Spatiotemporal properties of cortical haemodynamic response to auditory stimuli in sleeping infants revealed by multi-channel near-infrared spectroscopy

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Multi-channel near-infrared spectroscopy (NIRS) has been used as a neuroimaging tool to study functional activation of the developing brain in infants. In this paper, we focus on spatiotemporal dynamics of cortical oxygenation changes during sensory processing in young infants. We use a 94-channel NIRS system to assess the activity of wide regions of the cortex in quietly sleeping three-month-old infants. Auditory stimuli composed of a random sequence of pure tones induced haemodynamic changes not only in the temporal auditory regions, but also in the occipital and frontal regions. Analyses of phase synchronization showed that mutual synchronizations of signal changes among the cortical regions were much stronger than the stimulus-induced synchronizations of signal changes. Furthermore, analyses of phase differences among cortical regions revealed phase advancement of the bilateral temporal auditory regions, and phase gradient in a posterior direction from the temporal auditory regions to the occipital regions and in an anterior direction within the frontal regions. We argue that multi-channel NIRS is capable of detecting the precise timing of cortical activation and its flow in the global network of the developing brain.

Keywords: near-infrared spectroscopy; haemodynamics; developing brain

1. Introduction

(a) Near-infrared spectroscopy as a tool to explore the developing brain

The human brain is characterized as a complex system consisting of a large number of components and the networks among them. While the spatiotemporal activity of the brain is generated under the constraint of the anatomical structure, this activity shapes the structure over the course of development. A fundamental challenge is to elucidate how the structural and functional properties of the brain relating to motion, perception and cognition emerge over developmental time.

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Early theoretical studies predicted temporal dynamics as a key to understanding the functioning of the brain and its development [1–3]. Over the past decade, research on structural and functional brain imaging in infants has grown rapidly, and hypotheses concerning the role of brain dynamics in developmental processes are beginning to undergo rigorous testing.

Among modern neuroimaging techniques, multi-channel near-infrared spectroscopy (NIRS) is an important tool to study the functional development of the cortex in young infants. The application of NIRS to monitor cerebral oxygenation and haemodynamic changes non-invasively was first reported by Jobsis [4]. NIRS has been developed as a clinical tool to monitor steady-state cerebral tissue oxygenation in infants [5]. Since the finding that NIRS can detect cerebral blood oxygenation changes in response to sensory stimulation in adults [6–9] and infants [10], NIRS has been developed as a tool to assess cortical activation. In particular, advances in multi-channel NIRS systems using continuous-wave spectroscopy [11] have sparked neuroimaging studies in adults (for a review, see [12]) and infants ([13,14]; for a review see [15]). This paper focuses on how multi-channel NIRS can reveal spatiotemporal dynamics of the developing brain in young infants.

(b) Basis of near-infrared spectroscopy imaging for studies in infants

Neuroimaging techniques such as functional magnetic resonance imaging (fMRI) have been used to assess cortical activation by measuring haemodynamic changes due to neurovascular coupling. The cortical activation induces changes in vascular tone and oxygen consumption, which lead to increases in the cerebral blood flow, volume and oxygenation (for a review, see [16]). Although the mechanisms of the neurovascular coupling are not fully understood, animal studies have shown that neuronal excitation induces vasodilation and an increase in blood oxygenation, which leads to an increase in oxygenated haemoglobin (oxy-Hb) and a decrease in deoxygenated haemoglobin (deoxy-Hb), while neuronal inhibition induces vasoconstriction and a decrease in blood oxygenation, leading to a decrease in oxy-Hb and an increase in deoxy-Hb [17,18]. While decreases in deoxy-Hb are the major source of increases in fMRI blood oxygen level-dependent (BOLD) signals to show cortical activation ([19]; for a review, see [20]), increases in oxy-Hb and/or decreases in deoxy-Hb are regarded as cortical activation in NIRS imaging (for a review, see [12]). When we use neuroimaging techniques to study the developing brain, it is important to ensure neurovascular coupling during developmental periods. Studies showing negative fMRI BOLD responses to visual stimulation in sedated or sleeping infants [21,22] and deoxy-Hb increases in response to sensory stimulation in infants [10] have argued that neurovascular coupling might be immature in infancy. On the other hand, fMRI studies of infant macaques [23] and rats [24] showed no evidence of negative BOLD response to sensory stimulation. Although controversy remains as to whether neurovascular coupling is established in human infants, a number of NIRS studies with awake infants have shown that infants as young as two months show adult-like haemodynamic responses (increases in oxy-Hb and decreases in deoxy-Hb) to sensory stimulation [13,25]. Thus, multi-channel NIRS can provide information on the development of neurovascular coupling as well as information on the locus of activation.
(c) Developmental changes in spontaneous activity of the cortex

An important approach to elucidating cortical development is to observe changes in its spontaneous activity. In contrast with the traditional view that cortical development starts from stimulus-induced response or reflex, many studies in experimental animals have shown that spontaneous activity of the cortex commences at a time when sensory input through the thalamus has not yet become functional [26]. This suggests that spontaneous activity has a critical role in shaping the functional architecture and network of the brain in the early prenatal period, and this should be the case in humans [27].

The spontaneous fluctuation of activity in the brain is present during the resting state in adults, and this raises important issues as to brain energy metabolism and the functional topography of the brain [28]. Recently, a large number of fMRI studies have demonstrated that spontaneous low-frequency BOLD signal fluctuations (0.01–0.1 Hz) during the resting state show correlated changes among distributed but functionally related brain regions, providing us with a clue to understanding the network properties of the brain [29]. The emerging field of analysing cortical networks further raises a number of interesting questions about cortical development. The developmental emergence and changes of the resting-state functional connectivity of the brain have been explored from preterm periods to adolescence (see [30] for preterm infants, [31] for measurements of preterm infants at term-equivalent ages, [32] for two-week-old to 2-year-old infants, [33] for 7- to 30-year-olds). In particular, fragmentary elements of the repertoire of resting-state dynamics in the cortical network of adults is already present in the last trimester of gestation [30]. On the other hand, substantial changes in resting-state networks were observed over the course of development [32,33].

The spontaneous fluctuations of cerebral blood oxygenation have been studied by using NIRS [34–39]. In newborns, spontaneous changes in oxy- and deoxy-Hb signals were observed at the occipital cortex, suggesting that haemodynamic changes may reflect spontaneous activity of the cortex [40]. In accordance with the fMRI studies on resting-state functional connectivity in adults, several studies have demonstrated that multi-channel NIRS can detect correlated haemodynamic signals in structurally and functionally related cortical regions in adults [41–44].

Given the high time resolution of NIRS and quite a large number of channels (approx. 100), the current state of the art of the multi-channel NIRS technique has a great advantage in detecting detailed dynamics of spontaneous haemodynamic changes and network properties of the cortex. A multi-channel NIRS study of the functional connectivity of spontaneous activity in sleeping infants revealed drastic changes in the global cortical network during the first six months of life [45]. The development of functional connectivity included increased, decreased and U-shaped changes, depending on the cortical regions [45]. This finding suggests that dynamic processes for the generation of a functional cortical network are present in postnatal periods, whereas the cortical shape [46] and axonal organization [47,48] are well established at birth.

(d) Developmental changes in stimulus-induced activity

An important question about the development of the cortex considers how the functional specializations in relation to perceptual and cognitive ability emerge
over the course of development [49]. Although the use of fMRI with awake infants has a practical limitation in that the subjects need to remain still while lying flat in a scanner making noise, a small number of laboratories have successfully used it to demonstrate functional activation studies of young infants [50–52]. In contrast, NIRS has the great advantage of enabling investigation of stimulus-induced cortical activation in relation to perceptual and cognitive activity in awake infants [13,25,53–59,60,61]. Regarding the development of auditory and language perception, NIRS measurements have been performed during sleep [14,62–67]. Most of these studies have examined whether the cortical regions of interest in infants of different ages selected using behavioural studies showed hypothesized responses based on adult neuroimaging studies. The accumulation of knowledge about the neural correlate has formed the general view that the many regions of the cerebral cortex are functioning as early as a few months of age.

Studies comparing cortical response to the same stimulation between different age groups have shown developmental changes in activation patterns over months [25,57,59,64]. This approach sometimes reveals developmental changes that are beyond expectation based on hypothesis-driven neuroimaging. For example, functional activation patterns in response to audio-visual objects change between two and three months of age in a general-to-specific manner: increasing the localization of regions activated by a particular stimulus and increasing the exclusivity of the response to specific stimuli within a particular cortical region [25]. This study suggests that global interaction among the cortical regions may play an important role in developing localized functional regions.

NIRS has also been used to detect changes in stimulus-induced activity over a time scale of minutes. For instance, the prefrontal region of three-month-old infants showed habituation to repetitive stimuli and dishabituation to novel stimuli [68]. Thus, NIRS is a powerful tool for measurement of spatiotemporal patterns of cortical activity over multiple time scales.

**(e) Temporal dynamics of cortical activation in the developing brain**

While the vascular response to the onset of neural activity is consistently delayed by several seconds, the relative timing between the onset of fMRI BOLD signals in different brain regions is preserved with a temporal accuracy of tens of milliseconds [69]. Thus, precise characterization of haemodynamic responses allows ‘chronometry’ of information regarding the temporal sequence of events in neural activation during cognitive or perceptual tasks [69,70]. Unlike traditional functional imaging, a method to construct ‘chronoarchitecture’ was proposed based on time-series analyses of BOLD signals measured during natural conditions [71]. Despite the high sampling rate of NIRS, there are few studies of time-domain analysis of cortical response in infants. Recently, a NIRS study has shown temporal aspects of stimulus-induced cortical activation during audio-visual perception in three-month-old infants [72]. In the present study, taking advantage of a multi-channel NIRS system (94 channels) with a high sampling rate (10 Hz), we study phase relationships of haemodynamic signals among cortical regions of infants during sound stimulation to reveal the internal dynamics of activation in the global network of the developing brain.
2. Methods

(a) Participants

Eighteen full-term healthy infants participated in the study (six girls and 12 boys, mean age 111 days (s.d. = 5 days), range 98–116 days, mean birth weight 2980 g (s.d. = 288 g)). By using video recordings for each infant, we checked the state of the infants and the number of times they moved their heads, bodies, arms and legs. Only infants in the state of quiet sleeping were included for further analyses. If they moved more than four times during recording, even when they were sleeping, they were excluded from further analyses. We studied 20 additional infants but excluded them from the sample owing to their awakening during the experiment (n = 5) and active sleeping with their heads and body movements producing large motion artefacts (n = 15). Participants were recruited from the local Basic Resident Register. This study was approved by the ethics committee of the Graduate School of Education, University of Tokyo, and written informed consents were obtained from the parent(s) of all the infants prior to the initiation of the experiments.

(b) Stimuli

The infants were exposed to 5s auditory stimuli composed of a random sequence of 25 pure tones (C3 to F6) of 100ms. Fifteen cycles of stimulation with inter-stimulus intervals (ISI) of 10s and 15 cycles of stimulation with ISIs of 5s were separately presented for each infant. While 15s trial (10s ISI) was sufficient for a haemodynamic response to return to the baseline, 10s trial (5s ISI) was less than the time for the haemodynamic response to return to baseline. We performed the two different ISI conditions to examine the temporal properties of the responses.

(c) Procedure

We conducted the experiments in a noise-attenuating and dimly lit (180 lx) room. The infants were held in an experimenter’s arm during measurement and given auditory stimuli through a speaker system (Bose MMS-1). We examined the infants while they were in daytime sleep, and they were almost motionless and slept soundly throughout the experiment.

(d) Data acquisition

The multi-channel NIRS instrument used in the present study (ETG-7000; Hitachi Medical Corporation, Tokyo, Japan) can detect changes in the relative concentrations of oxy-Hb and deoxy-Hb with 0.1s time resolutions in multiple measurement channels. Two NIR wavelengths (785 and 830 nm) were used in this system. The intensity of the illumination of the NIR light at each incident position was 0.6 mW. On the basis of the Beer–Lambert law, we evaluated the relative changes in the oxy-Hb and deoxy-Hb signals from an arbitrary zero baseline at the start of the measurement period for the 94 measurement channels. Since the precise optical path length was unknown, the unit assigned to these values was derived by multiplying the molar concentration with the length (mM mm). The 94 measurement channels were arranged over the occipital, temporal and
Figure 1. Spatial configuration of the 94 measurement channels.

frontal cortices of each hemisphere, as shown in figure 1. Three pieces of incident and detection fibres (hereafter, triple-piece set) were arranged vertically, and the vertical inter-optode distance between the fibres was fixed at 2.0 cm. A previous study assessed haemodynamic responses to auditory stimuli over the temporal cortex in three-month-old infants by using multiple source–detector pairs of varying distances and found that a 2 cm source–detector distance provided the highest sensitivity to cortical responses [73]. We arranged 10 such triple-piece sets over each hemisphere at even horizontal distances. Thus, the horizontal inter-optode distance between the adjacent sets was approximately 2 cm, which varied depending on the infants’ head size. Each pair of adjacent incident and detection fibres defined a single measurement channel. The spatial positioning of the 94 measurement channels was determined by using the positions defined by a 10–20 electrode system for electroencephalography (EEG) as references [72]. It is practically important to attach optical probes quickly to an infant participant.

(e) Data analysis

Oxy- and deoxy-Hb signals were digitally band-pass filtered (0.04–0.2 Hz) to remove the effect of very low-frequency fluctuations and higher-frequency components such as respiratory and cardiovascular signals and measurement noise for each data point under different ISI conditions (10 s and 5 s). Next, the individual data points were split into data blocks of 15 s (ISI 10 s) or 10 s (ISI 5 s), by using the onset of each stimulus. When we detected a change in the sum of oxy-Hb and deoxy-Hb signals more than 0.15 mM mm between the mean of four successive samples and that of the next four successive samples (during 400 ms), the block that contained the rapid change in signals was eliminated from subsequent analyses. After removal of linear trends within the data blocks,
the changes in the oxy-Hb and deoxy-Hb signals were averaged over the data blocks for each infant to obtain the individual haemodynamic responses for each measurement channel for each ISI condition. Then, a grand average of the responses among infants was calculated on a channel-by-channel basis under each ISI condition.

To assess activation in response to the stimuli, we performed statistical analyses based on the general linear model (GLM) (see [25] for details). We used the same haemodynamic response function to 1 s stimulation as was used in an fMRI study [74]:

\[ h(t) = (t - c_1)^c_2 \exp \left[ \frac{-(t - c_1)^c_3}{c_3} \right]. \]  

(2.1)

where \( c_1, c_2 \) and \( c_3 \) are constant parameters. We then calculated a predicted time series for haemoglobin signals by convolution of a series of 5 s stimuli functions with this haemodynamic response function. Oxy-Hb and deoxy-Hb had generally different time lags for their responses depending on channels, participants and experimental conditions. To obtain the optimum parameter of time lag \( c_1 \) that maximizes the fitness of the response function in the present data set, we tested GLM analyses using different parameter values between 0.0 and 2.0 s. The number of activated channels was largest across the two ISI conditions when we chose \( c_1 = 0.6 \) s for oxy-Hb and \( c_1 = 1.5 \) s for deoxy-Hb. The parameter values of \( c_2 \) and \( c_3 \) were chosen (\( c_2 = 8.60 \) and \( c_3 = 0.547 \)) based on the literature [74]. The filtered data of oxy- and deoxy-Hb signals were fitted to the predicted time series with the weight parameter \( \beta \), which was calculated by the least-square estimate. To identify the activated channels, we considered individual \( \beta \) values as random effects and performed a t-test against a zero baseline for each channel. To take into account multiple comparisons among the 94 channels, we applied a false discovery rate (FDR) correction at \( Q < 0.05 \). Thus, we obtained activation maps based on oxy- and deoxy-Hb for each ISI condition.

To focus on the temporal aspects of the signal changes, an instantaneous phase of response \( \phi_i(t) \) was calculated by applying the Hilbert transform to the filtered data of channel \( i \) (\( i = 1, \ldots, 94 \)) [40]. This is based on the assumption that the time series can be characterized as a circular process with variable amplitudes and phases. The phase was reset every 2 \( \pi \) and the period of oscillation was determined by the interval of adjacent cycles of motion of the instantaneous phase. We then performed two types of analyses.

First, to quantify the phase locking of response to the stimuli, we calculated the instantaneous phase difference \( \Delta \phi_{ri}(t) \) between phase \( \phi_r(t) \) of the reference time series based on the haemodynamic response function and phase \( \phi_i(t) \) of the measured time series of channel \( i \) (\( i = 1, \ldots, 94 \)) as

\[ \Delta \phi_{ri}(t) = \phi_r(t) - \phi_i(t). \]  

(2.2)

The time-averaged response phase difference was determined by vectorially averaging the instantaneous phase differences over time as

\[ \Delta \bar{\phi}_{ri} = \tan^{-1} \frac{\sum_{k=1}^{N} \sin(\Delta \phi_{ri}(k))}{\sum_{k=1}^{N} \cos(\Delta \phi_{ri}(k))}, \]  

(2.3)
where $N$ is the number of measurement time points. A length of the time-averaged vector is also given as

$$
\gamma_{ri} = \frac{1}{N} \sqrt{\frac{\sin^2(\Delta \phi_{ri}(k))}{N} + \cos^2(\Delta \phi_{ri}(k))}.
$$

(2.4)

Since this value ($0 \leq \gamma_{ri} \leq 1$) reflects the degrees of phase locking of response to stimuli at each channel, we refer to this value as the synchronization index [75]. We then obtained a group mean matrix of the phase difference $\langle \Delta \phi_{ri} \rangle$ by vector summation and a group mean matrix of the phase synchronization index $\langle \gamma_{ri} \rangle$ by scalar summation.

Second, to understand the phase relationship of activation among the cortical regions, we defined instantaneous phase differences of signals between channels $i$ and $j$ as

$$
\Delta \phi_{ij}(t) = \phi_i(t) - \phi_j(t).
$$

(2.5)

The time-averaged phase difference $\bar{\Delta \phi}_{ij}$ and synchronization index $\gamma_{ij}$ between channels $i$ and $j$ were calculated in the same manner as the previous ones. For each infant, we obtained a $94 \times 94$ matrix of the time-averaged phase difference and a $94 \times 94$ matrix of the phase synchronization index. Finally, we obtained a group mean matrix of the phase difference $\langle \bar{\Delta \phi}_{ij} \rangle$ by vector summation and a group mean matrix of the phase synchronization index $\langle \gamma_{ij} \rangle$ by scalar summation.

### 3. Results

**Figure 2a** shows the group-averaged time courses of changes in oxy-Hb and deoxy-Hb signals in response to 5s auditory stimulation under 10s ISI condition. The typical pattern of haemodynamic response with an increase in oxy-Hb and a decrease in deoxy-Hb was observed not only in bilateral temporal auditory regions but also in the occipital, parietal and prefrontal regions. The statistical analyses based on the GLM showed significant positive changes in oxy-Hb signals and negative changes in deoxy-Hb signals over the diverse cortical regions (figure 2b). **Figure 2c** shows the case under 5s ISI condition. Conspicuous changes in both oxy- and deoxy-Hb signals were observed in the channels of focal regions of the temporal cortices on both hemispheres. The response pattern showed a pseudo-pre-undershoot, which was predicted from the linear model of haemodynamic response when the ISI was less than the time for the haemodynamic response to fully return to baseline [76]. The statistical analyses demonstrated significant positive changes in oxy-Hb signals and negative changes in deoxy-Hb signals only at the bilateral temporal regions (figure 2d). The different results between the ISI conditions showed that the GLM-based model with a fixed time lag of responses was not sufficient to account for temporal aspects of the responses.

Regarding temporal features of the response, we focused on the phase relationship between stimulation and responses. **Figure 3a** shows circular and vectorial representation of the phase differences between the stimuli and the oxy-Hb signals of each measurement channel under the 10s ISI condition. Each bar in the circles shows individual data, where the angular direction of the
bar represents the time-averaged response phase $\Delta \tilde{\phi}_{ri}$ and the length of the bar represents the synchronization index $\gamma_{ri}$. It can be seen that $\Delta \tilde{\phi}_{ri}$ showed high variations, suggesting that the phase lags of the response to the stimuli were individually different. Most of the values of $\gamma_{ri}$ were less than 0.5, suggesting that there were trial-by-trial variations in the response to the stimuli in each infant. The group mean of synchronization index $\langle \gamma_{ri} \rangle$ revealed that only the channels of bilateral temporal regions showed values exceeding 0.3, which were illustrated as the functional connectivity between the cortical regions represented by the circles and the stimulus input represented by the central point, as shown in figure 3b. This suggests that the temporal auditory regions showed more phase locking response to the stimuli as compared with those of the other regions.

We further analysed phase differences in activity among the cortical regions. Figure 3c shows a circular and vectorial representation of the phase differences in oxy-Hb changes between the selected channel located on the temporal auditory region and channels on the other regions of the same hemisphere. The selected channel in each hemisphere is labelled with a single bar. Overall, the values of time-averaged phase difference $\Delta \tilde{\phi}_{ij}$ distributed around the zero phase difference and showed fewer variations as compared with those of $\Delta \tilde{\phi}_{ri}$, suggesting that there were fewer individual differences in the phase locking between the activity of the auditory regions and the other cortical regions as compared with the stimulus-induced phase locking. The values of synchronization index $\gamma_{ij}$ were generally larger than those of $\gamma_{ri}$, suggesting that there were fewer trial-by-trial variations in phase locking in each infant. The group mean matrix of synchronization index $\langle \gamma_{ij} \rangle$ further revealed the network properties of the functional connectivity. Figure 3d shows functional connectivity between the temporal auditory regions and other regions. Functional connectivity with different strengths was separately illustrated according to the $\langle \gamma_{ij} \rangle$ values. The selected temporal channels had higher connectivity ($\langle \gamma_{ij} \rangle > 0.5$) with the channels of the homologous regions of the temporal cortices as well as the adjacent temporal channels, middle connectivity ($0.4 < \langle \gamma_{ij} \rangle < 0.5$) with those distributed over the bilateral temporal cortices, and lower connectivity ($0.3 < \langle \gamma_{ij} \rangle < 0.4$) with those of the bilateral frontal and occipital cortices. This result indicates that the activities of the bilateral temporal auditory regions were highly synchronized with each other, whereas the activities of the frontal and occipital regions were loosely coupled with the activities of the temporal regions.

Finally, to reveal the properties of the global network of the cortex, we mapped functional connectivity based on the group mean matrix of synchronization index $\langle \gamma_{ij} \rangle$. We further mapped the phase gradient between the connected regions based on the group mean matrix of the phase difference $\langle \Delta \tilde{\phi}_{ij} \rangle$. Figure 4 shows functional connectivity whose synchronization index $\langle \gamma_{ij} \rangle$ was more than 0.6 and the phase gradient, represented by an arrow from a phase-advanced channel to a phase-delayed channel, with its length showing the value of the phase difference. A noticeable property of the functional network is that the connectivity between the homologous regions was strong over the frontal, temporal and occipital cortices, suggesting that both hemispheres work highly synchronously. Any other connectivity whose synchronization index was more than 0.6 was local; long-distance connectivity within the hemisphere was not observed. Another notable finding is that the phase gradient is present in a posterior direction from the
temporal auditory regions to the occipital regions and in an anterior direction within the frontal regions. These results suggest that stimulus-induced activity generated in the temporal auditory regions might be propagated to other regions through short-distance cortico-cortical connectivity.

4. Discussion

We have argued that multi-channel NIRS can detect spatiotemporal haemodynamic changes relating to cortical activation in young infants over various time scales. This paper has demonstrated real-time dynamics of
Figure 3. Stimulus-induced synchronization of haemodynamic changes. (a) Circular and vectorial representation of phase differences between oxy-Hb signals of each measurement channel and reference signals based on the presentation of stimuli. Each bar in the circles shows individual data. The bar in the central circle shows reference phase of stimulation. (b) Functional connectivity between the cortical regions and the stimulus input. The group mean of the synchronization index exceeding 0.3 is illustrated. (c) Circular and vectorial representation of the phase differences in oxy-Hb changes between the selected channel located on the temporal auditory region and channels on the other regions of the same hemisphere. Each bar in the circles shows individual data. (d) Functional connectivity between the temporal auditory regions and other regions. Functional connectivity with different strengths was separately illustrated according to the group mean of the synchronization index. The data under the condition of 10s ISI are shown.

Figure 4. Mutual synchronization of haemodynamic changes. Functional connectivity whose synchronization index is more than 0.6 is illustrated by a red line. Phase gradient from a phase-advanced channel to a phase-delayed channel is illustrated by a blue arrow, with its length showing the value of the phase difference. They were calculated from the oxy-Hb signals under the condition of 10s ISI.

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haemodynamic changes in response to simple auditory stimuli consisting of a random sequence of pure tones in three-month-old infants who were quietly sleeping by using a 94-channel NIRS system. We observed an increase in oxy-Hb signals and a decrease in deoxy-Hb not only in the bilateral auditory regions of the temporal cortices but also in the broad regions of the occipital and frontal cortices when the 5s stimuli were presented with the longer ISI (10s). In contrast, the haemodynamic response patterns of temporal and other regions were dissociated when the stimuli were presented with the shorter ISI (5s). The apparent difference in the haemodynamic response to the auditory stimuli between the ISI conditions was likely to be caused by the subtle difference in the response lag depending on the cortical regions. In particular, under the 5s ISI condition, only the responses of the temporal auditory regions fitted the predicted haemodynamic responses with a fixed lag, whereas the responses of the other cortical regions did not fit, owing to lagging behind the predicted responses and/or individual variations in response lag.

Despite no apparent differences in the group-averaged haemodynamic response patterns among the cortical regions under the 10s ISI condition, the analyses of the response phases revealed that the response properties to the stimuli differed between the cortical regions. We found that individual differences in the phase lag of the response to the auditory stimuli were smaller in the temporal regions than in the other regions. We also found that the responses to the stimuli were more synchronized in the temporal regions than in the other regions. These results suggest that only the temporal auditory regions showed stimulus-induced activation and that other cortical regions, including other sensory areas such as visual, association and higher association regions, had different functional roles in the processing of auditory stimuli.

In addition to the properties of stimulus-induced activation, we found that the mutual synchronizations of activity among the cortical regions were much stronger than those between stimulation and response. This result may be accounted for by the anatomical property of the cerebral cortex, in which the number of internal connections within the cortex is much larger than that of sensory input [77]. One of the conspicuous characters of synchronization within the cortex was that the haemodynamic changes of the bilateral homologous regions were highly synchronized. A similar phenomenon was observed in spontaneous changes without stimulation in sleeping infants of a similar age [45]. In theory, the synchronization of cortical activation of the homologous regions can be produced by either the same sensory input through the thalamo-cortical pathway or the cortico-cortical interaction through the corpus callosum. Given the fact that long-distance cortical connections are primarily established by term age [47,48], the robust synchronization of the homologous regions for both stimulus-induced and spontaneous activity suggests that the corpus callosum may play an important role.

Another conspicuous character is the phase advancement of the bilateral temporal auditory regions and the phase gradient in a posterior direction from the temporal auditory regions to the occipital regions, and in an anterior direction within the frontal regions. There was no strong long-distance connectivity within the same hemisphere of the cortex. The phase advancement of the stimulus-averaged response in the temporal regions was reported in previous studies in awake three-month-old infants during audio-visual stimulation [72] and in sleeping
three-month-old infants during speech sound stimulation \[78\]. An fMRI study in three-month-old infants showed the fastest responses to speech sound (3s after stimulus onset) in the Heschl’s gyrus and increasingly slower responses towards the posterior part of the superior temporal gyrus (5s), the temporal poles (5s) and the inferior frontal gyrus (7–9s) \[51\]; however, it is not clear why such a long delay was generated. In this study, the stimulus-averaged response did not show a conspicuous spatial pattern of response lags and the synchronization analyses showed that most of the phase differences were less than 10° (0.4s of a 15s stimulus cycle). To reveal subtle differences in response timing, the correlation analysis has a limitation, because time lag can only be assessed with a discrete interval of sampling rate (0.1s). In contrast, the phase analysis concerns the time evolution of analogue values of instantaneous phase of the signals. Thus, to obtain a phase lag between signals, averaging phase differences should be rather a simpler way than estimating time lag based on cross-correlation. Furthermore, the phase analysis is a straightforward method to assess nonlinear phenomena such as phase locking and mutual synchronization, which are not easy to elucidate simply by averaging signals or fitting signals to a GLM.

The present study revealed phase locking activity among the cortical regions during auditory stimulation. A possible mechanism for the phase gradient is as follows. Even when no stimulation is given, each cortical region generates spontaneous activity. When auditory stimulation is periodically given, it entrains the activity of the temporal auditory regions, leading to stimulus-induced synchronization in these regions. The activity of the temporal regions further entrains the spontaneous activity of the surrounding regions, which further entrains the activity of the occipital and frontal regions through a short-distance connectivity. Although a functional property of the global flows of activation over the cortex is not clear, this reminds us of an interesting hypothesis that infants’ perception may be synesthetic: stimulation in one modality causes percepts not only in the original modality but also in a second sensory modality \[79\]. It is open to future studies whether the phenomenon that we found in the present study undergoes developmental changes, which could be a key to understanding the developmental mechanism in auditory processing.

Our finding that multi-channel NIRS can detect precise spatio-temporal dynamics of cortical activity during stimulus processing has a profound implication for future studies exploring how spatiotemporal activity in the global network of the brain develops over time to perform adaptive behaviours in a complex environment.

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