Cortical neuroplasticity in patients recovering from acute optic neuritis
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Abstract
Patients with optic neuritis (ON) undergo cortical and subcortical neuroplasticity as revealed by functional magnetic resonance imaging (fMRI). However, the heterogeneity of scotomas has not been adequately addressed previously. We introduce a new method of modelling scotomas in fMRI, to reveal a clearer pattern of neuroplasticity, across a heterogeneous patient-population. A longitudinal fMRI-study of visual function in 19 ON-patients examined at four timepoints between presentation and six months was performed and four different models were used. The first model included the four different examination timepoints as separate explanatory variables without adjustment for visual field defects. The second model also included covariates reflecting subject-specific deviations in visual field defect from the average group value of the Humphrey mean deviation (HMD) at each examination timepoint. In the third and fourth models the four examination timepoints were not modelled explicitly, but entered vicariously through the associated changes in the HMD for each subject that marked their individual recovery. The results show that the third and fourth models were more sensitive to geniculate and visual cortical neuroplasticity during recovery. Moreover, inferences from the fourth model can be extended to the general population of patients recovering from ON. In conclusion, we present a method of accommodating subject-specific differences between patients with acute ON by inclusion of an HMD-index. This method is sensitive to the processes of neuroplasticity whilst the generalisation of inferences makes the method suitable for future studies of treatment.
**Introduction**

There is often a significant improvement in function following an injury to the central nervous system, including lesions from multiple sclerosis (MS). This is due to adaptive changes in uninjured cortical and subcortical regions as well as recovery at the site of the lesion. Functional magnetic resonance imaging (fMRI)-studies have demonstrated cortical adaptive changes in patients with MS that may compensate for lesions affecting the visual system (Werring et al., 2000; Toosy et al., 2002), the motor system (Lee et al., 2000; Reddy et al., 2000; Pantano et al., 2002a; Pantano et al., 2002b; Reddy et al., 2002; Rocca et al., 2003; Pantano et al., 2005; Rocca et al., 2005) and cognitive functions (Staffen et al., 2002; Mainero et al., 2004; Audoin et al., 2005). It is essential to understand these changes, not only because of what we can learn about repair mechanisms and neuroplasticity in the adult brain, but also to characterise properly the effects of treatment.

MRI has been widely used as a marker of disease and of treatment efficacy, especially in MS. However there are special factors in MRI-analysis and in fMRI that may undermine the validity or sensitivity of previous results. We will first consider the existing evidence for cortical and subcortical neuroplasticity, focusing on the changes that follow optic neuritis (ON). ON is the presenting symptom in approximately 20% of patients diagnosed with MS. It typically causes symptomatic visual impairment and retrobulbar pain with decreased visual acuity, abnormal colour vision, decreased contrast sensitivity, and visual field defects such as central scotomas. ON usually recovers symptomatically despite long-term atrophy of the optic nerve (Hickman et al., 2001; Hickman et al., 2002; Hickman et al., 2004; Trip et al., 2006) and persistence of abnormally prolonged visually evoked responses (VEP) (Halliday et al., 1972; Youl et al., 1991). As atrophy is an indicator of permanent local damage following ON, recovery of vision must on principle occur either because of redundancy in the anterior visual pathways or because of subcortical or cortical remodelling of visual function (Hickman et al., 2004).
fMRI-studies of patients with ON have shown that patients have a smaller extent of activation in primary visual cortex than controls in response to visual stimulation (Rombouts et al., 1998; Gareau et al., 1999; Langkilde et al., 2002; Russ et al., 2002). In addition, widespread extra-occipital abnormalities have been reported in patients with previous ON including the insula-claustrum, corpus striatum, the lateral temporal, posterior parietal, and orbitofrontal cortex, and the thalamus (Werring et al., 2000; Toosy et al., 2002). However, these extra-occipital BOLD-responses were characterised by a very long haemodynamic delay of approximately 15-40 seconds, undermining an interpretation in terms of evoked neural responses to visual stimulation.

Reproducibility would strengthen the interpretation of such fMRI changes in terms of neuroplasticity and support a role as an outcome measure of interventions. However, earlier reports have been difficult to reproduce for several reasons. One study used a small and heterogeneous patient population including both acute and recovered patients (Rombouts et al., 1998), while others covered a limited portion of the brain (Rombouts et al., 1998; Gareau et al., 1999; Langkilde et al., 2002) or performed only region-of-interest (ROI)-analyses (Rombouts et al., 1998; Gareau et al., 1999; Langkilde et al., 2002; Russ et al., 2002; Levin et al., 2006) thereby potentially missing effects elsewhere.

In a recent study by Toosy et al. (2005) both the size and quality of the patient group seem sufficient to replicate the extra-occipital changes from their previous studies (Werring et al., 2000; Toosy et al., 2002). However, different effects were seen, including cortical adaptive changes in the lateral occipital complexes (LOC). Levin et al. (2006) recently suggested LOC to be robust to disruptions of the visual input in patients after clinical recovery from ON, as they found normal activation in LOC upon stimulation of the affected eye opposed to decreased activation in early visual areas. In a recent fMRI-study of patients in the acute phase of ON we reported decreased activation in LOC upon
stimulation of the affected eye in the acute phase (Korsholm et al., 2007), after 6 months the LOC activation was normalised.

fMRI-studies of normal human vision and cognition typically study brain activations ‘voxel-wise’ looking at each small unit of brain tissue of a few cubic millimetres. For comparison between subjects, these small brain volumes are normalised in 3D to a standardised average brain shape. This is a powerful and unbiased method to detect subtle effects, and enables the results to be reported in an objective and comparable framework (Frackowiak et al., 1997). However, only one previous fMRI-study of acute ON has used this method (Toosy et al., 2005). One reason for the paucity of voxelwise group studies of patients with acute ON might be that differences in scotoma location between patients make analysis of primary and secondary visual cortices problematic because of their retinotopic organisation (Sereno et al., 1995). In the study by Toosy et al. (2005), this problem was accommodated by using Humphrey mean deviation (HMD) and visual acuity as outcome measures, and optic nerve integrity and BOLD-signal changes to predict visual outcome.

However, questions remain about the best way to incorporate visual performance in the analysis of fMRI data. For example, to what extent can the apparent cortical neuroplasticity be attributed to the changes in visual field defects, or is there evidence of cortical neuroplasticity independent of changing field defects? To investigate this further we performed a longitudinal, standard space, voxelwise group fMRI-study of 19 patients with acute ON and during recovery. We introduce a new method capable of modelling subject-specific scotoma locations. The aim is to reveal a clearer pattern of cortical adaptive changes in the acute and recovery phases suitable for comparison across a heterogeneous patient population. Such a method has greater potential for use as a marker of efficacy of therapeutic interventions.
**Materials and methods**

**Patients**

Nineteen patients with acute ON were included in the study (15 women, 4 men, median age 31.5 years, range 18-45 years). Fourteen patients had clinically isolated acute ON (11 women, 3 men), and five patients had acute ON as an isolated manifestation of relapse in relapsing remitting MS (RRMS). Patients with RRMS were eligible for the study as long as this was their first presentation with ON and the VEP-latency of their unaffected eye was within normal values (≤ 112 ms) at the time of inclusion. Detailed clinical information on each patient is given in Table 1.

The diagnosis of ON was verified by clinical examination and para-clinical visual testing. The patients were examined in the acute phase, and again two weeks, three and six months after the acute phase examination.

The study was approved by the local scientific ethics committee (protocol no. KF 01-115/04) and written informed consent was obtained from all the subjects.

**Visual tests**

Visual acuity was assessed monocularly by the Snellen chart at 6 m. Autoperimetry of the central 30° region of the visual field was performed on each eye with a Humphrey Field Analyzer. Monocular VEPs were recorded with whole-field (27°), pattern-reversal checkerboard (9 mm), and the amplitude and the latency of the major positive component (P100) were measured.

**MRI**

The MRI included a functional scan followed by a structural scan on a 3.0 T Siemens Magnetom Trio Scanner (Siemens, Erlangen, Germany). The fMRI was acquired with a standard single channel birdcage head coil (Siemens, Erlangen, Germany). T2*-weighted echo planar imaging (EPI) with 42 slices of 3 mm thickness positioned parallel to the calcarine sulcus were acquired with the following parameters: repetition time (TR)=2.49 s,
echo time (TE)=30 ms, flip-angle 90°, field of view (FOV)=192 mm and in-plane resolution 3x3 mm². For each eye a total of 121 volumes were acquired.

During the functional MRI acquisition the cardiac and respiratory cycles were recorded with an infrared pulse oximeter on the patient’s index-finger and a pneumatic thoracic belt, respectively.

Structural T1- and T2-weighted images for diagnosis were acquired with an eight-channel head coil (Invivo, FL, USA) and included:

(1) MPRAGE (magnetization prepared rapid acquisition gradient echo): voxel dimension=1x1x1 mm³, FOV=256 mm, matrix 256x256, TR/TE/inversion time (TI)=1540/3.93/800 ms, and a flip-angle of 9°, before and after a double dose of paramagnetic contrast-agent (0.2 mmol/kg Magnevist, 0.5 mmol/ml, Schering AG, Berlin).

(2) Axial FLAIR (fluid attenuated inversion recovery): 42 slices with a thickness of 3 mm, FOV=230 mm, matrix 256x256, TR/TI/TE=9000/2400/85 ms, and a flip-angle of 150°.

(3) Axial turbo spin echo: 42 slices with a thickness of 3 mm, FOV=230 mm, matrix 256x252, TR/TE1/TE2=8820/115/14 ms, and a flip-angle of 163°.

**Experimental procedure**

Visual stimulation was provided by means of an LCD projector (Canon LV7540) located outside the scanner room. A zoom lens (Buhl Optics 849MCZ087) projected the image onto a screen behind the patient’s head. The screen covered 24°x18° of the visual field and was visible to the patient through a mirror mounted on the head coil. The patient viewed a stimulus consisting of a full-field black and white circular checkerboard reversing at 8Hz with checkers of sizes reflecting the cortical magnification factor (Slotnick et al., 2001). Each eye was stimulated separately (block design, 10 s of flickering checkerboard, 10 s pause, total scan time per eye 5 min and 1s), while the other eye was covered by an eye-pad.

A fixation dot remained throughout the experiment and this randomly changed colour from red to green (with a random interval of 4–8 s, the colour green remaining for 500 ms). To
maintain attention to the central visual stimulus subjects 7 to 19 were asked to press a button each time the colour of the fixation dot changed. At inclusion, all patients were able to at least perceive light, and all patients could as such participate in the visual stimulation fMRI paradigm. However, in the acute phase some of the patients had central scotomas impairing central fixation. They were instructed to keep their eye open and to look at the centre of the screen, guided by peripheral visual cues including the circular chequerboard.

**Data analysis**

*Image pre-processing*

All image pre-processing and data analysis was performed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/).

For each patient all functional images were motion corrected and registered to the first same-session EPI (Friston et al., 1995). The EPI images were spatially normalised to the SPM2 EPI template in MNI space (Ashburner and Friston, 1999) and smoothed with a Gaussian filter with a full width at half maximum (FWHM) of 8 mm.

*Single subject analysis*

Statistical analysis of fMRI data was performed using a general linear model (Worsley and Friston, 1995) where the signal of interest was modelled as a box car convolved with the standard SPM2 canonical hemodynamic response function. Serial correlations were modelled using a nuisance variable regression approach (Lund et al., 2006). In addition to the SPM2 standard discrete cosine set high pass filter (128s cut off), this approach includes regressors based on cardiac and respiratory oscillations (Glover et al., 2000) and 24 expanded motion parameters (Friston et al., 1996).

*Group analysis*

To assess group differences in brain activation upon stimulation of the affected eye during recovery three different second level design matrices (DM) were constructed:

**DM1.** With the first DM we wanted to test for mean activation across sessions and for significant differences between sessions. This DM consisted of four columns indicating
images acquired during the four examinations (figure 1, top). Five different contrasts were applied to this DM: (a) a t-contrast testing for mean activation across sessions, (b) an F-contrast testing for significant intersession differences in brain activation (i.e. where one session differs from the mean of the remaining sessions), (c) a t-contrast testing for the specific case of greater activation during session 3+4 than during session 1+2 (session 1+2 and 3+4 were pooled to accommodate for differences in disease duration), (d) a t-contrast testing for a mean negative BOLD-response in session four (i.e. after recovery), and (e) a t-contrast testing for a mean negative BOLD-response in session 3+4. The latter two contrasts were applied to replicate the findings by Werring et al. (2000) and Toosy et al. (2002) who found maximum signal during baseline condition of the visual paradigm in a group of patients recovered from ON.

DM2. To account for a subject-specific offset in BOLD-signal and for session-specific variations in visual performance, a second DM was constructed. This matrix included, in addition to those columns described above, one column per subject indicating scans belonging to the specific subject, and four columns (one for each session) modelling deviations around the session-wise mean HMD (figure 1, mid). If effects of subject-specific offsets in BOLD-signal and session-specific dependence on HMD variations are present, then we would expect intersession differences to be more pronounced with this analysis than with the previous. Three different contrasts were applied to this DM: (a) an F-contrast testing for significant intersession differences in brain activation, (b) a t-contrast testing for the specific case of greater activation during session 3+4 than during session 1+2, and (c) four t-contrasts testing for an extra effect of HMD deviation from each of the session-wise mean HMD.

DM3. In patients with ON, scotomas can vary greatly and may be central, paracentral, quadrantic, or small defects in the periphery. The scotomas of the patients included in this study are displayed subject-wise in figure 2. From the figure it is seen that patients no. 10,
11 and 12 during recovery exhibit no changes in the area covered by fMRI, whereas in all the other patients visual performance in the central part of the visual field changes during recovery.

To test for increased BOLD-responses correlated with increased HMD in the patients with changes in their central vision, a third DM omitting patient no. 10, 11 and 12 was constructed. To account for subject-specific offsets in BOLD-signal and subject-specific dependencies on HMD due to different scotomas, this third DM included 16 columns indicating the four scans belonging to each patient and 16 columns indicating each patient’s deviation around the mean HMD at his/her four visual field examinations (figure 1, bottom). To equalise the efficiency of the regressors modelling the subject-specific changes in HMD, these regressors were all normalised to a standard deviation of 1. In this third model, session indicator regressors were not included due to the co-linearity between session indicator regressors and the subject-specific HMD.

A t-contrast was applied to the first 16 columns in this DM to test for mean activation across subjects. To test for an extra effect of HMD on brain activation another t-contrast was applied to columns 17-32 (columns representing subject-specific HMD changes). The latter contrast tests for areas where on average across subjects there is an increase in BOLD-signal with an increase in HMD. This corresponds to a fixed-effect analysis.

**DM4.** Finally, a third-level DM (one sample t-test) was constructed to test for an effect of HMD generalisable to the population (Holmes and Friston, 1998) of ON-patients with scotomas in the most central ±9° of the visual field.

For the unaffected eye all DMs and associated contrasts were constructed except for DM3 and DM4 as the unaffected eye had by definition no central scotomas. In addition, in DM1 it was tested whether any regions were more active during the three first sessions than the last session (t-contrast), as the lateral geniculate nucleus (LGN) in a previous ROI-analysis
of the same patient population was found more active in early sessions than after recovery (Korsholm et al., 2007).

**Thresholding**

Statistical maps from tests in DM1-DM3 were thresholded at a family wise error rate (FWE) threshold of \( p=0.05 \) derived using the Gaussian Random Field theory (GRF). To increase sensitivity we also used a false discovery rate (FDR) at \( p=0.05 \) together with a cluster size threshold of 15 voxels. As FWE thresholds provided by GRF have been demonstrated to be too conservative (Nichols and Hayasaka, 2003) for smoothness and degrees of freedom similar to those of the t-map from the third level analysis, this map was only thresholded using FDR.

**Results**

**Affected eye**

**DM1**

There was significant activation of the visual cortex and LGN (bilaterally) across all sessions (FWE=0.05). The F-test for inter-examination differences was not significant neither at FWE=0.05 nor at FDR=0.05 (cluster size=15 voxels), and we found no significant differences between mean of session 3+4 and mean of session 1+2 neither at FWE=0.05 nor at FDR=0.05 (cluster size=15 voxels).

The t-contrasts testing for a mean negative BOLD-response in session 4 and in session 3+4 were not significant at neither FWE=0.05 nor at FDR=0.05 (cluster size=15 voxels). The non-significant effect was further examined using posterior probability maps (Friston and Penny, 2003). Using this method it was found that with a posterior probability of 0.95, for any of these two contrasts no brain voxels had an effect above 0.37% of the whole-brain mean signal (0.37% equals 10% of the positive activation).

**DM2**

In this analysis where subject-specific offsets in BOLD-signal and deviation from session-wise mean HMD also were modelled, we found significant intersession differences in the
visual cortex (FWE=0.05) and in the left LGN (FDR=0.05, cluster size=15 voxels). This means that there was a difference in BOLD-response to stimulation during recovery. The direction of this change is clarified with one-sided t-tests: When testing specifically for differences between mean of session 3+4 and mean of session 1+2, the visual cortex (FWE=0.05) and LGN bilaterally (FDR=0.05, cluster size=15 voxels) were activated, i.e. more activation of visual cortex and LGN bilaterally in session 3+4 than in session 1+2, (figure 3a).

With the t-contrast testing for an extra effect of HMD deviation from the session-wise mean HMD a small cluster (73 voxels) in the most occipital part of the visual cortex was activated in session 1 (FWE=0.05). At FDR=0.05 (cluster size =15 voxels) the cluster is still only covering the most occipital part, but now includes 1969 voxels (figure 3b). This means that greater BOLD-signal is correlated with higher HMD in session 1. In the later sessions 2, 3, and 4 there were no extra effect of HMD deviation from the session-wise mean HMD on cortical or LGN activation neither at FWE=0.05 nor at FDR=0.05.

**DM3**

The fixed-effect analysis test (t-contrast spanning columns 1-16) for a, on average, positive effect of visual stimulation, showed significant activation of visual cortex and LGN bilaterally (FWE=0.05). The fixed-effect analysis test (t-contrast spanning columns 17-32) for a, on average, positive effect of subject-specific increases in HMD was significant in visual cortex (FWE=0.05) and LGN bilaterally (FDR=0.05, cluster size=15 voxels), (figure 4).

**Third level DM4**

The random-effects analysis, the results of which can be generalised to the population of ON patients with changes in central vision during recovery, showed a positive effect of HMD in a more restricted region of central primary visual cortex and LGN (FDR=0.05, cluster size=15 voxels), (figure 5).
**Unaffected eye**

No significant intersession changes were observed upon stimulation of the unaffected during recovery (tested with DM1 and DM2), and there was no effect of HMD on brain activation elicited by stimulation of the unaffected eye. The t-contrast testing for regions more active during session 1+2+3 than during session 4 was not significant at FDR=0.05 (cluster size=15 voxels).

**Discussion**

Due to the different scotoma locations across patients with ON, applying a voxelwise analysis to whole brain activation in standard space can be problematic. Our patients represent a typical spectrum of acute ON patients with highly variable disease regarding location of scotomas and recovery rates. They therefore challenge the ability of any method to capture and characterise these differences, while retaining sensitivity to generalised phenomena that might be the basis of group analysis as required of treatment studies.

In this paper we compared three second-level designs, and went on to a use a third-level design. This third-level design is able to capture the realistic expectation of differential recovery between different regions and across different subjects. However, it can also be used to study generalised effects.

We will first review the inferences drawn from the three second level designs, before discussing the advantages of a three-level approach. With our first and simplest second level DM (DM1) no differences were found between sessions. Neither did we find any differences in brain activation when testing for greater activation during session 3+4 than during session 1+2. This is most likely due to the rather large variance in location of scotomas between patients. DM1 was also constructed omitting patients no. 10, 11, and 12, who had no changes in central vision, however, the results remained unchanged.

With DM2 it was possible to model some of the changes in visual activation during recovery by including subject-specific off-sets in BOLD and subject-specific deviations around session-wise mean HMD. With this model intersession changes in brain activation
were located to visual cortex and LGN. Though this result is generalisable to a similar population of ON-patients, this model is not fully satisfactory as it is assumed that equal HMD scores correspond to equal location and size of scotomas.

The significant effect of session-wise HMD changes in session 1 and the absence of such changes in sessions 2-4 reflect that the variation in HMD at sessions 2-4 are similar to those observed on the unaffected eye, i.e. the effect of variation in HMD at the later examinations is likely to be hidden in measurement error.

By applying a subject-specific HMD regressor as we did in DM3 a more realistic modelling is obtained. With DM3 it was possible to carry out a fixed-effect analysis to look for a mean effect of subject-specific changes in HMD on brain activation. Particularly in patients with acute ON a fixed-effect analysis is relevant as we do not expect the same voxels to be affected in all subjects, but on average, across subjects, we do expect to see an effect in visual cortex and LGN (figure 4).

In order to test for a general effect across all subjects with deficits in central vision, we also carried out a third level random-effects analysis. With this random-effects analysis changes were restricted to the most central part of visual cortex (figure 5). This is consistent with the clinical pattern, in which the very central part of the visual field is affected in most patients.

To illustrate that DM3 can indeed model subject-specific changes in scotoma location we have included figure 6. The bar plot shows the distribution of subject-specific HMD changes across the 16 subjects included in the DM3-analysis. The maximum effect is found at MNI coordinate (-6,-104, 12) and is seen to be largely driven by subject 7. When testing for an effect of HMD across the four examinations specific to subject 7, the maximum is found in an adjacent voxel (-8,-106, 12). As seen from figure 6, this location is on the upper bank of V1 corresponding to the lower part of the central visual field. The lower part of the central visual field is where patient 7 has a scotoma which markedly reduced over the
following examinations. As the changes in HMD for subject 7 is mainly driven by changes in the area covered by the visual stimulation in fMRI in our study, this subject is indeed the one where we would expect to see the best relation between changes in HMD and BOLD-signal over sessions.

We found no evidence of activation outside the visual cortex and LGN, thus we were not able to reproduce the findings by Werring et al. (2000) and Toosy et al. (2002). However, in their analyses no measures of visual function were included. In addition, our patients were followed from onset until recovery (0-6 months) in contrast to the patients in the studies by Werring et al. and Toosy et al., who were included 0.5-14 years after ON. The extra-occipital changes could manifest themselves at a time point later than six months and therefore could not be demonstrated in our patient-population. However, functional recovery of vision is greatest in this early period, and trials of new therapies are likely to focus on this early recovery window.

In a previous ROI-analysis (Korsholm et al., 2007) we showed reduced activation of LGN upon stimulation of the unaffected eye during recovery which was interpreted as a shift away from early compensatory changes established in the acute phase of ON. In the present study we could not replicate these findings; this may be due to different methods used in the two studies: To minimise partial volume effects our previous study was an ROI-analysis with LGN delineated on individual anatomical images before statistical analysis. In the present study a whole-brain voxel-wise analysis was used and the images were therefore spatially normalised to a template and smoothed before statistical analysis. This would lead to partial volume effects in small areas like LGN and for such reasons it is a less precise method.

In the present study the patients were included in the acute or early phase of the disease, however with some variance in disease duration at the time of the first MRI (range 9-42 days). As the first scan was obtained at inclusion and the second scan was obtained 14 days
after the first scan some overlap in time between first and second scan could not be avoided. However, in DM1+DM2 we tested for greater activation during session 3+4 than during session 1+2 thereby accommodating problems with overlap in time. In addition, in DM2 we tried to replace the four columns modelling deviations around the session-wise mean HMD with four columns modelling deviations around session-wise disease duration and no significant intersession differences were found, suggesting that, in our and in similar patient-populations, moderate inter-subject differences in disease duration do not play an important role. This might make large group studies of patients with ON, e.g. treatment studies, easier to conduct as time of inclusion is not as important as severity of disease. In conclusion, incorporating increasing amounts of prior information improved the model: With the first model no changes between examinations could be detected. With the second model some of the variation in disease severity could be modelled thereby allowing us to detect a change between examinations reflecting patient recovery. In the third model subject-specific scotomas could be modelled making this model more sensitive and the most realistic. With the latter two models it was demonstrated that the changes in brain activation during recovery from ON are located to the visual cortex and LGN, and that deviations in HMD seem a more relevant variable than disease duration. With the final model, one can extend the inferences from model DM3 to the general population of patients with ON. To be able to generalise in this way is important for future studies of therapeutic interventions and generalised conclusions about neuroplasticity following ON.

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Legends
Figure 1. Design matrices.

Design matrix 1 (DM1) consists of four columns modelling the four scanning sessions, where the first column indicates images acquired during the first examination, the second column indicates images acquired during the second examination etc.

The second DM (DM2) includes, in addition to the four columns indicating session images, one column per subject indicating scans belonging to each specific subject (modelling subject-specific offset in BOLD-signal), and four columns (one for each session) modelling deviations around the session-wise mean HMD.

In the third DM (DM3), 16 columns indicating the four scans belonging to each patient and 16 columns indicating each patient’s deviation around the mean HMD at the four visual field examinations are included to account for subject-specific offset in BOLD-signal and subject-specific dependence on HMD (due to different scotoma location). Three patients (patients no. 10, 11 and 12) are omitted from this analysis as they had no changes in central vision during recovery (see figure 2).

As HMD score was missing for the second examination of subject no. 6, DM2 and DM3 include a scan nulling regressor. For display purposes individual columns are scaled to have a standard deviation of 1 in all three DM.

Figure 2. Visual field deficits across examinations displayed subject-wise.

The figure shows visual field deficits displayed subject-wise with a row for each examination. In addition, the last column (C) of the figure indicates the area (±30°) covered by the autoperimetry (grey) and the area (±9°) covered by visual stimulation in fMRI (red).

It is seen that patients no. 10, 11 and 12 exhibit no changes in central vision (in the area covered by fMRI) during recovery, whereas in all the other patients the central part of the visual field changes during recovery.
**Figure 3a. Differences between mean of sessions 3+4 and mean of sessions 1+2.**

Testing with DM2 for significant differences between mean of session 3+4 and mean of session 1+2, the visual cortex and LGN bilaterally are activated (FDR=0.05, cluster size=15 voxels).

**Figure 3b. An extra effect of HMD is seen in session 1.**

With the t-contrast testing for an extra effect of HMD deviation from the mean session HMD in DM2, the central part of visual cortex is activated in session 1 (greater BOLD-signal correlated with higher HMD) (FDR=0.05, cluster size=15 voxels).

**Figure 4. Fixed-effect analysis of HMD slope.**

Testing for significant subject-specific increased BOLD-responses correlated with subject-specific increased HMD in DM3 (fixed-effect analysis), visual cortex and LGN bilaterally are activated (FDR=0.05, cluster size=15 voxels).

**Figure 5. Random-effects analysis of HMD slope.**

The random-effects analysis applied to the subjects with changes in central vision during recovery shows a positive effect of HMD in central visual cortex and LGN (FDR=0.05, cluster size=15 voxels).

**Figure 6. Modelling of subject-specific scotomas.**

The bar plot shows the effect of subject-specific HMD changes across the 16 subjects included in this analysis (patients no. 10, 11 and 12 are omitted from this analysis). The maximum effect is found at MNI coordinate (-6,-104,12) and is seen to be largely driven by subject 7. When testing for a change of HMD across the four sessions specific to subject 7, the maximum was found in an adjacent voxel (-8,-106,12). As seen from the image in
figure 6, this is on the upper bank of the calcarine sulcus (CS) (here displayed on a T1 weighted image of the brain of subject 7 normalised to MNI space). This corresponds to the lower part of the visual field which is exactly where patient 7 has a scotoma that markedly reduced over the following examinations.

Table 1. Clinical characteristics of the patients.

Abbreviations: F=female; M=male; R=right; L=left; VA=visual acuity; AE=affected eye; UE=unaffected eye; ON=optic neuritis; RRMS=relapsing remitting multiple sclerosis.

VA is shown for first, second, third and fourth examination. CF=counting fingers; HM=hand movements; S=silhouette (patients able to silhouette of a person standing 1 m away); NLP=no light perception.
Table 1. Clinical characteristics of the patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Affected eye</th>
<th>Snellen VA (AE)</th>
<th>Snellen VA (UE)</th>
<th>Diagnosis</th>
<th>Days from onset to inclusion</th>
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<tbody>
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5. **Figure3a**

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5. Figure 3b
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5. Figure
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Patient #

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