Author's response to reviews

Title: Visual recovery after perinatal stroke evidenced by functional and diffusion MRI.

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Author's response to reviews: see over
Dear Editor,

Please find enclosed a revised manuscript entitled "Visual recovery after perinatal stroke evidenced by functional MRI and diffusion tensor imaging" by SEGHIER et al. that we would like to resubmit for publication in BMC Neurology. As mentioned in our previous Cover Letter, this report describes a new approach to clinical investigation with combined functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI) to assess visual system recovery in an infant with perinatal stroke. While both reviewers have found that our article is of importance in its field, they raised interesting questions about our findings. This new version addresses now all reviewers comments.

The current version of the manuscript was revised according to the two reviewers comments. The major sections concerned by this revision are listed below:
- the abstract has been modified;
- the third paragraph of the introduction section has been extended;
- a new figure (Figure 1) showing conventional MRI scans is added;
- more details about the diffusion experiments are added in the DTI methods section;
- the Methods section has been reorganized;
- a long paragraph about the inter-session variability is added in the Discussion section;
- a new paragraph related to brain maturation is added in the Discussion section;
- the last paragraph of the Discussion section has been modified.

The text has been now increased by 30% with more than 20 new references added in this new version of our manuscript.

This resubmission includes a manuscript file, a point-to-point response to the two reviewers comments (see below), and three color illustrations.

The work reported in the attached manuscript has not been, and is not intended to be, published anywhere except in BMC Neurology. All authors have approved the submission of our work to BMC Neurology for publication as Case Report. Our work is not sponsored by pharmaceutical companies.

We think that our findings will be of interest to readers of BMC Neurology and we look forward to a rapid revision process.

Yours sincerely
Petra S Huppi, MD, the corresponding author.
Major Compulsory Revisions:

1- we agree with the reviewer that the issue of the reproducibility and the reliability of functional maps in fMRI is a crucial issue in longitudinal case report studies. According to the work of McGonigle et al. (Ref [30]), it was argued that the inter-session variability was important even with motor and visual functions. Note that the “spectacular” results shown in the McGonigle’s study has been recently revised by the same group, showing that this inter-session variability is not “so big” as claimed initially (see Ref [32]) and, more importantly, its magnitude was similar to within-session variability. Although this issue of reproducibility is inherent to any fMRI experiment, we have taken into account some aspects to minimise its influence on the interpretation of our results. First, we suppose that inter-session variability with a very simple visual task (no implication of higher cognitive functions) that involves primary visual cortices is small. Second, the fMRI experiments at 3 months of age and 20 months of age were done independently with both block and event-related paradigms. The block paradigms were repeated three times during each experiment. The results of the block paradigms at 3 months of age (see Ref [7]) are comparable between them. Also, the results of the block paradigms at 20 months of age (results not shown in the current paper) are also comparable between them. Moreover, the event-related paradigms at each session confirmed the results obtained in the block paradigms. The convergence of all fMRI paradigms would therefore speak against inter-session variability. Third, the activated right visual cortex in the intact hemisphere could be considered a “witness” of the weak influence of inter-session variability. This means that, in the presence of possible inter-sessions variability, this variability should affect both hemispheres with similar amplitudes (at our knowledge, there is no evidences that the inter-session variability could have different magnitudes in primary brain regions). In our case, the right hemisphere activation was stable and present with comparable statistics across sessions. This could suggest that, with the same simple visual paradigm, the modifications in the activated pattern of the left injured hemisphere could not be ONLY explained by methodological or inter-session variability. In this context, we suggested that some recovery mechanisms could generate the observed significant activation in the left hemisphere at 20 months of age that was not observed at 3 months of age. This issue is now succinctly discussed in the Discussion section of the new version of our manuscript (page 9).

2- Previous studies have shown that the optic radiation tracts are clearly visible at very early age (even in premature newborns, as shown in Figures 1 and 2 of Ref [35]) and their maturation with myelination is largely achieved during the first year of life (e.g. Ref [36]). Therefore, the developmental structural modifications between 12 and 20 months of age in the optic radiation are small (even that some subtle maturation could occur after one year of age, according to the study of Mukherjee et al. 2001 Radiology). In our case, the optic radiation tracts in the intact right hemisphere were visible at 3 months of age, and reinforced at 12 and 20 months of age. In contrast, the optic radiation tracts were not visible at 3 months of age in the left injured hemisphere, visible at 12 months of age and reinforced at 20 months of age (see Figure 2). These structural modifications of the optic radiation appeared differently in the two hemispheres, suggesting that some recovery and plasticity mechanisms have occurred during the maturation of the optic radiation of the left hemisphere after perinatal stroke. This issue is now added in the Discussion section (page 10). On the other hand, the spatial resolution was approximately identical for both experiences, with voxel size of 10 micro-lit for DTI-12 and 11 micro-lit for DTI-20.
Initially, the DTI experiment at 3 months of age was done with a voxel size of 10 micro-lit. We believe that the detected structural modifications could not be due to these slight voxel size variations. Thank you for these two interesting points of debate.

**Minor Essential Revisions:**

1- the abstract is now rewritten (particularly the Methods and Results sections). Briefly, a first DTI experiment was performed at 12 months of age, and a second DTI experiment was performed and combined with an ER-fMRI experiment at 20 months of age.

2- the definition of FA was missed in our previous version. We used the definition of Basser and Pierpaoli (Ref [25]) for the FA calculation (now added in page 6 of the new version of our manuscript). Thank you for this remark.

3- this is an interesting remark. According to several functional studies (since the works of Fox et al.), the optimal value for visual flashing frequency was found to be about 8Hz for the adult visual cortex. To date, in newborns and infants, there are no functional descriptions of the evolution of the infant BOLD signal with the visual stimulus rate. In our study, we used a stimulus rate of 2Hz according to findings showing that more robust visual evoked potentials were obtained in infants when reducing visual stimulation frequency (see Ref [28]). This issue is now added to the new version of our manuscript (Methods section, page 6). Also, we haven’t used a stroboscope. Therefore, visual stimuli were presented to the infant via a video projector, a front-projection screen and a system of mirrors fastened to the head coil

4- all activations above p<0.001 uncorrected (T threshold = 3.1) were considered and illustrated in Figure 2. The size of activated regions was also given at p<0.001 uncorrected. To show the statistical robustness of visual activation we reported also the p values after correction (corrected for multiple comparisons of whole brain voxels). These corrected p values indicated well the statistical significance of these visual activated regions and are fundamentally more conservative than the uncorrected p values (see Friston et al. 1995, 1998 about the SPM processing approach).

5- the results of the fMRI examination at 3 months of age are summarized in the Case Report section (page 5) and described in the Discussion section (page 9). The first paragraph of the Results section has been modified (see page 8).

6- for each activated region in the visual cortex, we report the size, the statistical T value and the associated corrected p value. Basically, in SPM, a T test was performed and then Z values are deduced from the calculated T values under some assumptions. We agree that giving both T and Z values is redundant. For clarity, we chose to remove Z values from the terms in parenthesis. Note that Z infinite value means that the associated cumulative probability value was equal to zero when the value of this probability is under the floating-point accuracy (SPM software packages used MATLAB language based on machines with IEEE floating-point arithmetic; accuracy = 2^-52).

**Discretionary Revisions:**

1- more details about the DTI experiment in the Methods section are added. New references are now included (see Refs [23-27]).
Reviewer: Massimo Filippi

**Major Compulsory Revisions:**

None

**Minor Essential Revisions:**

1- we agree with the reviewer that this issue has been extensively addressed in several studies. In this new version of our manuscript, we have modified the two last sentences of page 3 and included new references (see Refs [12-18]). Initially, our allusion to little anatomo-functional evidences about brain recovery concerned principally studies in the pediatric population. Thank you for this important remark.

2- we added a new figure (labelled Figure 1) showing the lesion of this infant with conventional MRI scans at different ages. This Figure 1 is now cited in Case report section (page 5).

3- more details about the DTI experiment in the Methods section are added with additional references (see Refs [23-27]) in the new version of our manuscript. The Bzero image is an acquired SE-EPI image with non-diffusion weighting (b-factor = 0), according to the Basser’s scheme.

4- Animal studies are now removed in the new version of our manuscript. This last sentence of the discussion section was replaced by recent studies on the plasticity in adults and children.

**Discretionary Revisions:**

None