Variability of the hemodynamic response as a function of age and frequency of epileptic discharge in children with epilepsy

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Abstract

EEG-fMRI is a non-invasive tool to investigate epileptogenic networks in patients with epilepsy. Different patterns of BOLD responses have been observed in children as compared to adults. A high intra- and intersubject variability of the hemodynamic response function (HRF) to epileptic discharges has been observed in adults. The actual HRF to epileptic discharges in children and its dependence on age are unknown. We analyzed 64 EEG-fMRI event types in 37 children (3 months to 18 years), 92% showing a significant BOLD response. HRFs were calculated for each BOLD cluster using a Fourier basis set. After excluding HRFs with a low signal-to-noise ratio, 126 positive and 98 negative HRFs were analyzed. We evaluated age-dependent changes as well as the effect of increasing numbers of spikes. Peak time, amplitude and signal-to-noise ratio of the HRF and the *t*-statistic score of the cluster were used as dependent variables. We observed significantly longer peak times of the HRF in the youngest children (0 to 2 years), suggesting that the use of multiple HRFs might be important in this group. A different coupling between neuronal activity and metabolism or blood flow in young children may cause this phenomenon. Even if the *t*-value increased with frequent spikes, the amplitude of the HRF decreased significantly with spike frequency. This reflects a violation of the assumptions of the General Linear Model and therefore the use of alternative analysis techniques may be more appropriate with high spiking rates, a common situation in children.

Keywords

Hemodynamic response function; fMRI; Spike; Age

Introduction

Simultaneous electroencephalogram (EEG) and functional magnetic resonance imaging (fMRI), EEG-fMRI, is a non-invasive approach to investigate epileptogenic networks in

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patients with epilepsy. Using the interictal spikes detected on scalp EEG as events, we can identify hemodynamic changes in the whole brain. Several studies demonstrated that this method provides valuable information about the generators of interictal spikes (Gotman et al., 2006), i.e. the irritative zone (Rosenow and Lueders, 2001). EEG-fMRI studies allow us to look at positive changes in the blood oxygenation level-dependent (BOLD) signal, known to be coupled to neuronal activation, as well as negative BOLD changes. The physiological correlate of BOLD is not yet fully understood (Kobayashi et al., 2006a,b; Shmuel et al., 2002, 2006). There have been a few EEG-fMRI studies in children, demonstrating that it is feasible to use EEG-fMRI in the pediatric population (Archer et al., 2003; Boor et al., 2003; de Tiege et al., 2007). Our recent study demonstrated a good concordance between focal epileptic activity, pattern of BOLD response and lesion in children with symptomatic epilepsy (Jacobs et al., 2006). However, there has been evidence that the hemodynamic responses to interictal discharges in children might show different patterns compared to adults. In particular, the occurrence of negative BOLD in this area seemed to be more prominent (Jacobs et al., 2006).

Possible differences in hemodynamic responses between children and adults may explain these results. Therefore it remains unclear whether it is feasible to use the same paradigms for the statistical analysis in children as in adults. Most fMRI studies use a model of the hemodynamic response function (HRF) derived from the auditory response (or Glover response) in healthy adults (Glover, 1999), with a time-to-peak of 5.4 s. This model proved adequate to disclose BOLD responses to cognitive tasks in healthy volunteers, but it is still to be determined how appropriate it is to study patients with epilepsy. There is evidence that the actual HRF varies depending on the brain region, individual and time of the recording (Aguirre et al., 1998). The use of multiple HRFs (Bagshaw et al., 2004) and patient-specific HRFs (Kang et al., 2003) in EEG-fMRI indeed is more sensitive to detect positive and negative BOLD responses that would be otherwise missed with the standard model. We improved the sensitivity of EEG-fMRI with the use of four different HRFs (peaking at 3, 5, 7 and 9 s) in a group of children with symptomatic epilepsy (Jacobs et al., 2006), and as in adults, the responses seem to be specific, as demonstrated by their concordance with the anatomical areas related to the generation of the spikes, as well as with the potentially epileptogenic lesion/region. The impact of age and brain maturation on the HRF is, however, unknown. This may explain differences in BOLD pattern between adults and children. An HRF that is appropriate to age-related changes may improve the yield of EEG-fMRI in children.

Another particularity of EEG-fMRI studies in children is the higher number of discharges per recording and different background activity due to sedation-induced sleep, compared to awake adults. The effect of these two variables on the shape of the HRF is unknown. However, there is evidence that studies with larger numbers of spikes are more likely to result in BOLD responses than those with small numbers (Gotman et al., 2006). On the other hand, the general linear model (GLM) used for statistical analyses in EEG-fMRI may fail or be inaccurate with high event numbers, as this model postulates that the response of two closely spaced events will sum linearly (Friston et al., 1998). Therefore it is important to investigate the impact of high number of events on the GLM more closely and ensure that this model can be used in children.

In the present study we analyzed the HRF shape and its changes due to age and the number of spiking events. HRFs were calculated in negative and positive BOLD responses in 37 children with epilepsy, aged between 5 months and 18 years. We hypothesize that the shape of HRFs might change during brain maturation and therefore the use of an age adapted HRF might be more appropriate in children. Additionally we assume that even if EEG-fMRI in children was possible with a very high number of events, the assumption of the GLM may fail in these cases and therefore lead to inaccurate results.

Materials and methods

Patients with different epileptic syndromes, 5 months to 18 years of age, were included in this study. All of them were treated at the Department of Neuropediatrics of the University of Kiel/Germany, in cooperation with the Northern German Epilepsy Centre in Raisdorf. The patients had an informed consent form signed by their legal guardians and the study was approved by the Research Ethics Committee of the University of Kiel, Schleswig-Holstein.

EEG-fMRI data were only acquired in children who fulfilled the following criteria:

1. indication for an anatomical scan on the basis of the necessity to investigate a lesion seen on a prior anatomical MRI scan or to diagnose their epilepsy syndrome and exclude pathological changes, and
2. frequent spikes (>10 in 20 min) recorded on routine EEG outside the scanner, without occurrence in bursts.

Clinical EEGs recorded prior to the exam were used to exclude patients with infrequent spikes and patients in which the spikes occurred in cluster. The patients were grouped into four age groups: group A (0–2 years), group B (3–5 years), group C (6–11 years), group D (12–18 years). In group D, the 16- to 18-year-old patients were also evaluated separately, as growth and puberty are certainly completed at that age and maturation processes of the brain should be completed (group D–, 12–15 years; group D+, 16–18 years).

Data acquisition

Patients were sedated with either chloral hydrate (Chloralhydrat®; average dose 50 mg/kg) or chlorprotixen (Truxal®; average dose 20 mg/kg). The sedative for the EEG-fMRI session was chosen according to the tolerance of each individual patient, as experienced on a prior routine EEG sleep study. The EEG was continuously recorded inside the MRI scanner (3-Tesla Philips Achieva, 8-channel SENSE head coil, Philips Medical Systems, Best, the Netherlands) from 30 scalp sites (10–20 system plus FC1, FC2, CP1, CP2, FC5, FC6, CP5, CP6, TP9, TP10) with a reference located between Fz and Cz. Sintered Ag/AgCl ring electrodes were attached using a “BrainCap” (Falk-Minow Services, Herrsching-Breitbrunn, Germany), which is part of the MR-compatible EEG recording system “BrainAmp-MR” (Brainproducts, Munich, Germany). Electrode impedance was kept below 7 kΩ. Two additional electrodes were placed on the infraorbital ridge of the right eye for recordings of the vertical EOG and perivertebrally on the left for acquisition of the electrocardiogram. Data were transmitted from the amplifier (5 kHz sampling rate, 250 Hz low pass and 0.03 Hz high pass filters) via an optic fiber cable to a computer located outside the scanner room.

A 3D-T1 weighted anatomical acquisition (FOV=224 mm, Matrix 224×224, 150 sagittal slices, 1 mm slice thickness, TR= 8.4 ms, TE=3.6 ms, flip angle 8°, Sense factor 2) was performed for coregistration with the functional images, followed by continuous fMRI data acquisition for 20 min (T2* weighted single-shot EPI sequence, FOV=200 mm, 30 slices, Matrix 64×64, 3.5 mm slice thickness, TR=2250 ms, TE=45 ms, flip angle=90°, 540 frames).

EEG processing

EEGs were filtered offline using Brain Vision Analyzer software (Brain Products, Munich, Germany). One of the important prerequisites of successful correction of the scanner gradient artifact was a phase synchronization of scanner (sampling rate 10 GHz) and EEG amplifier (sampling rate 5 kHz). In order to control for appropriate spike frequency and acquisition time, online gradient artifact correction was performed using RecView software (BrainProducts, Munich, Germany). This tool allowed us to prolong the fMRI session in case of a very low spike frequency (<5 in 20 min).

The gradient artifacts were corrected offline according to the methods of Allen et al. (1998, 2000). The pulse artifact was then removed from the EEG signal using an averaged artifact subtraction method (Allen et al., 2000), followed by an Independent Component Analysis-based procedure (Srivastava et al., 2005), and special filters based on artifact and brain signal topography (Ille et al., 2002). These correction methods were used as implemented in the software BrainVision Analyzer and BESA (Brain Electrical Source Analysis, Megis Software GmbH, Gaeffing, Germany). Two experienced neurophysiologists reviewed the filtered EEGs and jointly marked the epileptic discharges, which were classified into distinct types for each patient according to spatial and temporal topography (if more than one type was present). In some patient, an additional spatio-temporal pattern search was performed using BESA. The results of this automatic spike detection were reviewed visually and false detections were deleted. As the exact shape of an interictal discharge might change as a result of the artifact correction and filtering, spikes at a given location were not separated according to their shape.

A separate EEG-fMRI study was defined for each spike type; in patients with more than three spike types, the three most prominent spike types in terms of numbers were chosen for further analysis. This restriction was made to avoid biasing the study by patients with many different spike morphologies.

The event types were divided into three groups according to the number of spikes during the 20-min recording time: group 1 with very few spikes (1–20/20 min or 60–1200 s mean interspike interval), group 2 (21–100/20 min or 12–57 s mean interspike interval) and group 3 with frequent spiking (above 100/20 min or less than 12 s mean interspike interval). In a second analysis, group 3 was subdivided into group 3a (less than 200 spikes/20 min or 6–12 s mean interspike interval) and group 3b (more than 200 spike/20 min or less than 6 s mean interspike interval). The purpose of this was to look more closely at those event types for which the inter-spike intervals could be shorter than the time required for the HRF to return to its baseline. This would be the case in at least a few spikes in all event types of group 3 since 100 spikes/20 min corresponds to one spike every 12 s if the spikes were evenly

spaced (of course some are closer and some are further apart). It would be the case much more often in group 3b than in group 3a.

fMRI processing

The fMRI images were motion corrected and smoothed (Gaussian kernel, FWHM=8 mm) using SPM 2 software (Statistical parametric mapping, <http://www.fil.ion.ucl.ac.uk/spm/>). Data were analyzed using fMRIstat software (Worsley et al., 2002). Each spike that was noted in the EEG was included in the analysis as an event, with spikes differentiated into different event types based on their spatial morphology. A general linear model was constructed in which each spike type was entered as a separate contrast of the same analysis. In the case of events occurring in close temporal proximity, the model HRFs were assumed to summate linearly. Thus, a close succession of spikes would be modeled as a larger and longer BOLD change than an individual spike. We performed four separate analyses each using a different model HRF. All models consisted of a single GAMMA function with a FWHM of 5.2 s, with peaks 3, 5, 7, or 9 s after the event, resulting in four separate statistical maps. A single combined statistical map was then created by taking the highest absolute value of each voxel. This allowed some variation in the latency of the BOLD response (both between patients and within regions of each patient) while retaining information about its expected shape (Bagshaw et al., 2004).

Significant responses were defined as 10 or more contiguous voxels with $|t| > 3.1$ ($p=0.05$, corrected for multiple comparisons, Worsley et al., 2002, and for four analyses; Bonferroni correction). We examined the volumes of positive and negative BOLD responses and the maximum t -value of each cluster.

Calculation of the patient-specific HRF

An HRF was calculated for each cluster containing more than 10 contiguous voxels within the brain. The HRF was projected for a region of interest of 10 significantly activated voxels, centered on the voxel with the highest t value in the cluster. An HRF was projected over a 64-s time window (from 15 s prior to the EEG spike to 48 s after the spike) using a Fourier basis set of 20 sine-cosine waves (Josephs et al., 1997; Kang et al., 2003). Projected HRFs were sampled at one data point per second (Fig. 1).

For each calculated HRF, we determined the peak time, the signal to noise ratio (SNR) and the relative amplitude (in percent signal change). The SNR was defined as the amplitude of the peak of the HRF (defined as the largest value from 0 to 15 s after the spike) divided by the standard deviation of the baseline, defined as the data from 15 to 5 s before the event and from 30 to 48 s after the event.

Statistical analysis

In a first step, we excluded HRFs with an SNR lower than 3 to reject data in which the HRF did not clearly differ from the noise in the baseline (Bagshaw et al., 2005). This was done to ensure that the calculations of the peak time and amplitude would not be contaminated by noise. As the patients had a highly variable number of clusters of significant BOLD responses, some patients would have many more HRFs than others. To reduce bias due to an

unusually large number of clusters from a single patient, we limited the number of HRFs included in the analysis to a maximum of three negative and three positive HRFs per patient. The calculated HRFs of the three clusters with the highest t -statistic score in the fMRIstat analysis were included in the statistical analysis. We intentionally did not select the cluster visually by its localization, or relationship to the source analysis of the spike or the location of the lesion in symptomatic patients, as the major goal of this study was to evaluate the impact of age and spiking rate on the HRF. We aimed to include activity from various regions relative to the irritative zone given the number of positive BOLD responses distant from the EEG spike fields and the presumed epileptic focus, and that these distant HRFs are presumed to be a part of the patient's epileptogenic network because they occur as a result of spikes.

We used the parameters of the calculated HRFs to perform a one-way ANOVA followed by Bonferroni correction for multiple comparisons using the SPSS software. HRFs deriving from positive and negative BOLD responses were analyzed separately. Two different statistics were performed for the independent variables "age group" and "number of spikes". Peak time, amplitude and SNR of the HRF and the t -statistic score of the cluster in which the HRF was projected were used as dependent variables. The level of significance was defined at $p=0.05$ after correction for multiple comparisons.

Assessment of intrasubject variability

In a second statistical analysis, all HRFs were used to assess intrasubject variability. This was to compare the variability in the shape of the HRF between patients at different stages of brain maturation. Standard deviations of the peak times and amplitudes were calculated for every patient. A one-way ANOVA was performed with the age group as independent variable and the standard deviations of each patient as dependent variables.

Results

Patients

Thirty-seven consecutive unselected patients were included in this study. According to seizure semiology, MRI results, and EEG parameters, 20 were classified as symptomatic, seven as idiopathic and 10 as cryptogenic. Symptomatic epilepsy was caused in eight patients by malformations of cortical development. Twelve gliotic changes were observed, which resulted from trauma in one, a cerebral insult in 4 and asphyxia in 7 patients. There were nine patients in age group A (0–2 years, mean: 12.8 month), eight in age group B (3–5 years, mean: 4.6 years), eleven in age group C (6–11 years, mean: 8 years), and nine in age group D (12–18 years, mean: 16.3 years) (see Fig. 2). All patients needed sedation for the study, as they were unable to remain still because of their age or mental disability and developmental delay, caused by their severe epilepsy and concomitant structural brain damage. There was no significant difference between the age of the 12 patients receiving Chlorprotixen (Truxal®) and the 25 patients receiving chloral hydrate (Chloralhydrat®).

EEG parameters

All patients had focal or multifocal epileptic discharges: nine patients with two types of spike and nine patients with three. In all patients, the distinct spike types could be clearly differentiated by their spatial pattern (Table 1). This resulted in a total of 64 distinct fMRI event types. The number of spikes ranged from 6 to 735 (mean 90.8, ± 18.3 SEM). According to the number of spikes, 17 event types belonged to group 1 (mean 11, ± 1.03 SEM), 32 to group 2 (mean 43.6, ± 2.6 SEM) and 15 to group 3 (mean 276.7, ± 54.8 SEM) (8 in group 3a, 7 in group 3b). The number of spikes within each age group did not vary significantly. Nevertheless age group C (180, ± 51 SEM) showed a tendency ($F(3,33)=2.5$, $p=0.08$) to have higher numbers of spikes than age group A (43.3, ± 8.7 SEM). Further details regarding clinical information and spike field can be found in Figs. 2A and B and Table 1.

fMRI results and HRF projection

BOLD responses were found in 59 event types (92.2%), with only positive BOLD responses in two (3%), only negative ones in four (6%) and both positive and negative responses in 53 (82.8%). In the patients with symptomatic epilepsy, 36 different spike types were analyzed. A BOLD response was observed within the lesion as a positive response in 19.5% of event types, as a negative response in 44.5% and both responses in 27.8%. An HRF was calculated for each significant BOLD response resulting in a total of 402 positive HRFs and 394 negative HRFs: 241 (60%) positive and 214 (54%) negative HRFs passed the SNR criterion. For each patient, the three HRFs from positive BOLD responses with the highest t -statistic score were retained for each study, resulting in 126 positive HRFs; similarly 98 negative HRFs were included in the subsequent statistical analyses.

Comparison of negative and positive HRFs

As the SNR for positive HRFs (60%) was slightly but not significantly higher than for negative HRFs (54%), more positive HRFs could be included in the statistical analysis for all groups. A significant difference between positive and negative HRFs was observed in the peak time ($F(1,240)=11.7$, $p<0.001$), with a mean of 6.1 s ($SD\pm 2.3$ s) for positive and 7.2 s ($SD\pm 2.9$ s) for negative HRFs. Therefore, the mean difference in the peak time compared to the Glover response (5.4 s) was higher for negative than for positive HRFs.

HRF parameters according to age

There was no significant difference in the rate of HRFs exceeding the SNR threshold related to the age. The distribution of the number of HRFs per age group can be seen in Fig. 3A. Fig. 4 shows positive and negative HRFs in one patient per age group, as an example of our data.

Significant differences were observed in the peak time ($F(3,123)=8.8$, $p<0.001$) and amplitude ($F(3,123)=7.7$, $p<0.001$) for positive HRFs between the age groups. The amplitude of the HRFs was significantly higher in age group A ($p<0.001$, mean 0.65% signal change) and age group D ($p<0.004$, mean 0.62% signal change) than in age group C (mean 0.27% signal change). The peak time was significantly later in age group A (mean 7.74 s) compared to age group C ($p<0.001$, mean 5.1 s) and D ($p<0.001$, mean 5.3 s). Fig. 5

gives an example of a 10-month-old patient in whom we observed two HRFs with late time to peak.

A significant difference between the age groups for negative HRFs was only found for the score of the t -statistic ($F(3,111)=3.9$, $p=0.01$). The t -statistic of group C had a mean of 5.5 ($SD\pm 1.8$) and was significantly higher ($p<0.005$) than in age group A (mean 4.3, $SD\pm 0.4$). Graphs with results are summarized in Part B of Fig. 3 and Table 2. For negative and positive HRFs, no statistical difference between age group D- (12–15 years old) and age group D+ (16–18 years old) could be found.

HRFs and number of spikes

The number of HRFs passing the SNR criteria did not vary significantly for the different spike groups. The distribution of HRFs for each spike group can be found in Fig. 6A.

For positive HRFs significant changes in the amplitude throughout the spike group could be found ($F(3,123)=12$, $p<0.001$). The amplitude was significantly higher in group 1 ($p<0.0001$, mean 0.7% signal change) and 2 ($p<0.002$, mean 0.42% signal change) than in group 3 (mean 0.22% signal change).

In negative HRFs, significant changes in the amplitude ($F(3,111)=7.8$, $p<0.001$) as well as the t -statistic score ($F(3,111)=4.2$, $p=0.007$) could be found. The amplitude was significantly higher in group 1 (mean 0.92% signal change) than in all other groups (group 2: $p<0.001$, mean 0.31%; group 3: $p<0.02$, mean 0.29% signal change). The t -statistic score was significantly higher in group 3 ($p<0.01$) than in group 1.

No differences could be seen in SNR and peak times. Mean values and graphs of the significant findings can be seen in Fig. 6B and Table 3. Fig. 7 presents examples of the HRFs of patient # 25, a 5-year-old child with idiopathic epilepsy.

Comparison of spike groups 3a and b

There was no statistical difference when comparing groups 3a and 3b. However, the amplitude in group 3b was even lower than in group 3a for positive and negative HRFs. Therefore, it was not only significantly lower compared to spike group 1 ($F(3,123)=11.9$; $p<0.001$) but also spike group 2 ($p=0.03$) for positive HRFs. The same was observed for negative HRFs ($F(3,111)=7.75$), the amplitude of spike group 3b being significantly lower than in spike group 1 ($p=0.005$). The t -statistic score was no longer rising with the number of spikes, as for groups 1 to 3a, but dropping (see Fig. 6).

Intrasubject variability

The intrasubject variability of peak time and amplitude did not vary significantly across the age groups.

Discussion

In the present study, we calculated HRFs of 64 different spike types from patients of different age groups. The goal of the study was to evaluate if there were age-related changes

of the HRF following interictal epileptic discharges. A second objective was to investigate changes in the HRFs caused by increasing numbers of spikes.

Methodological issues

A Fourier basis set of 20 sine–cosine waves was used to calculate the shape of the HRF, as described by Josephs et al. (1997). This method allows an evaluation of the HRF by assuming that it is the same for each event, but without constraining its shape. However, for the purpose of this study, the method was used to refine the calculation of the delay of the HRF in areas that showed BOLD effects in the primary statistical analysis, used as regions of interest (Kang et al., 2003). The use of a single canonical model HRF in the primary analysis would result in an estimated HRF that is relatively similar to the model. We were expecting that the actual HRF of our patients could vary from the shape of the canonical HRF or the Glover response for three reasons:

1. We found many negative BOLD responses among our studies, which tend to peak later than positive responses (Bagshaw et al., 2004).
2. The use of multiple HRFs improved our results in a previous study, allowing us to detect additional BOLD responses (Jacobs et al., 2006).
3. There is evidence that the HRF might not be stable and show age-dependent peak time differences (Huettel et al., 2001).

Therefore, we decided to use multiple HRFs peaking at 3, 5, 7 and 9 s after the event to calculate the BOLD response. It could be argued that the BOLD responses we are calculating are likely to have a shape similar to those used in our four models, since we calculate HRFs for the regions showing a significant response when using these as models. This is indeed the case, but we feel that this bias only has a minor impact on our study for the following reasons; (i) we have shown that even when using a canonical HRF, calculated HRFs in activated regions can depart significantly from the model (Bénar et al., 2002); (ii) we were not trying to estimate precisely the shape of the HRF, but rather to estimate the latency and amplitude of the main peak; (iii) it has been shown that HRFs having a shape that deviate significantly from the canonical shape are unlikely to be related to the primary epileptic activity (Lemieux et al., in press).

Our results corroborated the above hypotheses: the calculated HRFs showed a wide variety of peak times, with a significantly later peak time in negative BOLD than positive BOLD responses. We excluded all HRFs with a signal to noise ratio <3 to detect real changes and minimize contamination by noise (Bagshaw et al., 2004). This ensured a high specificity, with exclusion of 40% of positive and 46% of negative HRFs, a rate similar to that described in adults (Bagshaw et al., 2005). There were no significant age-related changes in the SNRs, suggesting that the data in children, independent of their age, show a similar amount of noise than in adults. Peak time, amplitude and t -statistic were evaluated for all HRFs. We did not, however, calculate the duration of the HRFs and therefore cannot comment on whether it changes with different peak times.

In fMRI studies in healthy subjects using motor or sensory paradigms, it is straightforward to select an area of the brain to calculate the HRF, as the areas of interest are mostly known

beforehand and were studied with similar paradigms before. This has been done by several groups to either confirm the similarity of the HRF of the study with the canonical HRF or look at the age-related changes in adults (Huettel et al., 2001; Buckner et al., 2000; Brodtman et al., 2003). An exact prediction of the area of interest is not possible in EEG-fMRI in epilepsy patients, as patterns of BOLD response are highly different across patients, even if their epilepsy syndromes are similar. We did not select the HRF according to the brain region or pathology of the patient, as it is known that different brain regions show variable shapes of HRFs (Aguirre et al., 1998; Miezin et al., 2000) and our patient group was too small to allow the subdivision of the HRFs into more subgroups without losing statistical power. Since our major goal was to evaluate age-related changes independently of the brain region or epileptic syndrome, we do not think this imposes any limitations to the study. It would, however, be of interest to address this issue in a separate publication, evaluating the influence of different brain regions on the HRF in children of different age groups.

HRFs used in EEG-fMRI for epilepsy patients

The neurovascular coupling appears preserved in patients with epilepsy (Stefanovic et al., 2005), and many studies showed different patterns of BOLD responses related to interictal (Salek-Haddadi et al., 2006; Federico et al., 2005; Kobayashi et al., 2006a) and ictal (Salek-Haddadi et al., 2003; Kobayashi et al., 2006b) discharges. An unsolved problem of EEG-fMRI has been its low sensitivity, as only 40–60% of studies show a BOLD response when analyzed with a single HRF (Gotman et al., 2004). This low sensitivity could result from an HRF that differs significantly from the canonical or Glover response. Kang and coworkers demonstrated that the variability of the HRF to interictal discharges is quite high and that the use of a patient-specific HRF improves EEG-fMRI results (Kang et al., 2003). The use of four different HRFs further improved the detection of BOLD responses and can be implemented as a standard procedure for the analysis of EEG-fMRI studies (Bagshaw et al., 2004). This technique was applied to data from children with a similar effect, but the patterns of BOLD response differed from those in adults (Jacobs et al., 2006). When using the canonical HRF in the pediatric series, negative BOLD responses predominated in areas of presumed epileptogenicity (lesional areas/areas of spike generation), and additional areas of positive BOLD responses could be observed when using multiple HRFs (Jacobs et al., 2006). Though there were other influencing factors such as sleep and sedation, we wanted to assess whether age-related changes in the HRF could be an important cause of different patterns in children.

HRFs correlation with age

The impact of age on the BOLD response and the HRF to epileptic discharges is unknown. Language, motor and visual fMRI studies in children under 5 years showed age-related BOLD responses (Martin et al., 1999). Whereas awake adults showed consistent occipital positive BOLD responses to visual stimulation (Belliveau et al., 1991), children presented either positive or negative BOLD changes, depending on their age (Morita et al., 2000; Yamada et al., 2000; Marcar et al., 2004). However, normal children above eight years have showed no differences compared to adults in sensory and cognitive studies (Casey et al., 1997; Thomas et al., 1999). This suggests that the HRF may vary with age and that

hemodynamic coupling may be different in very young compared to older children (Yamada et al., 2000).

In our study, we classified children in age groups with regard to maturations processes in the brain as described in MRI developmental studies. Myelination should be complete in several brain areas after the second year of life (age group A) (Dietrich et al., 1988; Gogtay et al., 2004). However, some cortical areas especially in the frontal lobes are known to mature in the early twenties. Additionally, differences in the BOLD responses compared to those in adults have been observed in physiological studies until the early school age (age group B). Age group C and D distinguished pre- and post-puberty children, as we know that EEG patterns and epilepsies change dramatically during puberty. We expected that children above age 12 would behave similarly to adults. In fact, we could not find significant differences in the shape of the HRF between groups C and D. Even when dividing group D into two subgroups, no changes with age could be detected. The peak time of positive HRFs in these groups was very close to the Glover response. Interestingly, the peak time of negative responses differed from the standard response in all age groups but was closest to the standard response in age group C, which also showed a significantly higher *t*-statistic score for negative BOLD responses. We do not have an explanation for this phenomenon but it is notable that negative BOLD responses are important in this age group.

Several studies evaluated age-related changes in the HRF to motor and cognitive tasks in young and elderly adults. They mainly observed age-related changes in amplitude and peak time between younger and older healthy adults (Huettel et al., 2001; Buckner et al., 2000).

We also found significant changes in amplitude and peak time of positive HRFs with age. However, the changes in amplitude might be related to the average number of spikes per age group, which was lower in age group A and D compared to age group C. Similar changes in the amplitude have been found with rising spike numbers, as reported later in this paper.

The positive HRF of children under the age of two peaked significantly later than in older children. In early fMRI studies in children, it has been hypothesized that age-related increases in oxygen consumption are due to changes in synaptic density or to high-energy consumption by ineffective recruitment of synapses or the presence of superfluous synaptic connections (Huttenlocher, 1979; Yamada et al., 2000; Marcar et al., 2004). As a result, higher levels of deoxyhemoglobin following an event are expected in very young children. If no other parameter is changed, a higher level of oxygen consumption would lead to a lower value of the BOLD change. This could explain a suppression of the early part of the BOLD response but would not be sufficient to explain the later peaking of this change. We therefore hypothesize that, in addition to increased oxygen consumption, there is a change in coupling between CMRO₂ and blood flow, leading to a later peak of the BOLD response.

One study suggested that older adults show a higher intrasubject variability than younger adults (Huettel et al., 2001). We could not observe changes in the standard deviations of peak time and amplitude with respect to age and therefore this observation seems to be limited to adults.

In adults, it was concluded that even if changes in the HRF shape were observed with age, it is safe to use the same HRF for all age groups as the t -values and cluster sizes remained similar throughout aging (Brodman et al., 2003). In this study in epilepsy patients, it was not possible to predict the cluster size beforehand and perform a group study. Therefore we cannot safely conclude that the same HRF results in the similar responses in each age group. However, we did not find significant differences in HRF shape after the age of two, which suggest that the fMRI results are not altered by the use of the same HRF for older children. Additionally no age-specific HRF could be identified. It may be reasonable to take a closer look at the HRFs in very young children and perform their statistical analysis with late peaking HRFs. A closer look at hemodynamic coupling in this age group should be a topic of future investigation.

HRFs limitation due to number of spikes -GLM limitations

Interictal EEG patterns differ in children compared to adults. Even under medication, children show a higher rate of interictal discharges, and even more so during sleep. In our study, the mean number of spikes is higher than in most adult studies (Salek-Haddadi et al., 2006; Kobayashi et al., 2006a). This allowed us to reduce the scanning time to 20 min, which minimized the sedation time. Some patients (e.g. event type # 32 in patient 18 and # 45 in patient 24, who had 435 and 400 spikes/20 min; see Table 1) had exceptionally high spike rates. All patients in group three had high numbers of spikes, with a mean inter-spike interval of less than 12 s; in group 3b, the interval was even shorter with an average of less than 6 s. This implies that in all these patients, most of the spikes follow each other faster than it takes for the HRF to come back to baseline, which is approximately 12 s. The mean number of spikes in group 2 was 44, corresponding to an average inter-spike interval of 27 s (range of the means across subjects: 12–60 s). Since spikes are not equally distributed over the time of the EEG, some spikes were separated by less than 12 s in this group as well. In these situations, it is possible that the GLM, as the basis of our statistical analysis in event-related design, may no longer be valid (Friston et al., 1998). It assumes that if the inter-event interval is shorter than the HRF, the amplitudes of the overlapping responses will superimpose linearly. If the events appear in close succession, the GLM may no longer be a good representation of the BOLD signal (Mirsattari et al., 2006). Our study revealed that the amplitude of the calculated HRF, assuming the validity of the GLM, becomes significantly lower with increasing numbers of spikes. This can be explained either by the fact that the BOLD response to each spike is smaller in patients with frequent spikes or by a failure of the GLM. It has been shown that, past a certain point, the BOLD signal no longer increases linearly (Friston et al., 1998). Despite this, we could also observe in our study that a higher number of spikes results in higher t -statistics. No significant difference between the event types with less and more than 200 spikes per 20 min could be found, but in the group with more than 200 spikes the t -value was no longer rising with the number of spikes, as would be expected if the GLM was valid at such high spiking rates. A higher number of event types with very frequent spikes would have to be analyzed to make sure at which point the spiking rate is too high to result in rising t -values, which may provide more insight into the applicability of the GLM in these cases. In our study, spikes were distributed quite uniformly during the time course of the EEG, as we excluded patients with bursts of spikes. However, the GLM may fail even with lower spike numbers if the spikes are distributed very unequally

in the EEG time course. Therefore statistical approaches that do not assume linearity of the BOLD response may be better for the analysis of pediatric EEG-fMRI. One approach could be the use of an Independent Component Analysis, which does not employ a prior model of the brain activity as suggested in an animal model for epilepsy (Mirsattari et al., 2006).

Conclusion

We were able to perform EEG-fMRI on 37 patients between the age of 5 months and 18 years with a good sensitivity. By analysing a large number of HRFs from positive and negative BOLD responses, we could confirm the high inter- and intrasubject variability seen in adults. Age-dependent differences were mainly observed in the peak time, which differed considerably from the Glover response especially in negative BOLD responses. Positive HRFs in children under the age of 2 years peaked significantly later and the use of late peaking HRFs should be considered in these patients. We could not identify an age-specific HRF, which would allow the use of specific HRFs for certain age groups. Therefore, in children, the use of multiple HRFs is recommended.

Additionally, we demonstrated that the shape of the HRF changes with increased numbers of spikes, which indicated that the GLM may fail in studies with frequent events. Nevertheless the *t*-value of the BOLD response increased significantly with the number of spikes unless it exceeded a spike rate of 10 per minute. An approach that does not assume linearity of the BOLD response might be superior in patients with large numbers of events.

Acknowledgments

JJ was supported by a grant of the German Research Foundation (Deutsche Forschungsgemeinschaft (JA 1725/1-1)). This research was supported in part by grant MOP-38079 of the Canadian Institutes of Health Research.

Abbreviations

BOLD effect	blood oxygenation level-dependent effect
EEG	electroencephalogram
EEG-fMRI	simultaneous EEG and fMRI
fMRI	functional magnetic resonance imaging
HRF	hemodynamic response function
SPM	statistical parametric mapping

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Fig. 1.

Method to obtain the SNR of the calculated HRF. The HRF is calculated over a region centered on the voxel with the highest t -statistic within a cluster of significant BOLD responses. It is projected over a 64 s time window using a Fourier basis set of 20 sine-cosine waves. Signal-to-noise ratio was defined as the amplitude of the peak of the HRF (as indicated) divided by the standard deviation of the baseline. Only HRFs with a signal to noise ratio over 3 were used for further statistical analysis.

Fig. 2.

Overview of the selected patients and their epileptic syndromes. As the number of BOLD clusters and the SNR varied between patients, the number of HRF which could be included in the statistical analyses varied throughout the age groups. The epileptic discharges of each patient were grouped according to their topography into different spike types, which were analyzed separately (a single EEG-fMRI recording could therefore result in several studies, one for each spike type). In the younger patients, more different spike types could be identified per patient. (A) Number of patients, number of different spike types and positive and negative HRFs in the different age groups. (B) Distribution of epileptic syndromes in the different age groups.

Fig. 3.

Results for all positive and negative HRFs used for the statistical analysis. (A) Distribution of the HRFs throughout the age groups. (B) Results as boxplots. Significant results are indicated. The positive HRF of very young children (group A) peaks significantly later than in older children (groups C and D). The t -value for negative BOLD responses was significantly higher in group C than in the young children of group A. The significant decrease of amplitudes in group C compared to groups A and D may be caused by the number of spikes, which was higher in group C (see Discussion).

Fig. 4.

No age-specific HRF could be defined. This figure shows all positive and negative HRFs of one representative patient per age group. Positive HRFs are shown on the left and negative on the right. HRFs with signal to noise ratios above 3 are demonstrated in the upper graph and those which were excluded from further analysis due to low signal to noise ratios in the lower graph. The HRFs calculated from the three BOLD clusters with the highest t -statistic are drawn in red and a small green circle indicates the actual peak of each HRF. HRFs calculated in negative had a significantly later peak time than in positive BOLD responses.

Fig. 5.

Patient # 9 (event type # 18), 10 months old, presented with multifocal symptomatic epilepsy. The discharges were located over the left temporal and occipital lobes. Four clusters of positive and five of negative BOLD responses were observed. Three positive HRFs and one negative HRF with good signal to noise ratios could be used for statistical analysis. This figure illustrates one positive and one negative HRF and the BOLD response for which they were calculated. As observed in age group A, the positive HRF peaked late, at 10 s. The red line is the estimated HRF and two blue lines are the standard deviation of this estimate. The visualization of the fMRI results was done using Brain Vision Anatomist. The right side of the image corresponds with the right anatomical side of the patient.

Fig. 6.

Results of the statistical analysis of changes in the shape of the HRF related to increasing numbers of spikes. Results for positive HRFs are on the left and results for negative ones on the right. (A) Distribution of the HRFs in the different groups (group 1: <20 spikes, group 2: 21–100, group 3: >100, group 3a: 101–200, group 3b: >200). (B) Box plots, significant values are indicated. HRFs calculated in positive and negative BOLD responses show significant decrease in amplitude with increasing numbers of spike. This indicates that the HRFs resulting from spikes may not superimpose linearly with increasing numbers of events. This is in violation of the GLM assumptions when calculating the fMRI results. Nevertheless, the t -value increased significantly with the numbers of spikes, as observed in previous studies.

Fig. 7.

In the EEG of patient # 25 (event type # 46), 109 spikes could be marked during a period of 20 min. Three positive HRFs and three negative HRFs could be included in the statistical analysis. The amplitude of the positive HRF in this figure was 0.25% signal change and the corresponding BOLD response had a t -value of 5.45. The negative HRF had an amplitude of 0.30% signal change and the t -value of the BOLD response was 11.13. The amplitudes of the patient's HRFs in total were low with a median of 0.2% ($SD \pm 0.03$) for positive and 0.21% ($SD \pm 0.08$) for negative HRFs. However, the t -statistics of his BOLD responses were high for positive (5.1 ± 0.6) and negative BOLD responses (8.1 ± 2.7). This figure shows two of his typical HRFs with their corresponding BOLD responses (displayed as in Fig. 5).

Table 1

Clinical and EEG findings of all patients at the time of the EEG-fMRI assessment

Patient #	Event type #	Age	Age group	Gender	Epileptic syndrome	Spike localization	Number of spikes	Spike group
1	1	6 months	A	f	i	OL	148	3a
2	2	24 months	A	f	c	PL	36	2
3	3					PR	48	2
4	4	5 months	A	m	s	PL	12	2
5	5					TL	51	2
6	6					TR	33	2
7	7	7 months	A	f	s	PL	19	1
8	8					PR	27	2
9	9					CR	10	1
10	10	14 months	A	m	s	PL	117	3a
11	11					PR	59	2
12	12	6 months	A	f	c	OL	57	2
13	13					PL	46	2
14	14					TR	9	1
15	15	14 months	A	f	c	O/P Bi	41	2
16	16	19 months	A	f	s	TL	14	1
17	17					TR	9	1
18	18	10 months	A	m	s	T/O L	44	2
19	19	3	B	m	s	CL	105	3a
20	20					FL	50	2
21	21					C	124	3a
22	22	5	B	m	c	F Bi	109	3a
23	23	5	B	m	s	TL	35	2
24	24	5	B	m	c	CL	45	2
25	25					CR	297	3b
26	26	5	B	m	s	C Bi	40	2
27	27	4	B	m	s	OL	34	2
28	28					TR	49	2

Patient #	Event type #	Age	Age group	Gender	Epileptic syndrome	Spike localization	Number of spikes	Spike group
	29					P	6	1
16	30	5	B	f	c	O Bi	43	2
17	31	5	B	m	s	O/P L	9	1
18	32	8	C	m	s	F Bi	400	3b
19	33	8	C	m	s	F Bi	712	3b
	34					FR	735	3b
	35					PR	63	2
20	36	6	C	f	s	CR	49	2
	37					T L	28	2
	38					C L	51	2
21	39	10	C	f	i	F Bi	72	2
22	40	10	C	m	s	P/O R	161	3a
	41					F/C R	333	3b
	42					FR	17	2
23	43	6	C	m	s	T L	132	3a
	44					TR	233	3b
24	45	8	C	m	s	C L	435	3b
25	46	6	C	m	i	FR	109	3a
26	47	6	C	m	i	FR	12	1
	48					F Bi	35	2
	49					FL	12	1
27	50	11	C	m	c	F	6	1
28	51	10	C	m	s	T L	7	1
29	52	18	D	f	c	T L	32	2
	53					FL	9	1
30	54	17	D	m	s	P/T r	7	1
31	55	15	D	m	c	FL	11	1
32	56	18	D	m	s	T/O L	17	1
33	57	15	D	m	c	F/C Bi	57	2
34	58	18	D	f	i	C L	9	1
	59					CR	22	2

Patient #	Event type #	Age	Age group	Gender	Epileptic syndrome	Spike localization	Number of spikes	Spike group
35	60	18	D	f	c	F Bi	78	2
36	61	14	D	m	s	C L	60	2
	62					F/C R	19	2
37	63	14	D	m	c	O R	31	2
	64	14				T L	49	2

Abbreviations: c=cryptogenic, C=central, Bi =bilateral, f=female, F=frontal, I=idiopathic, L=Left, m=male, O=occipital, P=Parietal, R=right, s=symptomatic, T=temporal.

Table 2

Descriptive statistics for all age groups

Age	Activations				Deactivations					
	A	B	C	D	All groups	A	B	C	D	All groups
Peak time (s)	7.74±1.97	6.13±1.83	5.10±2.15	5.30±2.58	6.06±2.30	7.52±2.80	7.06±2.54	6.33±2.54	8.00±3.76	7.23±2.96
Amplitude (% signal change)	0.65±0.56	0.38±0.23	0.27±0.21	0.62±0.40	0.46±0.39	0.59±0.55	0.42±0.35	0.73±1.34	0.51±0.39	0.55±0.75
SNR	4.88±1.39	4.83±1.54	4.29±1.51	5.60±3.74	4.87±2.19	4.48±1.53	5.07±1.38	4.41±1.08	4.29±1.06	4.59±1.3
t-statistic	4.50±0.77	5.22±1.67	5.26±2.40	5.27±1.20	5.08±1.66	4.25±0.38	5.24±1.46	5.47±1.82	5.24±1.62	5.06±1.49

Numerical values of the statistical analysis for all age groups. Mean and standard deviation for peak time, amplitude, and signal to noise ratio of the HRFs and t-statistic value of the BOLD responses.

Table 3

Descriptive statistics for all spike groups

Spikes	Activations						Deactivations					
	1	2	3	3a	3b	All groups	1	2	3	3a	3b	All groups
Peak time (s)	5.60±2.20	6.28±2.27	6.26±2.47	6.56±2.47	5.93±2.52	6.06±2.30	7.07±2.81	7.30±3.12	7.38±3.07	7.79±3.48	6.77±2.35	7.23±2.96
Amplitude (% signal change)	0.70±0.51	0.42±0.26	0.22±0.18	0.30±0.2	0.14±0.13	0.46±0.39	0.93±1.06	0.31±0.17	0.29±0.22	0.37±0.23	0.19±0.16	0.55±0.75
SNR	5.05±2.73	4.70±1.44	4.97±2.58	5.67±3.3	4.18±1.01	4.87±2.19	4.54±1.18	4.74±1.53	4.47±1.22	4.58±1.42	4.31±0.90	4.59±1.31
t-statistic	4.96±1.45	5.10±1.44	5.22±2.27	5.70±2.62	4.67±1.7	5.08±1.66	4.61±0.77	5.04±1.02	5.74±2.31	5.92±2.78	5.49±1.45	5.06±1.49

Numerical values of the statistical analysis for all spike groups. Mean and standard deviation for peak time, amplitude, and signal to noise ratio of the HRFs and t-statistic value of the BOLD responses.