Illusory Stimuli Can Be Used to Identify Retinal Blind Spots

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Illusory Stimuli Can Be Used to Identify Retinal Blind Spots

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Background. Identification of visual field loss in people with retinal disease is not straightforward as people with eye disease are frequently unaware of substantial deficits in their visual field, as a consequence of perceptual completion (“filling-in”) of affected areas. Methodology. We attempted to induce a compelling visual illusion known as the induced twinkle after-effect (TwAE) in eight patients with retinal scotomas. Half of these patients experience filling-in of their scotomas such that they are unaware of the presence of their scotoma, and conventional campimetric techniques cannot be used to identify their vision loss. The region of the TwAE was compared to microperimetry maps of the retinal lesion. Principal Findings. Six of our eight participants experienced the TwAE. This effect occurred in three of the four people who filled-in their scotoma. The boundary of the TwAE showed good agreement with the boundary of lesion, as determined by microperimetry. Conclusion. For the first time, we have determined vision loss by asking patients to report the presence of an illusory percept in blind areas, rather than the absence of a real stimulus. This illusory technique is quick, accurate and not subject to the effects of filling-in.

INTRODUCTION

The induced twinkle after-effect (TwAE), first described by Ramachandran and Gregory in 1991, is a compelling illusion observed in normally sighted subjects who are deprived of visual input to a restricted area of retina using an artificial scotoma [1, 2]. Subjects adapt to a dynamic noise stimulus containing a small mean luminance region (the artificial scotoma). When the adaptation pattern is replaced with a uniform screen, the area that contained noise appears blank, while the area formerly occupied by the artificial scotoma appears to contain “twinkling” noise: the TwAE.

The locus of the TwAE within the visual pathway is not known. Ramachandran and Gregory proposed that “whatever mechanism is responsible for this induction of twinkle...is unlikely to be very different from the process causing the filling in of the scotoma in the first place” [1]. Electrophysiological and imaging studies indicate that filling in is likely to be mediated by higher level cortical areas [3–5]. Hardage and Tyler identified several key differences between filling-in and the TwAE including the larger area over which the TwAE can be observed (at least 20º, compared to 1.5º for filling-in), the absence of any chromatic component to the TwAE, and the absence of the TwAE when the temporal frequency of the dynamic noise is below 10Hz [6, 7]. Functional MRI experiments add weight to their hypothesis that the effect is mediated by an inhibitory rebound of high level large receptive fields rather than a persistence of filling in [8].

The most common cause of retinal scotomas, and indeed the principal cause of blindness, in Europe and the USA is age-related macular disease (AMD) [9, 10]. In advanced AMD central visual field is lost through an atrophic or neovascular process [11]. Whilst atrophic forms of macular disease remain untreatable, angiostatic and antiangiogenic agents can be used to retard the development of neovascularisation in AMD [12, 13]. The gold standard technique for identifying scotomas in people with AMD is retinal specific microperimetry: a time consuming procedure requiring the skilled use of specialised equipment limited to few clinical or research centres. Conventional perimetric tests, whilst more widely available, are not appropriate due to the poor fixation stability [14, 15] and noncentral fixation locus [16–18] of many people with retinal disease. Campimetric techniques involve patients identifying regions of absence within a homogenous field [19–21], but perceptual completion may limit the ability of many patients to identify visual field defects using this technique. Those at risk of scotoma development are given a simple grid chart to observe on a daily or weekly basis [22]: however this technique has poor sensitivity, again due to perceptual completion over the scotoma [23–26].

Given the difficulty of identifying retinal scotomas in people who fill-in their blind spot, we sought to determine whether the TwAE might be adapted to identify scotomas in people with macular disease. To this end, we identified eight patients with central and paracentral scotomas caused by macular disease. The location, shape and extent of these scotomas was carefully mapped with retinal specific microperimetry. Next we had subjects view a large field of dynamic (60Hz) noise followed by a homogenous grey test screen in thirty second cycles (figure 1). Patients were asked to fixate a cross that was continuously present, although eye movements during the noise phase were unimportant since the stimulus was a uniform, spatially extensive noise field. During the test phases, subjects were asked to identify areas of any perceived anomaly in the blank screen by tracing around them with a stylus on a touch-sensitive screen mounted over the computer monitor. All participants were unfamiliar with the TwAE before the study.

The most common cause of blindness is age-related macular degeneration, followed by diabetic retinopathy and glaucoma [1]. In the USA, estimated new cases of AMD are 200,000 per year with the condition affecting 1.7 million people over the age of 50 years [11]. Disease progression in AMD is described into dry form (ATM) and wet form (wet AMD). Dry AMD is the most common form of AMD, accounting for about 90% of cases and includes the milder forms of the disease. It is characterized by the presence of drusen, which are yellow or yellowish deposits under the retina. The presence of large drusen is associated with a higher risk of developing wet AMD. Wet AMD, which affects about 1% of the population over the age of 50 years, is characterized by the development of neovascularisation in the macula. Neovascularisation is a new blood vessel growth that can cause scarring and vision loss. Neovascular AMD is the principal cause of vision loss in AMD [11]. Neovascular AMD is initiated by the release of vascular endothelial growth factor (VEGF) from the retina into the subretinal space. VEGF is a protein that stimulates new blood vessel growth and is associated with AMD. VEGF binds to its receptor on the surface of cells, and this binding triggers a signal that stimulates cell proliferation and migration. This process continues until the cells reach a point where they are in contact with each other, forming a new blood vessel. Once the new blood vessel is formed, it can leak fluid and blood into the retina, causing vision loss. Currently, the only treatments for wet AMD are anti-VEGF agents, such as ranibizumab (Lucentis) and aflibercept (Eylea). These agents work by blocking VEGF from binding to its receptor, preventing new blood vessel growth and reducing exudation. The treatment is typically administered by an ophthalmologist in an office setting, and the patient is asked to return every few months to repeat the injections. This is an open-access article distributed under the terms of the Creative Commons Public Domain declaration which stipulates that, once placed in the public domain, this work may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose.

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RESULTS
Four of the eight subjects reported that the noise pattern appeared to fill in across their scotoma. Six of the eight experienced a TwAE during the test phases and could easily trace around its perimeter. The effect was variously described as an “aberration”, a “twinkling”, or most descriptively, as a “moving cumulus cloud” (table 1). Of the four subjects who experienced filling-in (who would not respond to conventional campimetric tests), three experienced the TwAE.

All subjects who experienced the TwAE were able to fully describe the boundary of their scotoma within the test phase. For the two subjects who did not experience the TwAE immediately, the size of each square element in the noise pattern was varied to have side of between 0.05° and 0.8°, and the stimuli were presented many times under these different conditions. Neither subject reported any TwAE even after these manipulations.

Figure 2 compares the location of visual field loss defined by microperimetry with the location of the TwAE mapped by the observers. The white lines show the boundary of scotomas defined by microperimetry. The red lines show the geometric mean of the apparent perimeter of the TwAE mapped by the observers. The shaded grey areas indicate 95% confidence intervals on this mean, based on a minimum of five repetitions. There was excellent agreement between both measures of the scotoma.

DISCUSSION
We have demonstrated that the induced TwAE can be used to map retinal scotomas accurately in some patients with central and paracentral scotomas arising from macular disease. Our findings build on earlier anecdotal reports of the TwAE in patients with retinal scotomas [2,27], and provide the first quantification of the TwAE in patients with eye disease. We believe that this is the first time patients have been asked to report the presence of an illusory stimulus, rather than the absence of a real stimulus, to determine the area of non-seeing retina.

Although it is difficult to determine exactly what participants perceived during the test phase, their eloquent and unprompted descriptions (table 1) indicate that they were experiencing the TwAE. No participants perceived any irregularity on a uniform background.

Table 1. Patient characteristics and results

<table>
<thead>
<tr>
<th>Initials</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>Visual acuity (logMAR)</th>
<th>Filling in</th>
<th>Description of TwAE</th>
</tr>
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<tr>
<td>PF</td>
<td>82</td>
<td>AMD:CNVM</td>
<td>0.92</td>
<td>No</td>
<td>‘aberration’</td>
</tr>
<tr>
<td>KJ</td>
<td>78</td>
<td>AMD:CNVM</td>
<td>1.50</td>
<td>Yes</td>
<td>No effect</td>
</tr>
<tr>
<td>SP</td>
<td>35</td>
<td>Traumatic maculopathy</td>
<td>0.32</td>
<td>No</td>
<td>‘twinkling’</td>
</tr>
<tr>
<td>EP</td>
<td>79</td>
<td>AMD:CNVM</td>
<td>0.82</td>
<td>No</td>
<td>‘dark patch’</td>
</tr>
<tr>
<td>TK</td>
<td>77</td>
<td>AMD: GA</td>
<td>0.30</td>
<td>Yes</td>
<td>‘mistiness’</td>
</tr>
<tr>
<td>GW</td>
<td>69</td>
<td>AMD&amp;IPCV</td>
<td>1.36</td>
<td>Yes</td>
<td>‘cumulus cloud’</td>
</tr>
<tr>
<td>VA</td>
<td>76</td>
<td>AMD:CNVM&amp;GA</td>
<td>1.06</td>
<td>Yes</td>
<td>‘grey shimmer’</td>
</tr>
</tbody>
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neurones have very large receptive fields, frequently extending 10°.

Figure 2. Scotomas identified by the dynamic after effect. Red line represents mean scotoma boundary; grey region indicates 95% confidence interval of estimate. White line represents boundary of dense scotoma identified from microperimetry assessment (except subject TK where scotoma is larger than the area measured using microperimetry, and GW and MK where only the upper boundary of the scotoma could be identified). Where present, white cross indicates centre of fixation. EP, PF: microperimetry performed using Rodenstock SLO-101 scanning laser ophthalmoscope. TK, SP, GW, MK: microperimetry performed using Nidek MP-1 microperimeter.

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Unlike retinal ganglion cells, MT+ neurons do not show any preference for the eye of stimulation, so should the effect be cortical in origin we would expect the TwAE to transfer between the eyes. Whilst Hardage and Tyler [6] found no interocular transfer of the TwAE in normally-sighted observers, recent work by Morgan and colleagues demonstrates interocular transfer of a facilitatory effect induced under similar circumstances to the TwAE [32]. To determine whether the effect could be transferred in patients with visual field loss, we performed further examination of one of our subjects who had highly incongruous scotomas: MK. We did not attempt to induce a transferred TwAE in any other observer. MK’s right eye had small areas of paracentral scotoma caused by geographic atrophy, whereas his left eye had a large central scotoma. We presented the adapting stimulus to his poorer (left) eye whilst occluding the right eye. On a given signal, the test screen was displayed, the left eye was uncovered and the right eye was covered. MK then reported a robust TwAE, but only in the areas corresponding to the locations of scotoma in his left eye (figure 3).

The reason that MK experienced the induced twinkle only in the areas of scotoma in his left eye (and not within the region of scotoma in the right eye) may explain the lack of transfer experienced by control subjects. We speculate that rivalry is introduced between the salient test screen in one eye and the TwAE in the other. Such rivalry could suppress the presence of the TwAE in normally-sighted observers and limit inter-ocular transfer of the TwAE. However, patient MK’s vision contains regions where no retinal input is present in the test eye: that is, within the scotoma. These regions of sighted and non-sighted vision in the test eye set up “piecemeal” rivalry across his visual field[33]. Under these conditions, perception of the test screen dominates in sighted areas of the test eye but the TwAE dominates perception in the non-sighted areas of the test eye. We conclude that the TwAE is likely to manifest within MT+.

It is not clear why two of our participants (KJ and VA) did not experience a TwAE. No apparent differences were identified in their scotoma properties, duration of disease, fixation locus or grey field without the period of adaptation, indicating that the effect described was not simple mapping of the apparent scotoma boundary. As the adaptation and test stimuli were of equal mean luminance, it is unlikely that patients were reporting a simple luminance afterimage during the test phase.

Three of our participants did not experience perceptual completion over their scotomas yet did experience a TwAE. This observation supports Hardage and Tyler’s proposal that filling-in and the TwAE are complementary mechanisms. More encouragingly, three of our patients described the TwAE despite experiencing completion of the dynamic noise pattern and not responding to a standard campimetric test.

What is the mechanism driving this phenomenon? As the TwAE cannot be induced at temporal frequencies of less than 10Hz, a location within the magnocellular pathway seems likely [7]. The large area over which the TwAE can be experienced (up to 20°) suggests that a likely locus would be a later area of the visual pathway such as the middle temporal complex (MT+) where neurones have very large receptive fields, frequently extending 10° into the ipsilateral visual field[28]. Further, MT neurones in the macaque have been shown to respond to stimuli presented well outside their classical receptive fields (by distances of >15°) even without stimulation within the receptive field[29]. An alternative candidate location is far earlier in the visual pathway; j-type retinal ganglion cells are known to have extensive horizontal connections and which respond preferentially to dynamic stimuli [30,31].

Figure 3. Transferred after image for subject MK. Red line indicates mean scotoma boundary assessed using microperimetry in the right eye but paracentral areas of relative scotoma were present.

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visual function. Nor was it related to the occurrence of filling in—
one did and one did not experience filling-in. In order to determine whether visibility of the noise was the limiting factor, 
the size of the inducing noise stimulus was varied by a factor of 16: 
from squares of side 0.05° of visual angle to 0.8°. One explanation 
could be that these two patients were unable to divide their attention 
between the fixation point and the region of the TwAE. People with 
macular disease are known to adopt a preferred retinal locus (PRL) 
for fixation in peripheral retina and, in some respects, reference their 
eye movements to this “pseudofovea.” [16, 17, 34]. It is not clear 
whether plasticity exists in the primary visual cortex in humans with 
macular disease [35, 36]. It is possible that different levels of cortical 
reorganisation had occurred in patients who did experience the 
TwAE compared to those who did not.

Unlike subjects with artificial scotomas [27] our patients did not 
 systematically underreport the size of their scotoma. Although the 
95% confidence interval on our estimates of scotoma size are 
large, it took under three minutes to perform five trials on the 
TwAE test, compared to around 20 minutes per eye for microperimetry. If determining scotoma size is critical then further 
 repetition of our test would reduce the size of the confidence 
 intervals. Alternatively, the TwAE test could be used as a screening 
test to identify scotomas prior to detailed microperimetric testing 
within this region. It should be noted that confidence limits of 
scotoma size are not produced by commercially available 
 microperimeters, yet quantification of uncertainty in the measure- 
ment of the scotoma boundary are critical for assessing changes in 
 scotoma area during disease progression or following treatment.

We do not aim to suggest that the limited number of subjects in 
 this study is sufficient to imply that this type of test should be 
 introduced into routine clinical practice: nor do we suggest that a test 
 with sensitivity of 75% is suitable for screening for this condition. 
 However, we feel that the concept of using illusory stimuli to 
determine the absence of function is appealing and novel. We 
 suggest that similar illusory techniques could be used by colleagues in 
clinical ophthalmology and other areas of the cognitive neurosciences to determine and investigate the absence of function.

MATERIALS AND METHODS

All subjects had age-related macular disease causing a central 
scotoma diagnosed by a consultant ophthalmologist (other than SP 
who had a traumatic maculopathy caused by a road traffic 
accident). No subjects had any other eye disease or any history of 
nerve disease. All patients had binocular scotomas except SP, who had one eye enucleated in adulthood due to ocular 
trauma. The study was approved by the Camden & Islington PCT 
Local Research Ethics Committee of the UK National Health 
Service. Subjects gave their informed consent prior to data 
collection and the study conformed to the tenets of the Declaration 
of Helsinki.

Microperimetry

Microperimetry was performed using either the SLO-101 
Scanning laser ophthamoscope (Rodentstock, Germany) or the 
Nidek MP-1 microperimeter (Nidek, Italy). In both cases, subjects 
were asked to observe a central fixation cross whilst retinal specific 
perimetry was performed of the central retina. Stimuli were 
Goldmann III targets presented against a dark background. In the 
MP-1, the automated 10-2 strategy was used to measure threshold 
retinal sensitivity. In the SLO, manual perimetry was performed at 
one intensity (200 cd/m²). In both cases microperimetry maps 
were automatically superimposed on the retinal image by tracking 
a retinal landmark.

Determining the TwAE

Stimuli were created on an Apple computer using custom functions 
written in Matlab (v.7.3; Mathworks, Natick, MA) based on elements of 
the Psychophysics toolbox [37] [38] and were presented on an 18” 
CRT monitor (Ultrascan P991; Dell, Round Rock, TX) with a 60Hz 
refresh rate. The peak screen luminance, measured using a photom- 
eter (Minolta CS-100, Konica Minolta, Japan) was 145 cd/m² when 
viewed through the touch screen panel.

Subjects observed the stimuli monocularly with their better eye, 
whilst the contralateral eye was occluded with an opaque eye 
patch. Subjects sat 50cm from the computer screen. Appropriate 
refractive correction was worn using full-aperture trial lenses.

The experimental procedure is illustrated in Figure 1. First, a 
mean luminance grey screen was presented for a period of 15 
seconds. Subjects were asked to identify any missing or irregular areas on the screen. Next, dynamic greyscale noise was 
presented for a period of 15 seconds. Each element of noise was 
square, of side 12 pixels (29 min arc) and was randomly assigned 
a new luminance value between 1 and 145 cd/m² every 17 msec 
(60Hz). Finally, an isoluminant grey screen was presented for 15 
seconds. Subjects were asked to draw around the edge of any 
area of irregularity with a stylus using a touchscreen (MagicTouch 
KTMT-1921; Keytec Inc., Garland, TX). Screen coordinates of 
the stylus location were recorded at 60Hz. A central fixation cross 
of arm length 1° was displayed throughout, and subjects were 
asked to maintain fixation on this point. The adaptation and test 
phase was repeated to a total of five presentations.

Additional testing for inter-ocular transfer of the TwAE was 
performed on subject MK. He viewed the 15 sec dynamic noise 
stimulus with his poorer (left) eye. At the onset of the adapting 
noise, an audible stimulus was heard, the test screen was displayed, 
his left eye was uncovered and his right eye was occluded. As 
before the subject was asked to indicate any anomalous areas of 
the display by marking the touch screen as above.

All data were analysed with Matlab. The Cartesian pixel 
coordinates of cursor position were converted to polar values with 
respect to the centre of the drawn area for each trial. The orientation of 
each co-ordinate (θ) was rounded into 30° bins from 0 deg. The mean 
distance from the centre and standard deviation (σ) of that value were 
calculated for each of the 12 polar coordinates. A dodecagon was 
constructed for the mean and mean ± 1.96σ of the data at each 
coordinate. Using Photoshop (v.7.0; Adobe, San Jose, CA) these 
dodecagons were flipped vertically to correctly represent visual field 
space. For each subject, the dodecagons were resized to the retinal 
image obtained by the microperimeter/SLO by equating 5° of visual 
field space to the horizontal extent of the optic disc [39]. The resized 
dodecagons were superimposed on the fundus image. For clarity, the 
dodecagon representing the mean position was drawn in red, and the 
area between the 5% and 95% dodecagons was shaded grey.

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Author Contributions

Conceived and designed the experiments: SD PB. Performed the 
experiments: MC. Analyzed the data: MC. Contributed reagents/ 
materials/analysis tools: SD PB. Wrote the paper: SD PB MC. Other: 
Contributed reagents and acquired support for Matlab programming: PB SD. Revised the 
manuscript: PB SD. Performed Matlab programming: MC. Responsible for the experimental design: PB SD MC.
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