Grey matter heterotopia: what EEG-fMRI can tell us about epileptogenicity of neuronal migration disorders

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Grey matter heterotopia are commonly associated with refractory epilepsy. Depth electrodes recordings have shown that epileptiform activity can be generated within these lesions, and also at a distance in the neocortex. Heterotopia seem to be part of a more complex circuitry involving also the surrounding and distant cerebral cortex. Blood oxygenation level-dependent (BOLD) changes to interictal spikes using continuous EEG and functional MRI (EEG-fMRI) can help to understand non-invasively the mechanisms of epileptogenicity in these patients. We studied 14 patients with epilepsy and heterotopia using simultaneous recording of EEG-fMRI. EEG was continuously acquired from inside the scanner during 2 h sessions. Epileptic spikes were visually identified in the filtered EEG and each type of spike determined one EEG-fMRI study. We looked at positive (activation) and negative (deactivation) changes in the BOLD signal. Eleven patients had nodular heterotopia and three band heterotopia. Four patients had more than one type of spikes, with a total of 26 EEG-fMRI studies. We excluded three with less than three spikes, and therefore a total of 23 studies (12 with nodular and 11 with band heterotopia) were analysed. Nodular heterotopia: Activation was present in nine studies, with involvement of the heterotopia or surrounding cortex in six, three of which had concomitant distant activation. Deactivation was also observed in nine studies, with involvement of the heterotopia and surrounding cortex in four, three of which had concomitant distant deactivation. Band heterotopia: Activation was present in all 11 studies, and always involved the heterotopia and surrounding cortex, 9 of which had concomitant distant activation. Deactivation was also observed in all 11 studies, with involvement of both the heterotopia and surrounding cortex, in addition to distant deactivation in 5 studies. EEG-fMRI studies reveal, non-invasively, metabolic responses in the heterotopia despite the fact that spikes are generated in the neocortex. The responses, activation or deactivation, had different correlation with the lesion and surrounding or distant cortex, activation reflecting intense neuronal activity, or excitation, and deactivation a possible distant (extra-lesional) inhibition. EEG-fMRI may become a useful tool to understand the epileptogenicity of such malformations.

Keywords: EEG-fMRI; interictal epileptiform discharges; malformations of cortical development

Abbreviations: BOLD = blood oxygenation level-dependent; fMRI = functional MRI

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Introduction

Grey matter heterotopia are malformations of cortical development due to abnormal neuronal migration (Rakic, 1995) or due to a failure of programmed cell death of neuroblasts within the periventricular matrix (Kuida et al., 1996). They can be found anywhere from the subependyma along the lateral ventricles to the cortical mantle (Barkovich et al., 2001). The most common form is the periventricular nodular heterotopia, but clusters of ectopic neurons may also form nodules in the subcortical white matter or form continuous bands of variable thickness separated from the cortex by a white matter layer and constituting subcortical band heterotopia (Barkovich et al., 2001). Both the nodular and band heterotopia can be associated with an abnormal overlying cortex, localized or diffuse (d’Orsi et al., 2004).
Patients with heterotopia show a wide spectrum of clinical manifestations, from being asymptomatic to presenting with intractable seizures and intellectual impairment (Dubeau et al., 1995; Raymond et al., 1995; Sisodiya, 2004; Guerrini, 2005). The clinical and electroencephalographic features of epilepsy in patients with heterotopia are variable and seizures may be generalized or focal (Raymond et al., 1994). Human studies using acute or chronic intracranial EEG recordings in these groups of epileptic patients showed that heterotopia can generate normal electrical activity but also spikes and seizures. Electrophysiological and histological data suggested that connections exist among the heterotopia and between them and the cortex creating a circuit responsible for the generation of epileptic discharges (Hannan et al., 1999; Barkovich and Kuzniecky, 2000). It is not clear which part of this circuitry is most critical; however, the epileptic focus has been demonstrated to involve independently or simultaneously the heterotopia, the overlying cortex, the mesial temporal structures and other distant neocortical regions (Morrel et al., 1992; Francione et al., 1994; Preul et al., 1997; Kothare et al., 1998; Mai et al., 2003; Aghakhani et al., 2005). Although depth electrode recordings can provide precise information about these structures, this technique has risks and limitations due to its invasiveness and limited coverage of the brain. On the other hand, combining scalp EEG and functional MRI (fMRI) may provide an interesting non-invasive alternative tool to understand those mechanisms since it can give information about changes in neuronal activity occurring at the same time in different structures. These studies have been so far performed only in isolated cases of epileptic patients with heterotopia (Krakow et al., 1999; Al-Asmi et al., 2003; Bagshaw et al., 2004; Diehl et al., 2003). We report EEG-fMRI findings in a large series of patients with grey matter heterotopia and focal epilepsy.

**Methods**

We studied 14 epileptic subjects with either nodular or band heterotopia. All patients underwent a 2 h recording session, after giving informed consent. The study was approved by the Research Ethics Committee of the Montreal Neurological Institute and Hospital. EEG was continuously recorded inside the MRI scanner (Siemens Sonata, 1.5 T) using 21 MRI compatible electrodes (Ag/AgCl) placed on the scalp according to the 10–20 system. Subjects’ heads were immobilized with a pillow filled with foam microspheres. Data were transmitted from an EMR32 (Schwarzer, Munich, Germany, 1 kHz sampling rate) or a BrainAmp (Brain Products, Munich, Germany, 5 kHz sampling rate) amplifier via an optical fibre cable to the EEG monitor located outside the scanner room. An anatomical acquisition (1 mm slice thickness, 256 × 256 matrix, TE = 9.2 ms, TR = 22 ms, flip angle 30°) was performed for superimposition with functional images. Blood oxygenation level-dependent (BOLD) fMRI data were collected in runs of 6 min with the patient in the resting state (voxel dimensions 5 × 5 × 5 mm, 25 slices, 64 × 64 matrix, TE = 50 ms, TR = 3 s, flip angle 90°), with a total of 6–14 runs per scanning session.

We post-processed the EEG acquired inside the scanner, with offline MRI artefact removal and filtering using FEMR or Vision Analyzer software. Filtered EEGs were reviewed by an experienced neurophysiologist and spikes marked according to spatial distribution, morphology and duration. An EEG-fMRI study was defined for each type of spike in a given recording, so that discharges with different spatial distribution or morphology in the same patient were analysed separately. Studies with less than three spikes during the scanning session were excluded from further analysis.

The fMRI images were motion corrected and smoothed [6 mm full width at half maximum (FWHM)] using in-house software. Temporal autocorrelations were accounted for by fitting an AR model of order 1 according to the methods of Worsley et al. (2002), and low frequency drifts in the signal were modelled with a third-order polynomial fitted to each run. Maps of the t statistic (t maps) were created using the timing of each spike as an event in the fMRI analysis (fMRIstat, Worsley et al., 2002). At each voxel, the maximum t-value was taken from four individual t maps created with four haemodynamic response functions with peaks at 3, 5, 7 and 9 s (Bagshaw et al., 2005).

EEG-fMRI responses were classified into positive (activation) and negative (deactivation). Significant responses required the following criteria: minimum of five contiguous voxels with a t value > 3.1, corresponding to P = 0.05, corrected for the multiple comparisons resulting from the number of voxels in the brain and for the use of four haemodynamic response functions (Cao, 1999). Localization of responses was determined by superimposition of the anatomical MRI acquisition and t-statistic maps using in-house software.

We evaluated the IMRI responses, activations and deactivations to scalp interictal spiking, in the heterotopia, in the perilesional areas and in distant cortical regions. The weak spatial resolution of IMRI data associated with the 6 mm FWHM smoothing performed during the analysis could cause difficulty in determining whether a response was exclusively lesional or exclusively perilesional or both. Whenever it was not clear if only the heterotopia, or the overlying cortex, or both regions were involved in the response, due to this smoothing effect, we considered that both regions were involved.

In nodular heterotopia the distinction between lesional, perilesional and distant areas was easy to determine. We defined perilesional areas as the cortical region overlying or surrounding the heterotopia and other cortical regions as distant areas. In band heterotopia, the distinction between perilesional and distant areas was more difficult, since the lesions were quite diffuse. We had three patients with band heterotopia, one bilateral periventricular and two bilateral subcortical. In the two patients with a subcortical band, the malformations showed in one an anterior preponderance and in the other a diffuse band involving both the anterior and posterior quadrants. Since the perilesional and the distant cortices were determined according to the vicinity of the band, they could not be clearly distinguished in the patient with a diffuse band. Finally, in the unique patient with a periventricular band, the malformation was surrounding entirely both lateral ventricles. We could not, also in this case, make a distinction between surrounding and distant cortex.

In this study we did not specifically address the significance of the involvement of the cerebellum, brainstem and diencephalic structures.

**Results**

The 14 patients (9 women) had focal seizures and heterotopia: 11 with nodules and 3 with bands. A total of 26 EEG-fMRI were acquired: three studies in two patients were not analysed because less than three spikes were recorded during the
scanning period; four patients had more than one type of spikes, which were analysed separately, giving a total of 23 EEG-fMRI studies (Table 1). Twelve were obtained from the 11 patients with nodules and 11 from the 3 with bands. Spikes per study ranged from 4 to 320 (median, 35). In 21 studies spiking was focal, in one it was bilateral focal and in one it was generalized.

A BOLD response was observed in 22 of 23 studies (95.5%): the majority, 18, had both responses, activation and deactivation, two showed activation only, and two showed deactivation only (Table 1). One study, from a patient with nodular heterotopia, showed no response. We analysed the patients with nodules separately from those with bands.

**Nodular heterotopia group.** Activation was present in nine (75%) studies. Six showed involvement of the heterotopia or surrounding cortex: in two studies both heterotopia and surrounding cortex were involved (Figs 1 and 2), in three only the surrounding cortex and in one (Fig. 3) only the heterotopia was involved. In three of these six studies concomitant activation was also seen at a distance (Figs 2 and 3), and none of these distant regions had a clear functional relationship to the lesion. In the remaining three studies, there was no activation in the heterotopia and surrounding cortex. One study (no. 12, Table 1) showed only activation in the posterior quadrant contralateral to a giant heterotopia, and, therefore, was potentially related to the lesion. In a second study, from a patient with left trigone heterotopia, the activation involved bilaterally the frontal, parietal and temporal regions, with left side predominance. In the third study, from a patient with right trigone heterotopia (Fig. 4), activation was in the ipsilateral temporal lobe, insula and thalamus.

Deactivation was also observed in nine studies (75%). Four showed involvement of the heterotopia and surrounding cortex: in two only the surrounding cortex was involved and in two only the heterotopia. In three of these four studies concomitant deactivation was also seen at a distance, and only one of these distant deactivated regions could be related to the lesion (contralateral and homologous to the lesion, study no. 7, Table 1, Fig. 1C). In the remaining five studies, there was no deactivation in the heterotopia and surrounding cortex (Fig. 4), and only unilateral or bilateral involvement of the cortical areas that were not homologous to the lesion or to the cortex surrounding the heterotopia.

**Band heterotopia group.** Activation was present in all 11 studies and always in the heterotopia and surrounding cortex: in nine studies both heterotopia and surrounding cortex were involved, and in two only the surrounding cortex was involved. Nine showed concomitant activation at a distance, and none of these distant regions had a clear functional relationship to the lesion.

Deactivation was also observed in all 11 studies, with involvement of both the heterotopia and surrounding cortex,
in addition to distant deactivation in five studies. In the remaining six studies, deactivation was found only at a distance from the heterotopia and surrounding cortex.

Responses were therefore found in both groups of patients, and due to the more diffuse aspects of band heterotopia it was of course more frequent to find this correlation in this group.

Activations and deactivations showed different spatial patterns in both groups; activations were more likely to be observed in the lesional or perilesional areas, and deactivations were more frequently found at distance from the malformation. Independently of the pattern of the response, activation or deactivation, observed in the heterotopia or

Fig. 1 EEG-fMRI study (No. 7, Table 1) from a patient with a periventricular nodule located in the right occipital horn (arrow, A), related to right temporal spikes and polyspikes. Activation (B) was observed in the nodule, also involving the mesial aspect of the occipital lobe. Deactivation (C) was seen bilaterally in the mesial occipital regions, but did not coincide with activation.
surrounding cortex, bilateral homologous cortical involvement was frequently seen (Figs 1C, 3 and 5A). This was described in 10 studies, even though the great majority of studies were related to unilateral focal spikes.

Discussion

Our results clearly illustrate the potential advantages of the non-invasive EEG-fMRI method to understand the relationship between the malformations and other cerebral structures, and their respective role in the genesis of epileptic activity. The findings parallel those previously observed in other series of epileptic patients with nodular or band heterotopia and studied with acute or chronic depth EEG recordings, which showed that epileptiform discharges can arise from the heterotopia, the surrounding cortex and at a distance (Morrel et al., 1992; Francione et al., 1994; Preul et al., 1997; Kothare et al., 1998; Mai et al., 2003; Aghakhani et al., 2005).

In 22 of 23 EEG-fMRI studies, positive or negative changes in BOLD signal were observed in response to epileptic discharges. There was a better concordance of a BOLD signal increase (85% of studies with activation) with the heterotopia and surrounding cortex than with a BOLD signal decrease (45% of studies with deactivation). In addition to the responses related to the lesion, there were frequent cortical responses at a distance, and when studies involved only such distant responses they were much more likely to be deactivations than activations. The band heterotopia group showed, as expected, good correlation between the responses and the lesions, supporting in this condition diffuse epileptogenicity. Although the lesional and perilesional responses were not as frequent in the nodular group, there was clear involvement of these regions in half of the activations. FMRI responses involving deep-seated lesions, such as heterotopia (either periventricular or subcortical), are evidence that even though the spikes recorded on the scalp are most likely generated in the cortical surface, they may imply interactions between those lesions and the cortex. We assume that activation is caused by an increased neuronal activity at the time of EEG spike generation. The concomitant involvement of the heterotopia in that response should represent a similar cellular hyperexcitation and should support their role in the epileptogenicity.

In two studies (Figs 1 and 2) the fMRI response involved the whole or almost whole heterotopia. Most studies, however, showed responses in part of the malformation. Such partial involvement of the lesion in EEG-fMRI BOLD responses has been previously described with other types of malformations of cortical development (Kobayashi et al., 2004; 2005a; Federico et al., 2005); this could depend on their biological characteristics. Brain malformations have different embryogenesis and they are heterogeneous (Barkovich et al., 2001) with respect to their structure and with respect to their functional connectivity with the rest of the brain (Sisodiya, 1995). The wiring of the abnormal neurons may vary, affecting, for instance, the connectivity between the abnormal cells and the surrounding cortex, a heterogeneity that can possibly be disclosed by functional studies. On the other hand, because the spatial extent of the BOLD response depends on statistical thresholds and is affected by the number of events used for the analysis, it is also possible that the partial involvement of the lesion corresponds to the ‘tip of the iceberg’: a larger number of epileptic discharges might have caused a more extensive response within the heterotopia. It must be remembered however that even in this situation the areas with the highest t-values would remain the most significant.

Intracranial EEG recordings in patients with heterotopia have shown normal EEG patterns in the malformation (Preul et al., 1997; Aghakhani et al., 2005), epileptiform discharges arising independently from the heterotopia (Morrel et al., 1992; Francione et al., 1994) or synchronously in the ectopic neurons and surrounding cortex (Francione et al., 1994; Mai et al., 2003; Aghakhani et al., 2005). Epileptic activity has also been recorded independently in the surrounding
cortex (Preul et al., 1997; Aghakhani et al., 2005) and mesial temporal structures (Aghakhani et al., 2005). Four patients with nodular heterotopia had previous stereoelectroencephalography (SEEG) recording but only two of them had electrodes placed in the nodules. They were reported in Aghakhani et al. (2005). Patient 2 ictal onsets usually involved the hippocampi, the nodules and the temporo-occipital cortex. Activation was seen in regions that were involved in ictal activity (right temporal nodule) but also in regions not covered by the SEEG investigation. Deactivation was seen in the mesial aspects of the occipital lobes. In Patient 8, seizures started in the nodule and in the right temporo-occipital

Fig. 3 Activation (A) and deactivation (B) related to bioccipital spikes in a patient with bilateral periventricular nodular heterotopia (study no. 2, Table 1), with nodules in the temporal and occipital horns (white arrows). There was activation in the right temporal nodule, and deactivation was seen in the bilateral occipital lobes, therefore perilesional to the occipital nodules. For both types of responses, there was also involvement of distant brain areas.
region. In the three studies of this patient, BOLD responses were present in these regions, but were also found in regions in which there were no depth electrodes.

Experimental data derived from animal studies showed that these lesions exhibit an unbalanced excitation involving ion channel abnormalities (Castro et al., 2001) and GABA circuitry (Calcagnotto et al., 2002; Chen and Ropper, 2003). These animal models also demonstrated the existence of reciprocal connections between the heterotopia and the surrounding cortex (Schottler et al., 1998; Chen et al., 2000). It is possible that in some situations only a hyperexcitable surrounding cortex is sufficient to generate the spikes, while in others hyperexcitability needs to involve both ectopic neurons and perilesional cortex. Moreover, epileptogenicity could be dependent on the generation of discharges in the heterotopia and propagation to the neocortex, the latter acting as an amplifier and synchronizing the abnormal discharge. We analysed events recorded from the scalp, likely generated in the neocortex and for which cortical responses could be expected. Nevertheless, we found involvement of the heterotopia in the majority of the studies, reflecting the involvement and possibly the origin of the discharge in the lesion. The fMRI responses observed in our study often extended to the surrounding cortex and sometimes to cortical areas not directly related to the lesions. This is in agreement with the above-mentioned depth EEG studies and animal results. More experimental data combining electrophysiological and fMRI studies may confirm this correlation between the EEG and the metabolic changes we observe with fMRI.

Concomitant involvement of sites distant to the malformation was found in 70% of activations and 89% of deactivations. Bilateral involvement of these areas of signal change was usually seen in a homologous pattern despite the fact that most studies were related to focal unilateral spikes. They were usually at a distance from the lesion (in only two studies they were in the contralateral area homologous to the heterotopia or the cortex surrounding the lesion). This bilateral cortical implication, especially in homologous areas, has been observed previously in EEG-fMRI studies, not only in patients with brain malformations but also in other lesional and non-lesional patients with focal epilepsy (Kobayashi et al., 2004, 2005a; Federico et al., 2005). The simultaneous involvement of areas at a distance from the lesion probably reflects connections between them and represents an effect of the spikes in these distant regions.

Some of the maps were clearly not related to the lesion, and were not informative with respect to the involvement of the heterotopia, the overlying cortex or distant areas. This pattern...
was relatively frequent for deactivations (50% of studies with deactivation) and rare for activations (9% of studies with activation). The characteristics of deactivations in this group of patients further support the idea that negative BOLD changes tend to occur further away from the region generating the spikes, and might rather represent an effect (e.g. distant neuronal inhibition) than a cause (excitation) of the discharges. The pattern of response in these patients with heterotopia is again congruent with previous studies with lesional epilepsy, which have shown that activations are more correlated with the structural abnormalities than deactivations (Bagshaw et al., 2004; Kobayashi et al., 2004, 2005b).

It is too early to use EEG-fMRI data for the planning of surgical resections. However, it can provide information about the involvement of other brain areas, as to how focal or widespread the epileptogenic area might be. We believe that eventually these findings could be used for instance to define targets for intracranial recordings even in those patients with diffuse responses. Spike related EEG-fMRI in patients with heterotopia disclosed responses that support a relationship between these deep-seated lesions and the rest of the brain. This is in agreement with the findings obtained with intracerebral electrodes. The EEG-fMRI method, however, has the advantage of non-invasiveness and the further advantage of exploring the whole brain at the same moment. It remains unclear why heterotopia are the primary epileptogenic region in some patients while it seems to play a lesser role in others.

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