Prefrontal Cortical Activation Associated with Working Memory in Adults and Preschool Children: An Event-related Optical Topography Study

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It is well known that lateral areas of the prefrontal cortex (LPFC) play a central role in working memory (a critical basis of various cognitive functions), but it remains unknown whether the LPFC of children of preschool age is responsible for working memory. To address this issue, we adopted a recently developed non-invasive imaging technique, optical topography (OT), which can potentially be applied to functional mapping in childhood. We firstly examined changes of activity in the LPFC using OT while adult subjects performed an item-recognition task, which requires working memory, under different memory-load conditions. We observed activation in the bilateral LPFC during performance of this task, the magnitude of which differed depending on memory-load. Then, we applied the same technique on 5- and 6-year-old children and observed the activation associated with working memory in the LPFC. Areas and properties of such activity were similar in adults and preschool children. Thus, for the first time, we demonstrate that the LPFC of preschoolers is active during working memory processes, indicating that in 5- and 6-year-old children, the LPFC has already developed processing of this important cognitive function.

Keywords: cognitive development, NIRS, optical topography, prefrontal cortex, preschool children, working memory

Introduction

Working memory is a brain system involved in temporary storage and manipulation of information and is a key requirement for various cognitive functions such as planning and reasoning and, thus, everyday life (Baddeley, 1986, 1992). Study of its development at the level of the brain should be significant for the understanding of neural development associated with this important cognitive function (Huttenlocher, 2003). However, most studies of the neural basis of working memory have examined adults of human or non-human primates. For example, in non-human primates, both lesion and single-neuron studies using a spatial delayed-response task (a kind of working memory task) have demonstrated that lateral areas of the prefrontal cortex (LPFC) contain the neural substrate for visuospatial short-term (or working) memory (Goldman and Rosvold, 1970; Bauer and Fuster, 1976; Funahashi *et al.*, 1989, 1993; Sawaguchi and Yamane, 1999; Sawaguchi and Iba, 2001; for reviews, see Funahashi and Kubota, 1994; Goldman-Rakic, 1995; Fuster, 1997). In addition, in the adult human, many studies using neuropsychological and brain-imaging approaches have provided significant evidence of LPFC involvement in working memory (Freedman and Oscar-Berman, 1986; Jonides *et al.*, 1993; McCarthy *et al.*, 1994; Shimamura, 1994; Owen *et al.*, 1996, 1999; Sweeney *et al.*, 1996; Belger *et al.*, 1998; Courtney *et al.*, 1998; Leung *et al.*, 2002). Thus, the LPFC plays a central role in working memory.

A limited number of developmental studies have demonstrated that working memory performance improves with age from childhood to young adulthood (Kail and Salthouse, 1994; Luciana and Nelson, 1998; Zald and Iacono, 1998). In particular, Luciana and Nelson (1998) reported, using psychological approaches, that prefrontally guided working memory systems emerge at around the age of 4 years and improve substantially between the ages of 5 and 7 years. Furthermore, morphological studies have demonstrated that brain structure changes substantially around preschool age, in terms of the volume of grey and white matter (Giedd *et al.*, 1999) and the number of synapses in the PFC (Huttenlocher, 1979). Therefore, it is important to study the functional development of working memory in childhood, especially around preschool age; such an approach should extend our understanding of not only cognitive development, but also the neural mechanisms of cognitive functions (Casey *et al.*, 2000). However, although some fMRI studies of working memory development have been performed in children aged 7 years or older (Casey *et al.*, 1995; Thomas *et al.*, 1999; Nelson *et al.*, 2000; Kwon *et al.*, 2002), the neural basis of working memory development in preschool children is poorly understood. Particularly, it is still unknown whether, as in adults, the LPFC of preschoolers is active during working memory tasks. This deficit in understanding mainly results from the fact that many of the brain-imaging procedures are invasive or require extensive constraints and thus cannot easily be used to study normally developing children, in particular those of preschool age.

The aim of the present study is to reveal whether the LPFC of preschool children is active during working memory. To address this issue, we adopted a recently developed non-invasive imaging technique, optical topography (OT; Maki *et al.*, 1995; Yamashita *et al.*, 1996; Koizumi *et al.*, 1999, 2003). OT is a type of near-infrared spectroscopy (NIRS) imaging (Jöbsis, 1977) and has several advantages over other imaging methods such as fMRI or PET, e.g. OT does not require a head constraint and hence can potentially be applied to the developmental mapping of children and infants. Previous studies using OT have reported haemodynamic changes associated with various cortical functions such as motor action (Maki *et al.*, 1995, 1996) and language processing (Watanabe *et al.*, 1998; Sato *et al.*, 1999; Kennan *et al.*, 2002). Further, Noguchi *et al.* (2002) employed OT to examine event-related changes in activity of the cerebral cortex, including LPFC, during syntactic and semantic decision tasks and demonstrated that OT is useful for studying the higher cognitive functions, although studies using OT on LPFC-mediated cognitive functions have been limited.

In this study, we firstly examined changes in activity in the LPFC of human adults using OT with an event-related paradigm, while subjects performed an item-recognition task,

which requires working memory, under high and low memory-load conditions. Then, we used the same technique with preschool children (aged from 5 to 6 years). We report here that the LPFC not only of adults, but also of preschool children, is active during working memory, and the region and properties of such activity are similar in adults and preschool children.

Materials and Methods

Subjects

Seven right-handed healthy adults [aged between 22 and 28 years, 25.4 ± 1.9 (mean ± SD), six males and one female] and 16 healthy right-handed preschool children [aged between 5 and 6 years, 69.7 ± 6.7 months (mean ± SD), six males and 10 females] participated in this study. The children attended Shiratori Kindergarten, Saitama, Japan. All adult subjects provided informed consent for this study. For the children, parents provided written informed consent and were informed verbally of the purpose of the study and the safety of the OT experiment.

Behavioural Task

The behavioural task for adults was an item-recognition task with two memory-load conditions (i.e. HIGH and LOW conditions; see Fig. 1), which was controlled by a personal computer (PCG-505; Sony, Japan). A trial commenced when the central fixation spot (grey cross, 1 × 1°) turned to white (warning signal). After a warning period of a variable interval (1–3 s), two or four white squares (1.5 × 1.5°) were presented at the peripheral locations (two or four of eight locations) in the LOW and HIGH conditions, respectively (sample cue, 2 s), which was followed by a delay period of 8 s. Then, a white square (1.5 × 1.5°) was presented as a test cue at one of eight peripheral locations and, based on working memory of the locations of the sample cue, the subjects were required to report whether the location of the test cue was identical to the location of any of the sample cues. The response was reported by pressing a button: 'yes' was indicated by the right index finger and 'no' by the left index finger. When the subject responded, the test cue was turned off and the trial ended. A trial was followed by an intertrial interval (ITI) of 25 s, where a grey cross was presented in the centre of the display. Throughout the trial, the subjects were instructed to maintain fixation on the central cross. 'Yes' and 'no' trials were pseudo-randomized to be the same number (i.e. five trials for 'yes' and the other five for 'no').

Each adult subject performed two blocks of trials, each consisting of 10 LOW or HIGH trials. The order of conditions was counterbalanced among the seven subjects. Each child performed 10 trials for the LOW condition only, because of their difficulty in performing, or poor performance in, the HIGH condition. Children received sufficient training before the measuring session so that they were able to perform the task adequately (i.e. >80% correct response).

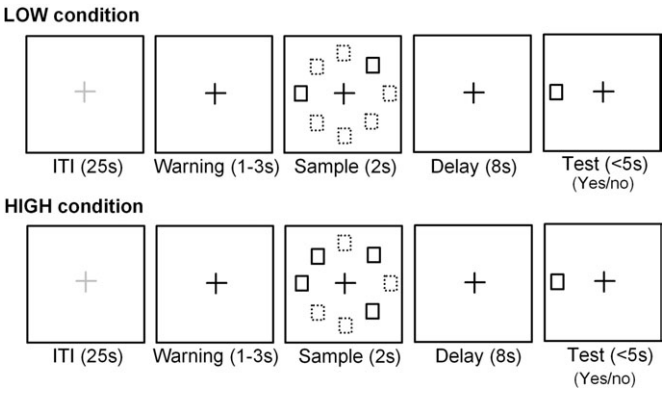

Figure 1. Schematic drawings of the item-recognition task with two memory-load conditions. The subjects remembered the location of two or four sample cues in the LOW and HIGH conditions, respectively. Then they were required to report whether the location of the test cue was identical to one of the sample cues.

Optical Topography Measurements

While the subjects performed the behavioural task, we measured haemoglobin oxygenation in the LPFC using an OT system (ETG-100; Hitachi Medical Corporation, Tokyo, Japan). Near-infrared laser diodes with two wavelengths (780 and 830 nm) were used as light emitters. The re-emitted lights were detected with avalanche photodiodes located 30 mm from the emitters. In the LPFC of each hemisphere, five emitters and four detectors were placed at alternate square intersection points on a 3 × 3 grid (Fig. 2). This configuration enabled us to detect signals from 12 channels in each hemisphere (i.e. a total of 24 channels from both hemispheres), which covered a 60 × 60 mm^2 area of the frontal cortex. We used two different optical probes, each of which was specialized for each subject group (i.e. adults and children) and, according to the landmarks such as the glabella and the temple, we positioned the optical probe carefully so that the position was similar among all subjects. The detected signal was separated into two components that corresponded to the two wavelengths, using lock-in amplifiers. Raw optical data for the two near-infrared lights were recorded every 100 ms, simultaneously for each of the 24 channels and sent to a data collection computer via an A/D converter. The timing of each task event, such as the onset of the sample cue, was also transmitted to the data collection computer from the task-control computer by serial communication via RS232C. These digitized data were stored on the hard disk drive and were eventually transferred to magneto-optical diskettes for further off-line analysis.

Although the spatial resolution of OT differs according to the definition, the spatial accuracy of OT has been suggested to be ~10 mm, as far as the experiment using phantom is concerned (Yamamoto *et al.*, 2003). Concerning the safety of this technique, a previous study showed that light absorption of OT leads to tissue heating well below accepted levels of risk and concluded that the OT system is completely safe (Ito *et al.*, 2000). Details of the OT system have also been described elsewhere (Koizumi *et al.*, 1999, 2003).

To identify the anatomical position of each recording channel, we obtained a magnetic resonance image for two adult subjects using 1.5 T magnetic resonance imaging (MRI) (Stratis II, Premium; Hitachi Medical Corporation, Tokyo, Japan). When scanning, alfacalcidol beads were positioned at each of the 24 channels as MR markers, which can be identified on the MR image as spheres (Noguchi *et al.*, 2002). Three-dimensional MR images with markers were reconstructed using MEDx for Linux software (Sensor systems, Sterling, VA).

Data Analysis

To analyse the raw optical data, we first defined a 20 s period after the aligned point, which was usually the onset of the sample cue, as the activation period. Second, we defined 5 s periods before and after the activation period as pre- and post-control periods, respectively. Then,

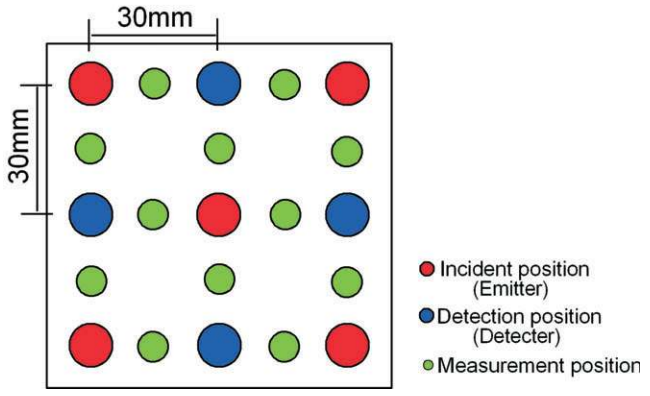

Figure 2. Configuration of emitters, detectors and measurement points for a hemisphere.

to determine the background transmittance, we applied linear regression by the least squares method on transmittance during the pre- and post-control period.

After averaging over 10 trials for each condition, the relative changes in the oxyhaemoglobin concentration (C_{oxy}) and deoxyhaemoglobin concentration (C_{deoxy}) were calculated using a modified Beer–Lambert law (Maki *et al.*, 1995). As the transmitted light detected by each detector reached the cerebral cortex after passing through the skin and the skull (McCormick *et al.*, 1992), transmittance $T(\lambda, t)$ for wavelength λ and measurement time t, is approximately:

$$-\ln[T(\lambda, t)] = \varepsilon_{oxy}(\lambda)c_{oxy}(t)d + \varepsilon_{deoxy}(\lambda)c_{deoxy}(t)d + a(\lambda, t) + sc(\lambda) \quad (1)$$

where ε_{oxy} and ε_{deoxy} are the molar absorption coefficients of oxy- (oxyHb) and deoxy-haemoglobin (deoxyHb), c_{oxy} and c_{deoxy} are the concentrations of oxyHb and deoxyHb, d is the effective path length in tissue, $a(\lambda, t)$ is attenuation due to other absorption such as cytochrome aa_3 except haemoglobin and $sc(\lambda)$ is attenuation due to scattering in tissue. When activation occurred

$$-\ln[T^s(\lambda, t)] = \varepsilon_{oxy}(\lambda)c^s{}_{oxy}(t)d + \varepsilon_{deoxy}(\lambda)c^s{}_{deoxy}(t)d + a^s(\lambda, t) + sc(\lambda) \quad (2)$$

where superscript 's' means the value during activation. We assume $a(\lambda, t) = a^s(\lambda, t)$ because the absorbance of haemoglobin is ~10 times larger than that of cytochrome aa_3, which dominates $a(\lambda, t)$ mainly in the near-infrared region. By subtracting equation (1) from equation (2), we obtain

$$\begin{aligned} -\ln[(T^s(\lambda, t))/(T(\lambda, t))] &= \varepsilon_{oxy}(\lambda)[c^s_{oxy}(t) - c_{oxy}(t)]d \\ &+ \varepsilon_{deoxy}(\lambda)[c^s_{deoxy}(t) - c_{deoxy}(t)]d \\ &= \varepsilon_{oxy}(\lambda)\Delta c_{oxy}(t) + \varepsilon_{deoxy}(\lambda)\Delta c_{deoxy}(t) \end{aligned}$$

where

$$\Delta c_{oxy}(t) = [c^s_{oxy}(t) - c_{oxy}(t)]d$$

and

$$\Delta c_{deoxy}(t) = [c^s_{deoxy}(t) - c_{deoxy}(t)]d$$

We defined

$$\Delta c_{total}(t) = \Delta c_{oxy}(t) + \Delta c_{deoxy}(t)$$

We evaluated the human brain oxygenation state by using these relative changes [$\Delta c_{oxy}(t)$, $\Delta c_{deoxy}(t)$ and $\Delta c_{total}(t)$], because it is difficult to determine the effective path length d inside human tissue. To produce the topographic images, the concentration in oxyhaemoglobin detected from each channel was spatially interpolated (Maki *et al.*, 1995).

To determine whether each recorded site was significantly activated, we compared the C_{oxy} during the pre-control period with that during the activation period using the student's t-test. To compare the activity level between different memory-loads, we applied a paired t-test on C_{oxy} during the activation period excluding the first 5 s following stimulus onset (i.e. from 5 to 20 s after the sample cue).

Results

Behavioural Performance

Seven adults performed 10 trials of each load condition and 16 children performed 10 LOW trials. To examine the behavioural performance, we calculated the reaction time and accuracy (percentage correct) separately for each condition. Figure 3 summarizes the behavioural data. For the adults, the mean reaction time (RT) was significantly faster in the LOW condition than in the HIGH condition [1077 ± 376 (SD) ms for the LOW condition and 1257 ± 486 ms for the HIGH condition; $P < 0.05$, paired t-test], indicating that the memory-load was significantly different between the two conditions. The children's RT was 1791 ± 419 ms, which was significantly slower than that for adults in both the HIGH and LOW conditions ($P < 0.01$, student's t-test). Figure 3*B* shows individual data for the percentage of correct responses. All adults showed ≥80% correct responses for both conditions and the percentage correct did not differ between the two conditions (91 ± 9 (SD) % for the LOW condition and 89 ± 7% for the HIGH condition; $P > 0.1$, paired t-test). Four children showed relatively poor performance (<75%). The mean percentage correct for the children was 84 ± 15%, which was slightly lower than that for the adults, although the difference was not statistically significant.

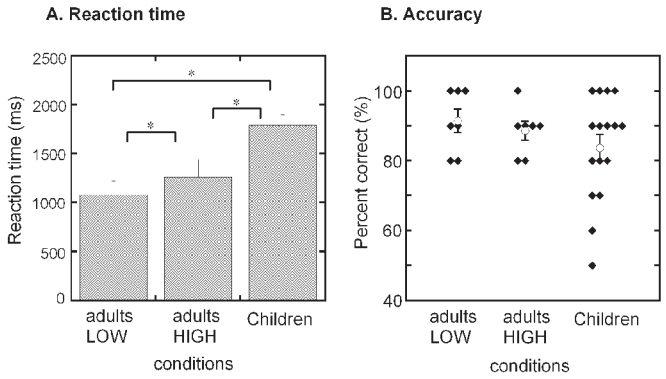

Figure 3. Behavioural results. (*A*) Mean reaction times are shown separately by condition. (*B*) Percentage correct for each individual subject for each condition. Filled quarries represent the data from each subject and open circles show mean percentage correct for each condition. Error bars indicate SE. *$P < 0.05$.

Optical Topography Results in Adult Subjects

To examine the spatiotemporal characteristics of changes in C_{oxy}, we generated dynamic topograms for each hemisphere, by interpolating the data from each channel. Figure 4 shows an example of the dynamic topogram, where changes in C_{oxy} in the right LPFC of an adult during HIGH condition are sequentially illustrated in 2 s increments. C_{oxy} was aligned with the onset of the sample cue. Specifically, as shown in Figure 4, C_{oxy} gradually increased ~5 s after the onset of the sample cue. This increase was sustained until 5–10 s after the response, and then decreased gradually to the background level.

Figure 5*A* shows a static topogram for the same subject represented in Figure 4, displaying the timing of peak C_{oxy} for each hemisphere (11.2 and 10.5 s from sample cue onset for right and left hemispheres, respectively), together with the position of each channel. The activated area was maximized at one channel from each hemisphere (i.e. channels 6 and 19 for the left and right hemisphere, respectively). In the HIGH condition, all seven adults showed a significant increase in C_{oxy} during the 20 s activation period for both channels, whereas in the LOW condition, four of the seven showed a significant increase for both channels, with two showing increases in the left hemisphere only and one in the right hemisphere only (t-test, $P < 0.01$; Table 1). According to the group-level analysis, both channels 6 and 19 showed significant activation in the HIGH condition (t-test, $P < 0.01$), although activation at only channel 6, but not channel 19, reached the significant level in

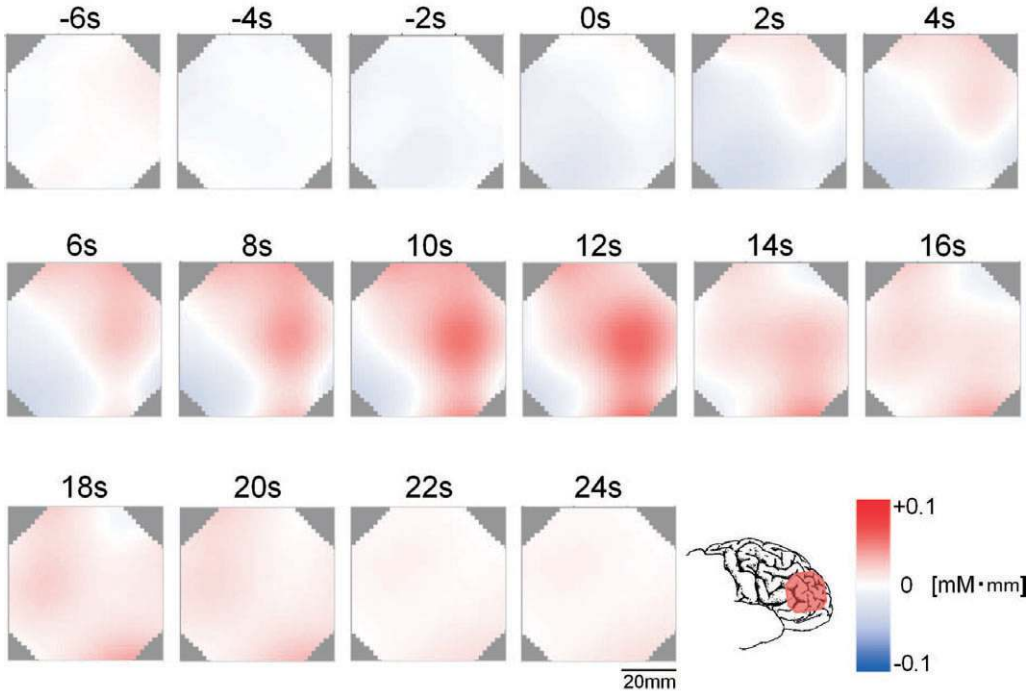

Figure 4. Dynamic topogram of changes in oxyhaemoglobin concentration in the right LPFC of an adult during a working memory task under the HIGH condition. Averaged data over 10 trials for a typical subject are shown for every 2 s, from –6 s to 24 s. The onset of the sample cue is represented by 0 s. The approximate recording area is indicated as a shaded area of the frontal cortex in the lower right illustration of this figure.

the LOW condition. Thereafter, we focused on these channels (i.e. Nos 6 and 19) for further analyses of each hemisphere.

To determine the precise anatomical position of these channels, we examined an MR image of the subject whose LPFC activity has been shown in Figures 4 and 5*A*. Figure 5*B* shows a 3D MR image of the subject for each hemisphere with MR markers for each measurement point. Channels 6 and 19, which are indicated by red arrows, were located in the dorsolateral area of the PFC (BA 9/46). We confirmed that the locations of channels 6 and 19 of the other subject whose MR image were obtained were similar to those of the subject shown in Figure 5*B*.

As shown in the dynamic topogram in Figure 4, the change in C_{oxy} appeared to be temporally associated with the onset of the sample cue. To examine this in detail, we generated event-triggered averaged plots of C_{oxy} aligned at the onset of the warning signal, the sample cue and the button press. Figure 6 shows such plots of data averaged across the seven adults. This figure shows that the peak value was highest when the time was aligned with the onset of the sample cue (blue line) compared with when it was aligned with either the warning signal (red) or the button press (green). Thus, the change in C_{oxy} appeared to be more nicely time-locked to the sample cue presentation, rather than either the warning signal or the button press, although the warning signal also appeared to provide a time-locked signal.

To test the effects of memory-load on LPFC activity, we compared C_{oxy} in the HIGH condition with that in the LOW condition. Figure 7 shows the time course of C_{oxy} and C_{deoxy} in the HIGH and LOW conditions, in which data averaged across the seven subjects are shown. In the HIGH condition, both right and left LPFC showed significant increase in C_{oxy} during the activation period compared with the pre-control period in the HIGH condition ($P < 0.001$ for both hemispheres). On the other hand, in the LOW condition, the magnitude of change in C_{oxy} was small relative to that in HIGH condition. According to a paired *t*-test, the mean C_{oxy} was significantly higher in the HIGH condition than in the LOW condition for both hemispheres ($P < 0.01$), indicating that the magnitude of activity in the LPFC was load dependent. For both the HIGH and LOW conditions, the activity level was not significantly different between the left and right hemispheres – although, especially in the LOW condition, the left hemisphere tended to show higher activity than the right hemisphere (paired *t*-test: $t = 1.46$, $P > 0.1$ for the HIGH condition; $t = 2.24$, $0.05 < P < 0.1$ for the LOW condition). This absence of significant hemispheric difference would be due, at least in part, to the high variability in hemispheric dominance across individuals, which could also be related to probe placement or folding/localization variability across subjects.

Contrary to C_{oxy}, C_{deoxy} showed a slight decrease after the sample cue onset, although this change was not significant. This tendency for a slight decrease is consistent with many previous NIRS studies (e.g. Maki *et al.*, 1995; Sato *et al.*, 1999).

Optical Topography Results in Preschool Children

Figure 8*A* shows a dynamic topogram of the right LPFC of a preschool-age subject (6 years old) during task performance. As shown in Figure 8*A*, C_{oxy} increased gradually after the onset of the sample cue. This increase was sustained until ~5 s after the response. Then, C_{oxy} decreased gradually to the background level. Compared with the adult subject shown in Figure 4, the overall pattern of changes in C_{oxy} was similar in adults

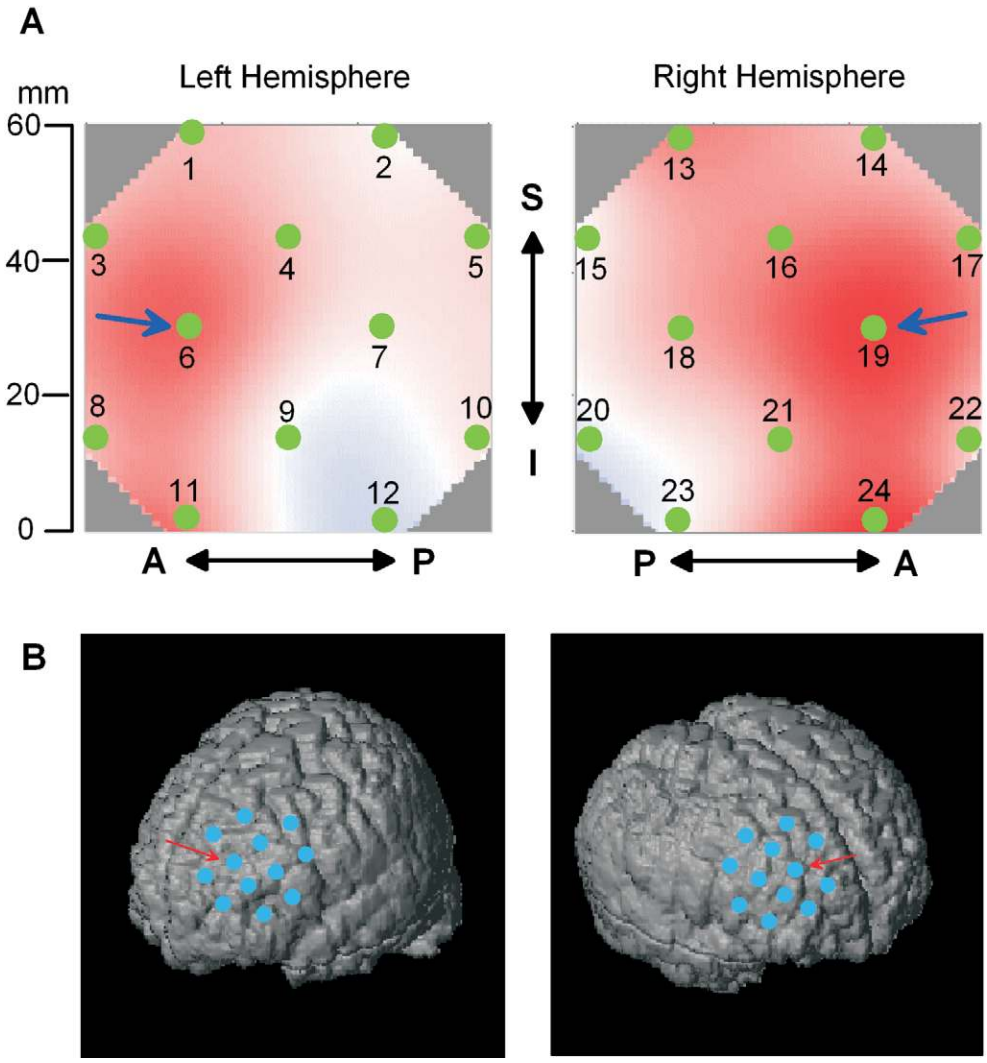

Figure 5. Static topogram and the anatomical sites of focused channels. (*A*) Static topograms at the peak time for each hemisphere are shown, together with the point of each of the 24 channels. The data from the same subject as in Figure 4. Blue arrows show the channels that showed highest activity in each hemisphere. (*B*) 3D MR image of the subject shown in (*A*). Blue dots indicate each recording channel shown. Channels indicated by a red arrow correspond to channels 6 and 19 in (*A*), for left and right hemispheres, respectively.

Table 1
The number of subjects showing a significant increase in C_{oxy}

	R only	L only	Both	Total
Adults HIGH ($n = 7$)	0	0	7	7
Adults LOW ($n = 7$)	1	2	4	7
Children ($n = 16$)	3	1	11	15

R and L indicate right and left hemispheres, respectively.

and children, although the magnitude of activity was larger, and active areas were more extended in children than in adults.

To examine the precise time course of changes in C_{oxy} for this subject, we plotted averaged C_{oxy} as a function of time triggered at the onset of sample cue for those channels that were associated with maximum activity in adults (i.e. channels 6 and 19 for the left and right hemispheres, respectively; see Fig. 5) (Fig. 8*B*). As shown in Figure 8*B*, the LPFC of both hemispheres showed a clear change in C_{oxy}, while C_{deoxy} slightly decreased. This change was characterized by a gradual increase that was time locked to the onset of the sample cue and that sustained activation until 5–10 s after the response. As summarized in Table 1, 11 of 16 subjects showed significant activation in both the right and left hemispheres, with one of the 16 and three of the 16 showing significant activation in the left or right hemisphere only, respectively (Student's *t*-test, $P < 0.01$).

To examine the time course of changes in C_{oxy} across our population of preschool children, we plotted mean C_{oxy} as a function of time at channels 9 and 16 for the left and right hemispheres, respectively, across all 16 children (Fig. 9*A*). Similarly to individual data from a typical subject (Fig. 8*B*), the group mean data (Fig. 9*A*) also showed a significant increase in C_{oxy}, but not in C_{deoxy}, for both hemispheres, which was time locked to the onset of the sample cue. Again, these characteristics of LPFC activity during the task were similar to those of the adults. However, unlike adults, children tended to show right-hemisphere dominance, according to the same analysis used for adult subjects (paired *t*-test: $t = 2.08$, $0.05 < P < 0.1$).

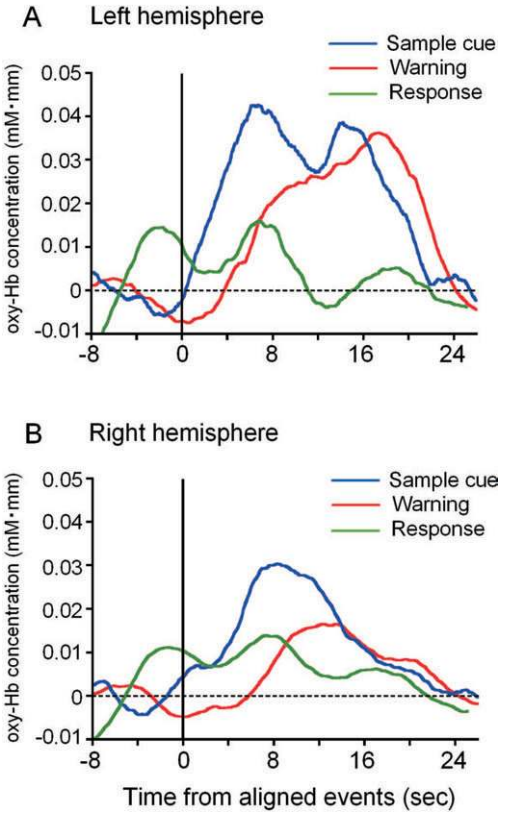

Figure 6. Temporal change in the oxyhaemoglobin (oxy-Hb) concentration across different task events. (*A*) and (*B*) show the left and right hemisphere, respectively. Data for the group mean ($n = 7$) in the HIGH condition are shown. The blue line shows the time course when aligned at the onset of the sample cue. The red and green lines show the time course when aligned at the warning signal and the button press, respectively. In each hemisphere, changes in oxy-Hb were temporally associated with the sample cue presentation.

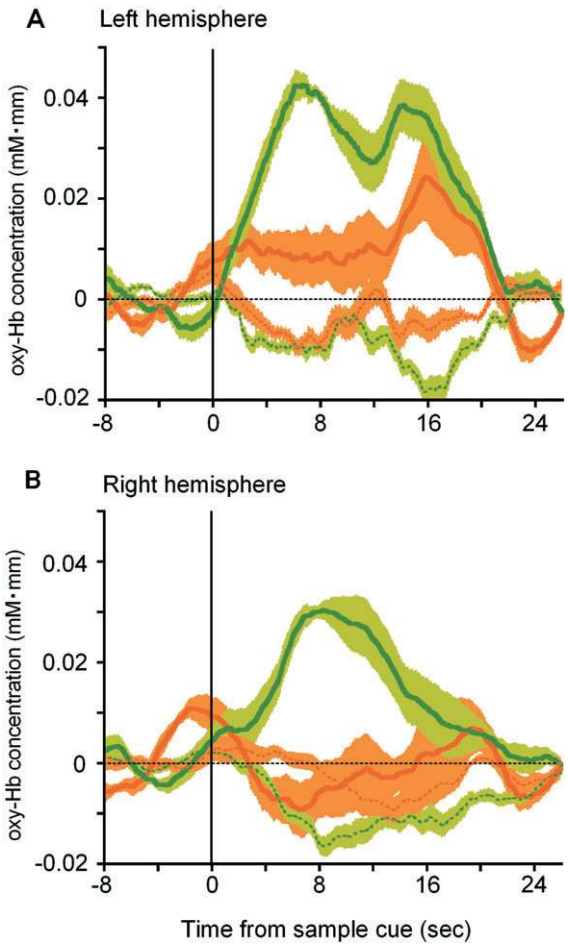

Figure 7. Load-dependent difference in changes in oxyHb and deoxyHb. (*A*) and (*B*) show the left and right hemispheres, respectively. Time courses of changes in C_{oxy} and C_{deoxy} for seven subjects are shown separately by HIGH and LOW conditions. The green and orange lines show the HIGH and LOW conditions, respectively, and the solid and dotted lines show C_{oxy} and C_{deoxy}, respectively. Shading indicates standard errors.

Since, as shown in behavioural results in Figure 3*B*, performance in some children were poor, we also plotted the histogram of mean C_{oxy} after excluding the data from subjects showing poor performance (i.e. <75%; Fig. 9*B*). However, there was no clear difference after excluding the data, which may be because incorrect response was primarily a kind of careless miss despite remembering the sample cue locations, as all subjects extensively practiced the task to be high performance (>80%) before the recording session.

Discussion

In the present study, we measured haemodynamic changes in the LPFC of adults and preschool children using an OT technique while subjects performed an item-recognition task, which requires working memory. We observed changes in C_{oxy} in the bilateral LPFC of adults during task performance. This change was characterized by a sustained increase that was temporally associated with the onset of the sample cue. The magnitude of activity differed between different memory-load conditions; the LPFC was more active in the high-memory-load than in the low-memory-load condition. In preschool children, we also observed haemodynamic changes in the LPFC during performance of the task. The overall pattern of these changes was similar to that of the adults, in terms of (i) a temporal association between the change in C_{oxy} and the sample cue presentation, (ii) a sustained increase in C_{oxy} and (iii) a decrease to the background level 5–10 s after the response. For both adults and children, the change in C_{oxy} was accompanied by a nonsignificant trend of decrease in C_{deoxy}, which has also been reported in many previous studies that used the NIRS technique (e.g. Maki *et al.*, 1995; Sato *et al.*, 1999). These results indicate that the LPFC is active in both preschool children and adults during working memory, suggesting that the LPFC has already developed functionally in 5–6-year-olds to enable processing of this cognitive function.

LPFC Activation Associated with Working Memory Measured by Event-related OT

The OT technique adopted here is a type of near-infrared spectroscopy (NIRS; Jöbsis, 1977), but it has an advantage over other NIRS measures: OT can measure temporal changes in haemoglobin oxygenation from multiple regions simultaneously, via multiple pairs of light emitters and detectors (Koizumi *et al.*, 1999, 2003), whereas other NIRS techniques measure spectroscopic reflection and scattering via a single emitter-detector (Chance *et al.*, 1993; Hoshi and Tamura, 1993; Kato *et al.*, 1993; Villringer *et al.*, 1993). Although OT also has

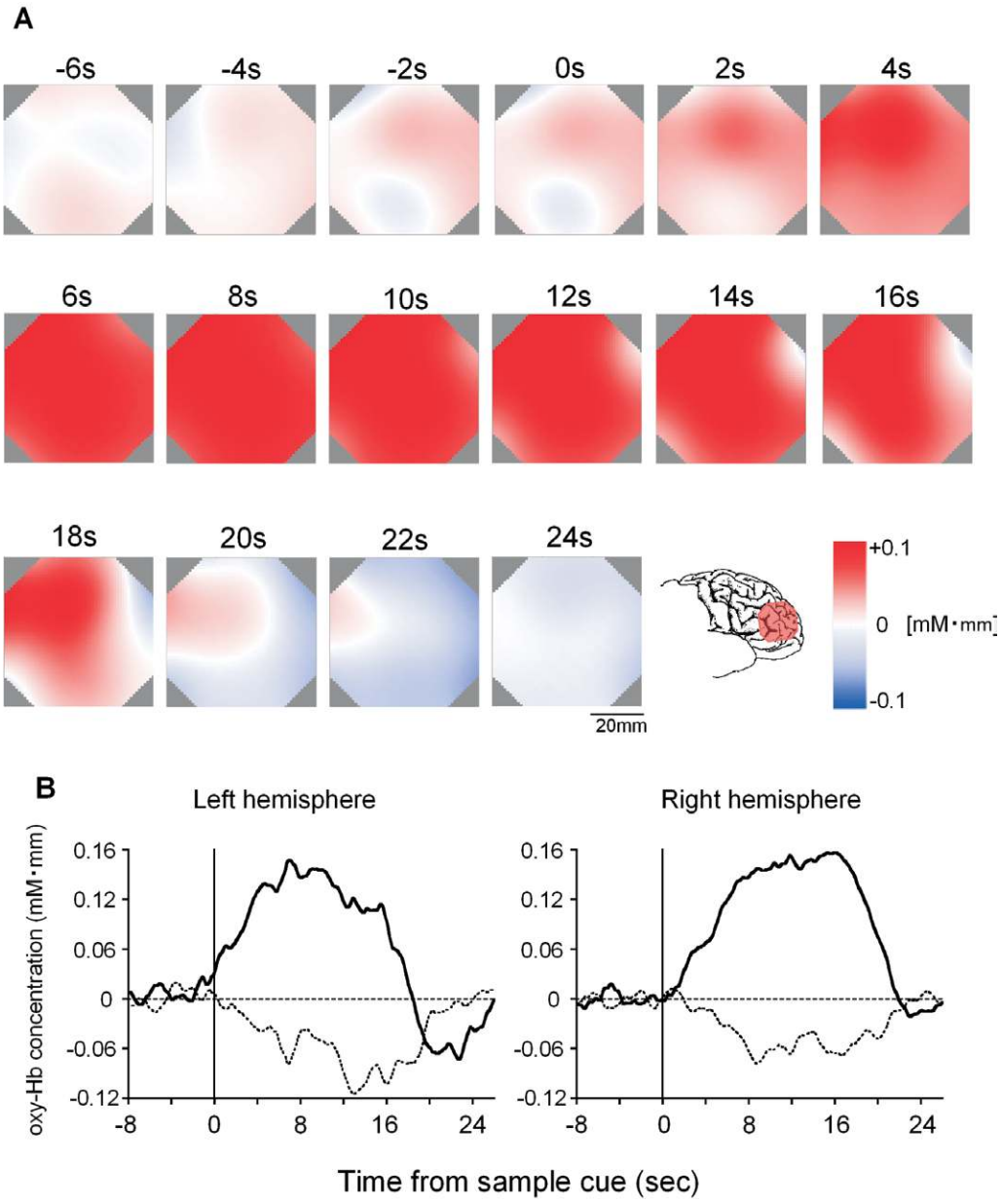

Figure 8. (*A*) Dynamic topogram of changes in the concentration of oxyhaemoglobin in the right LPFC of a 6-year-old child. The format is the same as that in Figure 3. (*B*) Time course of changes in oxy- and deoxyhaemoglobin concentrations in the LPFC of the child are shown in (*A*). Solid and dotted lines show oxy- and deoxyhaemoglobin, respectively. Data from channels 6 and 19 are shown for the left and right hemispheres, respectively.

some disadvantages compared with other methods (e.g. it can not measure the absolute value of each haemoglobin), it can potentially be a useful tool for functional mapping. Indeed, previous studies using OT have reported haemodynamic changes associated with various cortical functions such as motor action (Maki *et al.*, 1995, 1996) and language processing (Watanabe *et al.*, 1998; Sato *et al.*, 1999; Kennan *et al.*, 2002). These studies have adopted a blocked-task paradigm that measures accumulated activity over many trials in close succession. In contrast, Noguchi *et al.* (2002) recently demonstrated that an event-related design is useful in OT for studying higher cognitive functions. In the present study, we were the first to adopt such an event-related design for the study of function mediated by dorsolateral areas of the PFC and we detected event-related changes in C_{oxy} in the dorsolateral PFC.

Our behavioural task was a type of item-recognition task, which has been extensively used to study working memory processes, including functional activation studies with PET/fMRI (e.g. Jonides *et al.*, 1993, 1997; Belger *et al.*, 1998; Leung *et al.*, 2002; for reviews, see Smith and Jonides, 1999; D'Esposito *et al.*, 2000). While the subjects performed this task, we observed activation in the dorsolateral areas of the PFC (BA 9/46). This area of activation is consistent with those reported in many previous studies (e.g. Funahashi *et al.*, 1989; Jonides *et al.*, 1993; Belger *et al.*, 1998; Owen *et al.*, 1999; Zarahn *et al.*, 1999). Furthermore, the time course of haemodynamic changes appears to be similar to those reported in previous event-related fMRI studies (e.g. Zarahn *et al.*, 1999; Rypma and D'Esposito, 2000; Leung *et al.*, 2002). In addition, our task had two different memory-load conditions, which

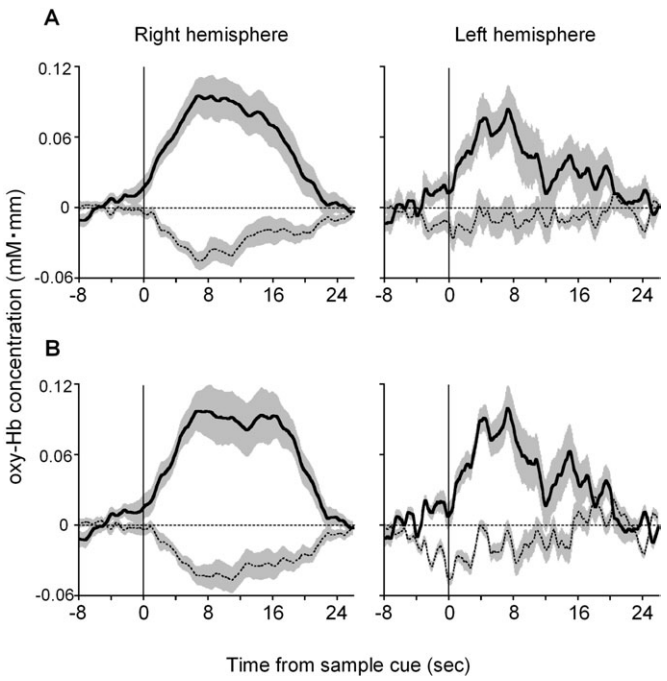

Figure 9. Time course of changes in oxyhaemoglobin concentration in the LPFC of children. (*A*) Mean concentration change across the population ($n = 16$) for the LPFC. (B) Data excluding subjects with poor performance (<75%) are shown ($n = 12$). Solid and dotted lines show oxy- and deoxyhaemoglobin, respectively. Shading indicates standard errors.

was confirmed by the differential behavioural data, and we observed load dependency in LPFC activation across different memory-load conditions. Such a load dependency in LPFC activation has also been reported in many previous studies that used PET/fMRI to examine working memory (Braver *et al.*, 1997; Cohen *et al.*, 1997; Jonides *et al.*, 1997; Manoach *et al.*, 1997; Rypma and D'Esposito, 1999). From the precise time course of changes in C_{oxy}, both the sample cue onset and the warning signal appeared to provide a time-locked signal. This may also be consistent with previous studies, because the LPFC has been suggested to play a role in task preparation (MacDonald *et al.*, 2000; Brass and von Cramon, 2002). Taken together, these data indicate that the changes in C_{oxy} observed here are associated with working memory.

Thus, event-related OT is useful for examining higher order cognitive functions that are mediated by the LPFC, although this method has some disadvantages – for example, relatively low spatial resolution and the restriction of measurements to the lateral surface of the cerebral cortex. Nonetheless, OT will become a new approach to the mapping of cognitive functions, since the method has some clear advantages, in particular its complete non-invasiveness and unobtrusiveness, which makes it usable with children.

LPFC Activation in Preschool Children and Possible Application of OT on Functional Developmental Study

Since OT could measure LPFC activity associated with working memory in normal adults, we applied the same procedure to preschool children aged from 5 to 6 years. We detected significant changes in C_{oxy} in the LPFC during the task performance. These changes were characterized by (i) a temporal association between the change in C_{oxy} and the sample cue presentation, (ii) a sustained increase in C_{oxy} and (iii) a decrease to the background level 5–10 s after the response. These characteristics were similar to those of adults. Therefore, it has been suggested that these changes in preschool children are associated with working memory. Thus, our findings are the first to suggest that the LPFC of 5- to 6-year-olds has developed functionally to enable processing of working memory and should make an important contribution to the understanding of the functional and/or structural development of the LPFC.

Previous psychological and morphological studies have suggested that a PFC-mediated working memory system has already developed by preschool age. For example, at the behavioural level, it was suggested that visuospatial working memory emerges at around the age of 4 years and improves between the ages of 5 and 7 years (Luciana and Nelson, 1998). With respect to brain structure, morphological studies have shown that the volume of white matter increases monotonically from the age of 4 years to young adulthood and that the volume of the frontal grey matter increases from childhood to adolescence (Giedd *et al.*, 1999). Furthermore, the number of synapses in the PFC increases from birth to around 7 years of age (Huttenlocher, 1979). The present findings are consistent with these studies and extend their results, by providing direct evidence that development of the LPFC in preschool children (aged from 5 to 6 years) already enables processing of working memory.

Recently, Kwon *et al.* (2002) showed an age-related increase in LPFC activity during a working memory task in subjects aged between 7 and 22 years. Together with their findings, we suggest that functional development of the LPFC for working memory has already commenced by the age of 5 years. However, in the present study, we could not examine age-related changes, because the age of our subjects did not vary enough to investigate the correlation between age and activity. Further studies using OT in children across a wider age range (e.g. from 3 to 6 years of age) will contribute to the understanding of age-related functional maturation over a range of preschool ages.

Comparison between Adults and Preschool Children

In the present study, according to detailed characteristics of the haemodynamic changes in adults and preschool children, the LPFC of children appeared to be more highly and broadly active than that of adults. This may reflect the developmental change of LPFC function to enable more efficient processing of such an important cognitive function as working memory (Casey *et al.*, 1997; Tamm *et al.*, 2002). However, the difference in magnitude and extension may simply reflect some structural differences between adults and children. Indeed, we could not directly compare the difference in activity between adults and children, owing to differences in brain structure, skull, hairline and so on, which can cause differences in light absorption between adults and children. This would also make it difficult to address whether our results are consistent with the morphological studies that have found protracted increase in white matter volume of the PFC from the age of 8 years to young adulthood (Giedd *et al.*, 1999).

Furthermore, an inverse tendency was observed with respect to hemispheric dominance in adults and children: the left LPFC tended to be dominant in adult subjects, especially in the LOW condition, while the right LPFC tended to be dominant in children. This difference might reflect developmental

changes in functional specialization of the LPFC for working memory, as reported in previous event-related potential studies of the development of language systems (Mills *et al.*, 1997; Neville and Bavelier, 1998). However, the hemispheric differences observed here were not very significant (i.e. $0.05 < P < 0.1$) and it is possible that adults and children might adopt different strategies for performing the present task. Therefore, these problems regarding hemispheric difference remain to be solved by further studies that use a more appropriate method.

Conclusions

We first demonstrated that OT with an event-related paradigm can examine the neural correlate of working memory mediated by the LPFC. This can introduce a new dimension to the mapping of LPFC function in normal children as well as in adults. In addition, for the first time, we demonstrated that the LPFC of preschool children is active during the operation of working memory. Although we could not comprehensively compare the activity of adults and children, or in children at different stages of development, our results extend the understanding of not only the functional development, but also the neural basis of cognitive functions mediated by the LPFC.

Notes

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