Neuro-Ophthalmology, 2013; 37(5): 192–197

ISSN: 0165-8107 print / 1744-506X online
DOI: 10.3109/01658107.2013.819581

informa
healthcare

ORIGINAL ARTICLE

Functional Magnetic Resonance Imaging in Patients with the Wet Form of Age-Related Macular Degeneration

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ABSTRACT

The study is designed to determine the relationship between the progress of the wet form of age-related macular degeneration and the activity of the visual cortex examined using functional magnetic resonance imaging. Ten patients with the wet form of age-related macular degeneration (9 female and 1 male) with a mean age of 74.7 years (58–85 years) at various stages of bilateral involvement of the disease were included. Patients did not suffer from any other ocular nor neurological disease. All the patients underwent functional magnetic resonance imaging examinations with stimulation of both eyes using a black-and-white checkerboard of size 25.8×16.2 degrees. The group was compared with a group of healthy subjects with an average age of 54.1 years (45–65 years). For statistical evaluation, the Mann-Whitney *U* test was used. Comparing the extent of visual cortex activations we found a statistically significant difference between both the groups ($p = 0.0247$). However, the dependence of functional magnetic resonance imaging activity on visual acuity was not statistically significant ($p = 0.223$). We conclude that in patients with the wet form of age-related macular degeneration, lower functional magnetic resonance imaging activity of the visual cortex was found compared with the control group of healthy subjects. Dependence of functional magnetic resonance imaging activity on visual acuity was not statistically significant.

Keywords: Brain fMRI, ganglion cells, wet AMD

INTRODUCTION

Age-related macular degeneration (AMD) is a sign of damage to choroidal vessels and Bruch membrane conditioned by age, genetic, and external factors. Lipoid infiltration and degeneration of collagen and elastic tissue affect mainly the richly vascularised macula. In exudative AMD, the choroidal capillary damage leads at elevated intracapillary pressure to proliferation of neovascularisation that penetrates through the defects in Bruch membrane under the pigment epithelium or eventually further, between the pigment epithelium and photoreceptors, and causes exudation and haemorrhage, and detachment of the pigment epithelium and photoreceptors. All of these processes result in not only destruction of the pigment epithelium and photoreceptors but in damage to bipolar and ganglion cells as well.

In our previous paper, using functional magnetic resonance imaging (fMRI), we showed changes in the visual cortex in primary damage to retinal ganglion cells and their axons in hypertensive glaucoma with defects in visual fields.[1]

The aim of this study was to use fMRI to investigate visual centres in patients with exudative form of AMD, where the retinal ganglion cells are damaged secondarily. We want to prove our hypothesis that there is a difference in fMRI activation between

Received 15 May 2013; revised 7 June 2013; accepted 10 June 2013; published online 19 September 2013
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patients with wet AMD and control group and also that a relationship between visual acuity and fMRI activity exists.

METHODS

Subjects

Criteria for inclusion: We included 10 patients with the wet form of AMD (9 female and 1 male), mean age 74.7 years (58–85 years) with various degrees of bilateral impairment. None of our patients underwent anti-vascular endothelial growth factor (VEGF) therapy. Patients had no other ocular or neurological disease. The group was compared with a group of 9 healthy subjects with an average age of 54.1 years (45–65 years). Minimum time of stable visual acuity was 3 years (Tables 1 and 2). None of our patients had any visible structural changes in cerebral tissue found in standard structural MRI examination.

Functional MRI

All fMRI measurements were performed on a Philips Achieva TX SERIES with a magnetic field strength of 3 Tesla. A standard 32-channel SENSE head radiofrequency (RF) coil was used for scanning. Optical stimulation for fMRI was performed with a commercially available Eloquence (InVivo) stimulus system. Size of the stimulation area consisting of black-and-white checkerboard (see Figure 1), was 25.8×16.2 degrees. Due to the low visual acuity of one or both eyes, the examination was performed after simultaneous stimulation of retinas of both eyes. For measuring fMRI with the BOLD (blood oxygen level dependent) technique, the gradient-echo EPI (echo-planar imaging) sequence was used with the following parameters: TE = 30 ms, TR = 3 s, flip angle of 90°. The measured volume contained 39 continuous 2-mm-thick slices. The voxel size was $2 \times 2 \times 2$ mm (field of view [FOV] = 208×208 mm, matrix 104×104, SENSE factor 1.8). During fMRI scanning, a checkerboard of alternating black and white was projected to all the subjects (see Figure 1). This alternation is a colour inversion with a frequency of 2 Hz. During the resting phase, a static crosshair situated in the centre of the visual field was projected and all subjects were instructed to look at the middle of the visual stimulation field. Each measurement consisted of a block scheme with five active intervals lasting 30 seconds (10 dynamic scans) and five resting intervals of equal duration. In total, every measurement included 100 dynamics and took 5 minutes.

The fMRI evaluation was performed with SPM8 software. During the preprocess, the data were motion corrected (realignment) and corrected for time-shift of individual slices (slice timing), smoothed with a Gaussian filter with FWHM (full width half maximum) $6 \times 6 \times 6$ mm, and finally normalized into the MNI_152 space. On the level of individual subjects, the general linear model statistic with canonical HRF (haemodynamic response function) applied to the block scheme of stimulation was used.

TABLE 1 Control group.

No.	Sex-Age	VA RE/LE	fMRI activation (number of voxels)
1	F-45	1.0/1.0	7 100
2	F-48	1.0/1.0	9 544
3	F-50	1.0/1.0	11 650
4	F-50	1.0/1.0	6 815
5	F-50	1.0/1.0	8 358
6	F-61	1.0/1.0	5 973
7	F-65	1.0/1.0	8 060
8	M-58	1.0/1.0	6 809
9	M-60	1.0/1.0	7 878

TABLE 2 Patients with AMD.

No.	Sex-Age	VA RE/LE	fMRI activation (number of voxels)
1	F-58	0.04/0.03	2 630
2	F-65	0.3/0.1	6 870
3	F-67	0.2/0.7	8 600
4	F-69	0.1/0.8	5 450
5	F-79	0.001/0.02	3 917
6	F-79	0.5/0.001	2 910
7	F-81	0.05/0.3	1 530
8	F-85	0.1/0.02	2 013
9	F-85	0.001/0.1	8 517
10	M-79	0.09/0.09	28

The fMRI activities in control person no. 4 and in patient no. 1 are shown in Figures 2 and 3, respectively.

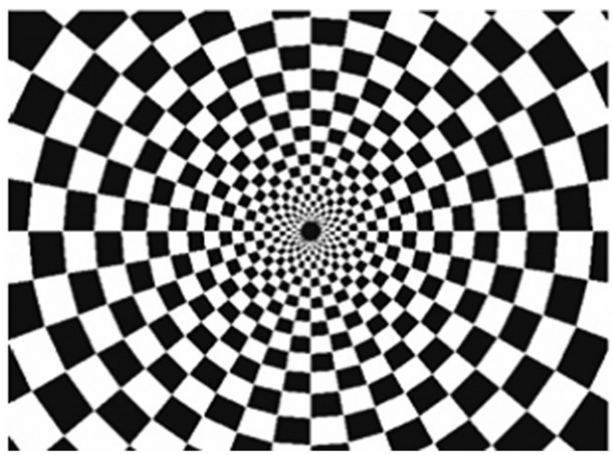

FIGURE 1 The black and white checkerboard (BW) used for stimulation (size: 25.8×16.2 degrees). During the stimulation, the checkerboard picture was alternated with its inversion with a frequency of 2 Hz. *Note*: Figures 1–6 of this article are available in colour online at www.informahealthcare.com/oph.

Statistical maps were thresholded at $p = 0.05$ with FWE (familywise error-rate) correction. For the statistical evaluation of group differences, the Mann-Whitney *U* test was used.

Ophthalmological Examination

All patients included in the study have been followed-up in our clinic for at least 5 years. Visual acuity was determined at the time of examination using the ETDRS charts and recorded as the decimal acuity (Tables 1 and 2). Refractive error was in the range of ±2 dioptres.

RESULTS

Position of subjects within the head coil was adjusted before start of fMRI in addition to ensuring that their visual field completely covered the stimulation checkerboard. After fMRI examination, all our subjects were asked to confirm whether the stimulation checkerboard was clearly visible and all of them responded positively.Comparison of the extent of visual cortex activation using Mann-Whitney *U* test showed a statistically significant difference between both the groups ($p = 0.0247$) in the number of an activated voxels. All measured values are shown in Tables 1 and 2. The fMRI activities in control person no. 4 and in patient no. 1 are shown in Figures 2 and 3, which demonstrate an example of difference in fMRI activation in case of healthy control and patient with AMD, respectively.

The difference in fMRI activation extent (number of significantly activated voxels) in the control and patients groups is shown in Figure 4. Wider box plot of the patient group is caused by higher variability of the visual acuity compared with controls.

However, the relationship between fMRI activity and average visual acuity was not statistically significant ($p = 0.223$), as shown in Figure 5.

Because of age difference between both studied groups, age dependence analysis was also performed. After this adjustment by multinomial regression analysis, the number of voxels in patient group was about 2144 lower than in controls. However, due to the small sample size, this difference is not statistically significant ($p = 0.267$), as demonstrated in Figure 6.

DISCUSSION

FMRI of the visual cortex is not a frequent clinical examination and there are only few studies dealing with fMRI findings in AMD. We included only patients with the wet form of AMD in our set of patients, i.e., the form of AMD that is in most patients and is difficult to treat, and the prognosis of which is generally worse than that of the dry form.

The decline in activity of visual cortex can result from lower input from retinal ganglion cells. The work of Boucard et al.[2] brings evidence in favour of this after investigation of grey matter density in visual

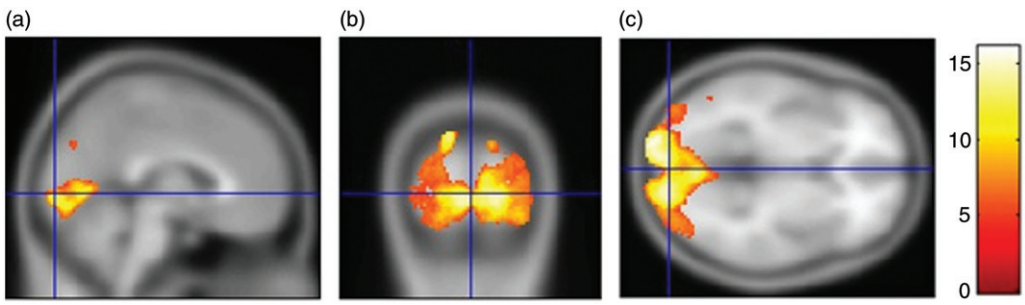

FIGURE 2 Usual activation of the visual cortex in control subject (no. 4). Findings on the sagittal (a), coronal (b), and transverse (c) sections are normal.

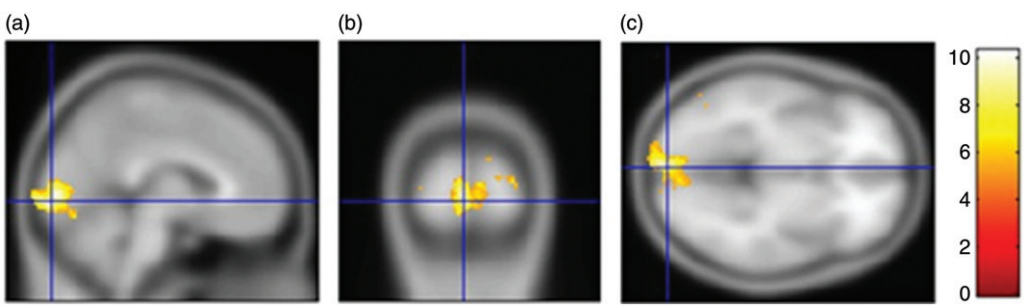

FIGURE 3 Activation in visual cortex of patient no. 1 (female, age 54, VA RE 0.04, VA LE 0.03). The sagittal (a), coronal (b), and the transverse (c) sections show a significant decrease in fMRI activity.

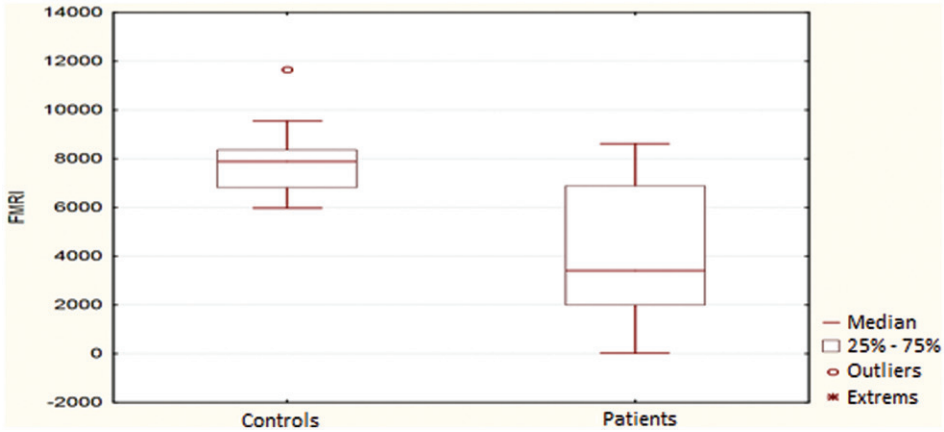

FIGURE 4 Box plot shows the difference in fMRI activity (expressed in the number of activated voxels) between the control group and the AMD patients.

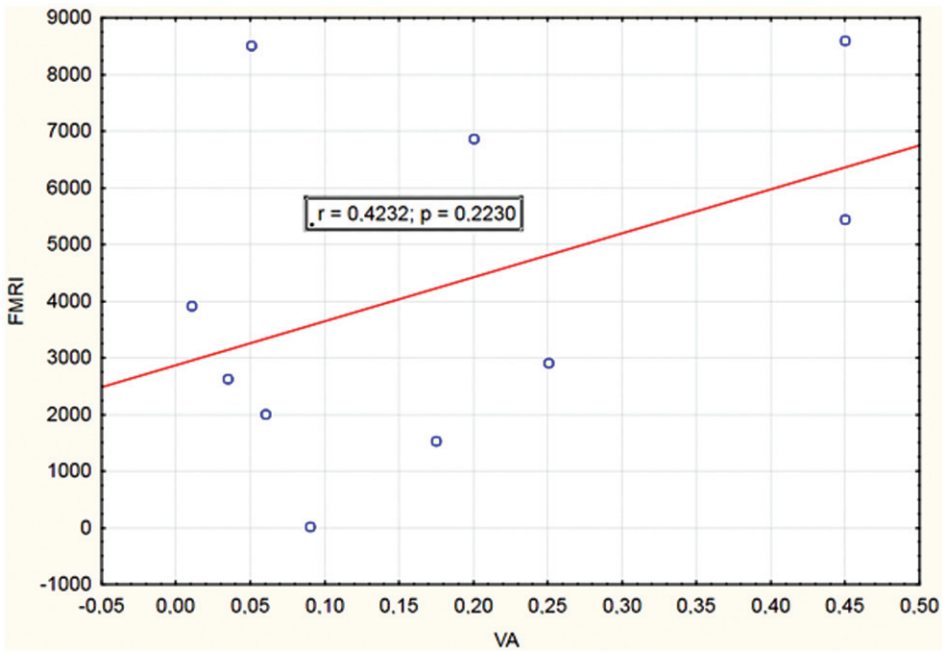

FIGURE 5 The graph shows the relationship between fMRI activity (expressed in the number of activated voxels) and the average visual acuity of both eyes.

cortex by means of MRI in glaucoma patients and patients with AMD. The main finding of their study is that visual field defects caused by longstanding retinal pathology due to glaucoma and AMD are associated with a reduction in grey matter density in occipital cortex. Moreover, in both the AMD and glaucoma patients, the location of the grey matter density reduction corresponded with the approximate visual field defect projections in visual cortex. The more central scotomas of the AMD subjects correlated with a reduction located more posteriorly in occipital cortex, corresponding to the location of the foveal representation in visual cortex. The difference was more pronounced in the left hemisphere. In agreement with this latter finding, the sensitivity deviation map shows a macular defect that was more pronounced in the right visual fields. Their results suggest that retinal visual field defects acquired later in life can lead to retinotopically specific grey matter density reduction in the visual cortex.

Sunnes et al.[3] examined fMRI in a female patient with geographic macular degeneration and similarly found loss of stimulation to the cortical areas representing the site of the atrophic lesion.

None of our two patient sets had any brain pathology found by structural MRI, in agreement with the finding by Grosso et al.,[4] who performed MRI examinations in 1684 AMD people aged 51–72 years and found no statistically significant association with brain changes.

Nguyen et al.[5] used light flashes as a paradigm in their first study. They proved significant haemodynamic response, even in the most damaged macula, in most of AMD patients. This finding has a basis in

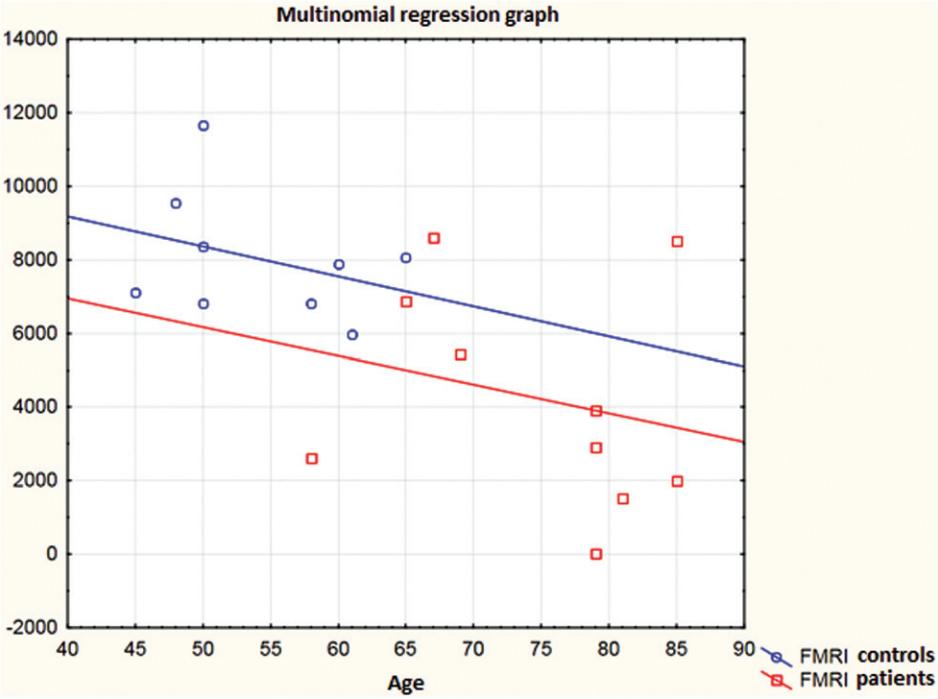

FIGURE 6 Multinomial regression analysis shows decrease of fMRI activation in patients with AMD despite of aging.

stimulation of photoreceptors by a light flash in the entire retina. In the following work, the same authors[6] proved a decrease of fMRI activity after stimulation of damaged eyes. Light flash stimulation activates photoreceptors of the entire retina and the response of the damaged part may not be become evident in the fMRI activity.

Baseler et al.[7] examined one patient with wet AMD before and after application of three injections of ranibizumab using fMRI. They used full-field flickering (6 Hz) white light alternating with a uniform grey background (18 seconds on and 18 seconds off) as visual stimulation during fMRI examination. The area of visual cortex activated increased significantly after the first treatment to include more posterior cortex that normally receives inputs from lesioned parts of the retina. Subsequent treatments yielded no significant further increase in activation area.

We used checkerboard stimulation, in which no brightness change of the stimulus occurs (Figure 1). The reason of this paradigm is targeted stimulation of parvocellular and magnocellular channels.

Our work had to deal with several shortcomings. The first was the age disparity of patients. AMD occurs mainly in patients older than 60 years. In our group, 6 patients were older than 70 years and the mean age of patients was 74.7 years. FMRI examination is time-consuming and takes up to 1 hour. It is difficult to persuade healthy people of similar age to undergo this examination. That is why our control group examinations were performed on persons of 54.1 average age. Another pitfall in the group of AMD patients was decreased central visual acuity. Most patients had a problem with central fixation with the diseased eyes. Therefore, we carried out tests on both eyes simultaneously. To distinguish subsequently the effect of each eye in fMRI activity was not possible. Therefore, we compared even in the statistical evaluation the results after stimulation of both eyes, although we know from our experience that surprisingly fMRI activity may have a higher value after stimulation of one eye than after stimulation of both of them.

In AMD, the relationship between retinal pathology and cortical degeneration might be slightly more indirect, in that photoreceptor damage may first lead to retinal ganglion cell loss. It has been shown that the retinal ganglion cell count is significantly lower in AMD than in control eyes, and more in wet AMD than in geographic atrophy (GA). In comparison with the control eyes, in GA a significant loss of retinal ganglion cells by 30.7% was observed, even when the number of ganglion cells neurons did not significantly differ.[8] In another study, wet AMD eyes had 47% fewer retinal ganglion cells than control eyes.[9] In agreement with the idea that cortical atrophy is associated with a reduction in retinal ganglion cell number and optic nerve damage, a voxel-based morphometry study reported abnormally reduced grey matter volume at the occipital poles of a group of human albinos.[10]

It can be assumed that, on the basis of possible feedback control of the visual centres of the brain, a lesion is localized at the level of the photoreceptors, which have neurotransmitter glutamate.[11,12] Glutamate as a neurotransmitter acts also on retinal ganglion cells,[13] lateral geniculate nucleus, and visual

cortex. To restore the number of action potentials entering vision centres, there are two possible options. The first is an increase in the washout of neurotransmitters, the second option is to restrict their absorption or metabolism from the synaptic cleft. Both lead, at prolonged stimulation of *N*-methyl-D-aspartate receptors, to a cascade of events resulting in the apoptosis of the cells at the end. The damage to retinal ganglion cells may not occur only in this way. Primary exudative retinal process may damage directly not only photoreceptors, but other cells as well, including retinal ganglion ones.

If retinal ganglion cells are damaged, transsynaptic neuronal degeneration and damage to ganglion cells of the visual cortex can be expected. Studies dealing with high-tension glaucoma describe the same phenomenon.[1,14,15]

One possible and currently implemented method of exudative AMD treatment is intravitreal application of anti-VEGF. Brar et al.,[16] who studied the effect of bevacizumab on retinal ganglion cells in animal models, found that the effect of retinal ganglion cell protection against oxidative stress was eliminated by bevacizumab. Also Avci et al.[17] demonstrated in rabbits that intravitreal application of bevacizumab and pegaptanib sodium caused a significant increase in apoptotic activity of retinal photoreceptors. On ground of this information, the possibility of further damage to retinal ganglion cells by anti-VEGF drugs and thus possible subsequent damage to ganglion cells of the visual cortex of brain cannot be completely ruled out. Therefore, we plan in our next work to concentrate on follow-up of patients with wet form AMD treated with anti-VEGF.

CONCLUSION

In patients with the wet form of AMD, lower fMRI activity of the visual cortex was found compared with the control group of healthy subjects. Dependence of fMRI activity on visual acuity was not statistically significant.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Note: Figures 2–6 of this articles are available in colour online at informahealthcare.com/oph

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