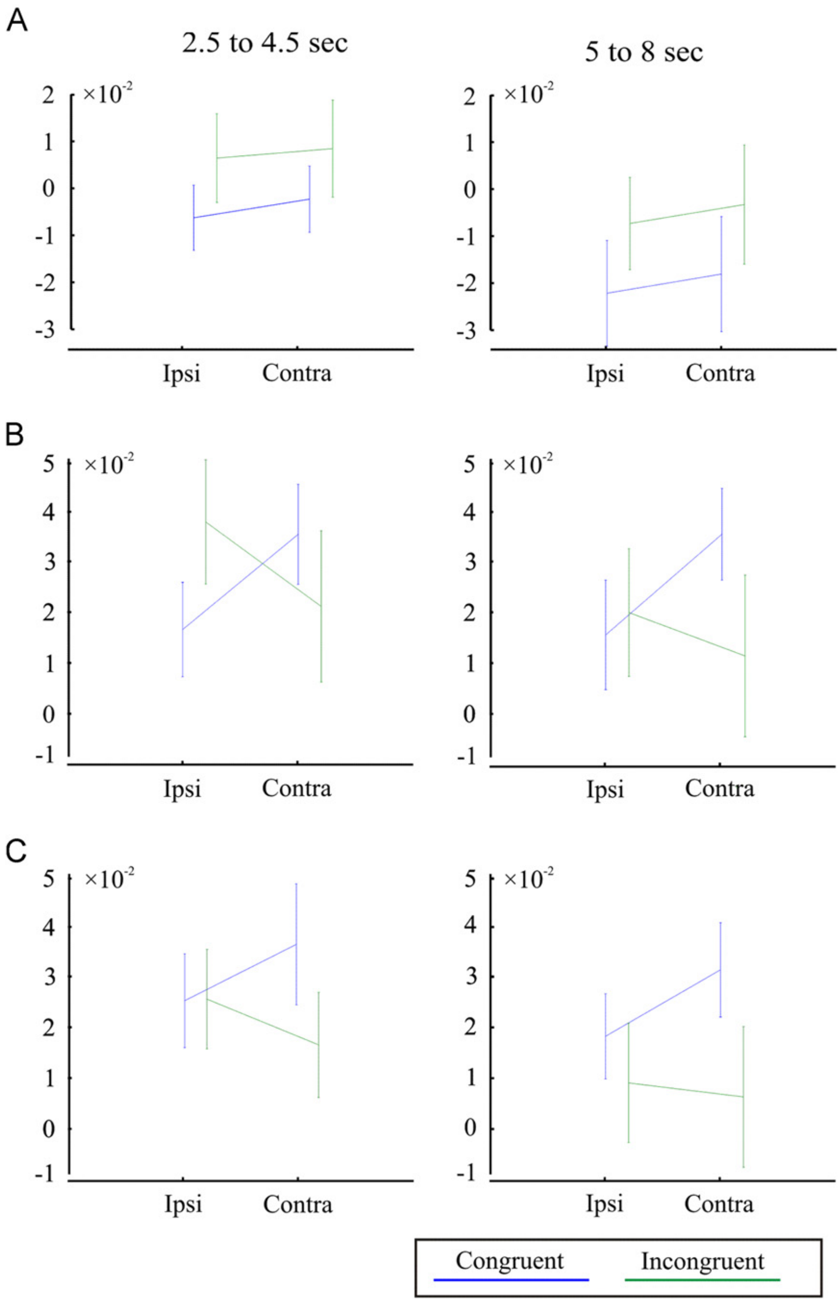

Fig. 2 – Mean amplitude and 95% confidence intervals of fNIRS measures in time intervals with significant Congruency × Hemisphere interactions in time intervals marked by boxes 2 and 3 in B. (A) 2.5–4.5 s and (B) 5–8 s.

the congruent condition and equal or higher signal amplitude in the hemisphere contralateral to the response hand for the congruent condition when compared to the incongruent condition. The early part (2.5 – 4.5 s) of the ΔHbO signal showed an excellent fit to the expected pattern of amplitude. This data suggest that there was incorrect motor response activation in the incongruent relative to the congruent condition. This confirms previous results from fast-optical imaging (DeSoto et al., 2001) and fMRI experiments (Cohen-Kadosh et al., 2007) as well as our previous LRP/EMG findings (Szűcs et al., 2009a; Bryce et al., 2011) and supports the motor response conflict theory of the Stroop effect by demonstrating in an explicit manner that task-relevant and task-irrelevant stimulus dimensions in incongruent trials activate opposing response tendencies at the level of the motor cortex.

The later part of the ΔHbO signal (5–8 s) still showed a Congruency × Hemisphere interaction but for a different reason than the early part of the signal: the amplitude was larger in the congruent condition than in the incongruent condition in the contralateral hemisphere while there was no difference in amplitude in the ipsilateral hemisphere. This observation is in very good agreement with the typical finding that the LRP is larger in the congruent than in the incongruent condition of Stroop tasks (Szűcs et al., 2009a; Bryce et al., 2011). A straightforward interpretation is that motor cortex activation is larger in the congruent than in the

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incongruent condition. This could happen because the motor conflict in the incongruent condition may result in some inhibition of correct response processes thereby decreasing the amount of activation over the contralateral hemisphere. For example, we have demonstrated that larger incorrect hand activation as determined by EMG is correlated with longer RT in the incongruent condition of a Stroop task (Szűcs et al., 2009b). Importantly, the higher temporal resolution of fNIRS relative to fMRI permitted a separation of the above described early and late stages of the Stroop conflict: an early, transient incorrect response activation followed by a later correct response activation. The separation of these stages is highly significant as they reflect functionally very different stages of the Stroop effect. Further, it is important to note that our interpretation is supported by the fact that Hemisphere × Congruency interactions were clearly restricted to the motor cortex, given that anterior and posterior channels did not exhibit the interaction. As our study focused on the motor cortex, channel locations did not extend to cover the frontal and parietal cortices; therefore, a comparison of data with previous fNIRS Stroop findings from those brain areas could not be assessed (Schroeter et al., 2002, 2003, 2004; Ehlis et al., 2005; León-Carrion et al., 2008).

Some methodological issues need to be discussed. First, concentration changes of hemoglobin are usually calculated by assuming specific differential path length factors (DPFs) which are known to be highly variable (e.g. Duncan et al., 1996). In the present study amplitude was standardized according to the mean and standard deviation determined in each individual in order to assure comparability across subjects. Such standardization resulted in DPF-independent hemodynamic values that have been reliably used in the statistical analysis.

Second, there were differences between ΔHbO and the other concentrations. Unlike ΔHbO, ΔHbR and ΔHbT did not show the same interaction observed for ΔHbO. The effect found for ΔHbR is of no interest to the response conflict theory, and is caused by an anomalous increase of HbR in incongruent trials. Usually, the ΔHbR effect that one should expect consists of a larger decrease of HbR in incongruent trials than in congruent trials (e.g. Schroeter et al., 2002). Furthermore, the normalized amplitude for HbR is roughly halved with respect to that observed in HbO hemodynamic response. Thus, it is very likely that the effect found for ΔHbR is caused by a sub-optimal SNR that substantially limits its interpretation. Consequently, it is reasonable to hypothesize that ΔHbR decreased the ΔHbT signal quality. However, this finding also raises the question about a possible differential sensitivity of fNIRS measures to separate cognitive processes. Currently, little is known about the differential sensitivity of fNIRS measures and reporting is not consequent across studies (sometimes only some measures being reported). Previous fNIRS studies of the Stroop effect reported various congruency effects in all three hemodynamic concentrations (see Introduction; Schroeter et al., 2002, 2003, 2004; Ehlis et al., 2005; León-Carrion et al., 2008). Although the studies used lights with different wavelengths (posing some limitations for a stringent comparison of the results) the results for ΔHbO were quite consistent across the studies; on the other hand, the results for ΔHbR were less conclusive for some studies (e.g. Ehlis et al., 2005; Ciftçi et al., 2008). Usually, ΔHbO has a higher SNR with respect to the other concentrations, but it is also thought to be less localized than ΔHbT and ΔHbR (Culver et al., 2005); however, recent investigations on neurovascular coupling (Berwick et al., 2008; Sirotin et al., 2009) showed that the half-width of HbO is comparable to that of HbT. Furthermore, the value of the information contained in HbO is confirmed by an elegant optical imaging investigation of the initial dip in alert monkeys (Sirotin et al., 2009). The initial dip consists of the observed initial darkening in intrinsic signal optical imaging (e.g. Chen-Bee et al., 2007), or in the temporarily reduced BOLD signal in fMRI (e.g. Menon et al., 1995). While the initial dip is usually interpreted as a local conversion of HbO to HbR caused by increased oxygen consumption by local neurons before any active vascular response, Sirotin et al. (2009) have shown that the initial dip largely reflects increases in HbO with no significant increase in HbR. They argued that the initial dip occasionally found with fMRI is likely to contain no privileged information about neural activity, while blood volume (HbT) signal “is as rapid and spatially focused and more than an order of magnitude stronger and longer lived” (Sirotin et al., 2009).

Taking these observations together, it is conceivable to hypothesize that HbO could provide further information with respect to HbR and the BOLD response (Das and Sirotin, 2010); this could explain why only one fMRI study (Cohen-Kadosh et al., 2007) was able to reveal a response conflict effect in the motor cortex. Indeed, if we consider this issue in conjunction with the temporally transient nature of response conflict effects in EEG studies (Szűcs et al., 2009a, 2009b; Bryce et al., 2011), the presence of only a single positive fMRI result supporting response conflict theory strongly suggests that relatively short-lived response conflict effects might not be easily observed by fMRI. In this regard, fNIRS might provide the optimal description of hemodynamic activity to highlight the neural effect of response conflict, by combining some particular advantages of EEG and fMRI. Indeed, the temporal subdivision of the Stroop effect in the motor cortex observed in the present study should be very difficult (but not impossible) to detect with fMRI, given the reasonably high temporal resolution required to observe such an effect. Conversely, recent fNIRS investigations (Cutini et al., 2011a; Minati et al., 2009) confirmed the ability to detect significant differences in hemodynamic peak latency across conditions.

In summary, our results provide explicit hemodynamic evidence supporting the response conflict theory of the Stroop effect and extend the consistency between hemodynamic results and electrophysiological evidence; furthermore, our results suggest that fNIRS is an excellent technique to reveal a possibly brief and transient increase in hemodynamic activity localized to the motor cortex, which in this study is related to subthreshold motor response activation.

4. Experimental procedures

4.1. *Participants*

Participants were 12 paid graduate and undergraduate students at the University of Cambridge, UK (mean age and

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range: 27 years; 23–36 years; 6 women). All subjects had normal or corrected-to-normal vision, and normal color vision; none of them reported a prior history of neurological or psychiatric disorders, nor was under medication at the time of testing. Participants gave a written informed consent and were paid 10 pounds per hour. The study was approved by the Psychology Research Ethics Committee of the University of Cambridge.

4.2. *Stimuli*

The stimuli and the task were the same as in Szűcs et al. (2009a). The stimuli pairs were combinations of 50 colored pictures of familiar animals. These were presented in the middle of a 17-inch computer screen with a white background. Two animal pictures were presented simultaneously, one to the left, the other to the right of the center. Subjects had to chose with a button press (left or right) which picture stimulus depicted the animal that was larger in real life. Subjects responded with their left or right thumbs using the buttons at the front of a gamepad controller. In the incongruent condition the physically larger stimulus picture represented an animal that is small in real life (e.g. small ladybug in large size vs. large tiger in small size). In the congruent condition the physical size of the stimuli and the real life animals they represented had the same relative relations (e.g. small ladybug in small size vs. large tiger in large size). On every trial the stimulus pairs were presented for 1 s followed by an inter-stimulus interval (ISI) of 12 s.

4.3. *Procedure*

Experimental trials were presented in four blocks with 40 trials in each block. Each block was made of intermixed congruent and incongruent trials. Trials were counterbalanced so that half of the congruent/incongruent trials required a left/right hand response. The trials were randomized whereby each subject had a different random order for the presentation of the trials. The following rules for the randomization were followed; congruent and incongruent epochs requiring either a left or a right hand response were preceded by epochs belonging to each response hand and congruency condition an equal number of times. This was to ensure that there would be no random effects due to one particular trial randomization sequence. Stimuli were presented by the Presentation program (version 13) of Neurobehavioral Systems. Data was recorded in an acoustically and electrically shielded testing booth. Only correctly responded trials were included in both the behavioral and fNIRS analysis.

4.4. *fNIRS data acquisition and analysis*

The hemodynamic activity was monitored using a continuous wave multi-channel fNIRS (ETG-4000, Hitachi Medical Co., Kashiwa, Japan). This device measured changes in attenuation at two wavelengths (695 and 830 nm), allowing the detection of HbO and HbR at a sampling rate of 10 Hz. The light that came out of the head after scattering through the brain was captured by the detectors and then was transmitted into a set of lock-in amplifiers limited to the particular frequencies of interest. Concentration changes (Δ) in HbO and HbR (mmol × mm) are calculated based on a modified Beer–Lambert approach (Cope and Delpy, 1988). Total hemoglobin (HbT) was calculated by summing up HbO and HbR.

In the present experiment we used 10 emitters and 8 detectors. Left and right motor cortices were covered with two arrays (one per hemisphere) of 9 optodes (5 emitters and 4 detectors). The distance between each emitter/detector pair (heretofore, channel) was 30 mm, so as to equate channels for optical penetration depth into the cortical tissue (about 20 mm; see Franceschini et al., 2000). The present configuration provided 24 channels (as in Herrmannet al., 2005), with 12 over the right and 12 over the left hemisphere. Fig. 3 illustrates the layout of optodes and channels.

Optodes were inserted into two (left and right) standard Hitachi holders. These holders were plastic rectangles with holes for the optodes and were affixed to participants' head by adjustable straps. The arrays of optodes covered an area of 6 × 6 cm over the left and right hemisphere. Holders were placed on the head so that their midpoint lay on electrode positions C3 and C4 as defined by the international 10–20 electrode system. The hypothesis driven primary analysis was focused on the 2 × 4 innermost channels in the left/right arrays (channels 4, 7, 9 and 6 over the left hemisphere and channels 16, 19, 21 and 18 over the right hemisphere; see Fig. 3). As defined above, these channels were positioned around C3 and C4 10–20 points. Both cranio-cerebral correlation (Okamoto et al., 2004) and probe placement methods (e.g. Cutini et al., 2011b) indicate that the cerebral regions underlying 10–20 points can be estimated with a precision that is well below the spatial resolution of fNIRS. Thus, we carried out a spatial conversion of C3 and C4 10–20 points to

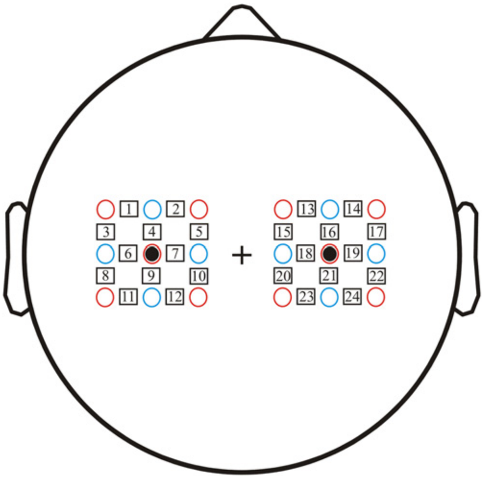

Fig. 3 – Schematic representation of the layout of the fNIRS emitters (red circles) and detectors (blue circles) over the scalp. The cross represents the vertex. The filled black circles represent 10–20 electrode positions C3 (left) and C4 (right). The four channels surrounding positions C3 and C4 were used for analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Talairach coordinates (Talairach and Tournoux, 1988) using the Talairach-to-10/20 Converter (T2T-converter; http://wwwneuro03.uni-muenster.de/ger/t2tconv/), as in Cutini et al. (2008). The resulting Talairach coordinates (x, y and z) were −56, −11, 46 for C3 and 57, −7, 47 for C4. Finally, we performed an anatomical labeling of the coordinates with the Brede Database location search (http://neuro.imm.dtu.dk/services/jerne/brede/) similar to previous studies (e.g. Cutini et al., 2011a); the anatomical labeling confirmed that both cerebral projections of C3 and C4 were located on the precentral gyrus.

The continuous data recorded at each sampling point was loaded into Matlab (version R2010B, The Mathworks Inc., Natick, Massachusetts, USA) and filtered by a 0.03–0.7 Hz second order Butterworth filter in order to remove physiological artifacts. The data was segmented into epochs extending -2 to 12 s relative to stimulus presentation. In order to assure comparability across individuals, amplitude was standardized according to the mean and standard deviation determined in each individual. Standardized values have the advantage of being differential path-length factor (DPF) independent. Epochs in each condition were baseline-corrected (baseline: −2 to 0 s). In order to enhance signal-to-noise ratio (SNR) epochs with excessive variability were rejected from analysis. This was done by determining the most extreme peak-to-peak signal amplitude differences within each epoch. Epochs where peak-to-peak variability exceeded the mean plus/minus two standard deviations of amplitude differences computed for each individual were rejected from the analysis. This procedure removed about 1–2% of epochs in each measure (HbO: 1.8%; HbR: 1.8%; HbT: 1.4%; range of removed trials in individuals per measure: 0–6). The remaining approximately 98% of epochs were left in the analysis.

The primary analysis focused on data recorded on the 2×4 innermost channels surrounding locations C3/C4. Data from these channels was averaged and treated as two single channels representing the left/right motor cortex (Left motor cortex: channels 4, 7, 9 and 6; Right motor cortex: channels 16, 19, 21 and 18). Furthermore, in order to test specific spatial localization of effects, channels anterior to motor cortex channels (left: channels 1 and 2; right: channels 13 and 14) and posterior (left: channels 11 and 12; right: channels 23 and 24) to motor cortex channels were also examined.

Data on left/right hemisphere channel pairs was organized according to two factors: one factor was Congruency (congruent vs. incongruent conditions), while the other factor was Hemipshere. Hemisphere had two levels: contralateral (the hemisphere on the contralateral side relative to the response hand) and ipsilateral (the hemisphere on the ipsilateral side relative to the response hand). Epochs were averaged for each individual participant in each condition and then group averages were computed. $\triangle$HbO, $\triangle$HbR and $\triangle$HbT values were tested by repeated measures Congruency (congruent vs. incongruent) × Hemisphere (contralateral vs. ipsilateral) ANOVAs run at each sampling point. Results were considered as significant if point-by-point ANOVAs passed the $p < 0.05$ significance threshold for at least 10 consecutive sampling points. Given that point-by-point analysis is a standard procedure in EEG, the high temporal resolution of fNIRS can be fully exploited with such analysis to provide more information on the temporal aspects of the hemodynamic response. A 10 sampling point threshold was used because our main interest was to detect effects on two optodes in the 2–8 s range. This range includes 60 sampling points on two channels, i.e. 120 sampling points. At an alpha level of 0.05, six of these points could show effects by chance. We chose a higher threshold of 10 points and in the data all relevant effects were much longer than this threshold. Furthermore, merely in order to be able to characterize statistical significance with a single alpha value the mean amplitude of intervals with significant findings were evaluated by overall Congruency × Hemisphere ANOVAs. From the point of view of the hypothesis the crucial question was whether a significant Congruency × Hemisphere interaction would appear and whether the pattern of this interaction would match the expected pattern. That is, we expected to have opposite patterns of signal amplitude in the contralateral (larger signal in the congruent than in the incongruent condition) and in the ipsilateral hemispheres (larger signal in the incongruent than in the congruent condition). In addition, in order to further specify interactions, pair-wise comparisons of Congruency × Hemisphere cells were done by post hoc Fisher-LSD tests. Statistical analyses were performed in Matlab and in Statistica (version 10, Statsoft, Tulsa, Oklahoma, USA).

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