HIGH-RESOLUTION IMAGING OF GUNN'S DOTS

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Background: In healthy fundi, glistening whitish dots (so-called Gunn's dots) can often be seen, especially in young subjects. They are commonly attributed to the reflectance of Müller cell's footplates. However, despite their potential interest as biomarkers of retinal diseases, Gunn's dots have received little attention in the scientific literature.

Methods: Scanning laser ophthalmoscope reflectance imaging and adaptive optics infrared flood imaging were performed in 18 healthy subjects (age range, 18–58 years) to analyze the localization, density, and shape of Gunn's dots.

Results: Gunn's dots were more easily observed in the midperipheral retina along temporal vessels, although in two subjects, they could be detected in the macula. The reflectance of Gunn's dots showed a strong directional variability, which paralleled that of the inner limiting membrane. The mean (±SD) diameter of Gunn's dots was 13.3 μm (±3.5). Their density peaked at ~120 per square millimeter and decreased with age to become barely detectable after 50 years.

Conclusion: Gunn's dots are highly anisotropic structures close to the inner limiting membrane. The density, size, and age-related decline are closer to the characteristics of hyalocytes than those of Müller cells. Further studies are necessary to progress in the determination of their origin and interest as biomarkers of retinal diseases.

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Ophthalmoscopic examination of healthy subjects often shows minute glistening white dots apparently located in the inner retina. These were originally described by Robert Marcus Gunn as "very minute yellowish-white shining dots for some distance around the disk, especially to the nasal side and below. In distribution, these dots are remarkably equidistant from each other and are situated anteriorly to the largest retinal blood vessels, each being less than one-fifth of the diameter of a large vessel. . .. This appearance is most easily seen when the light is thrown somewhat obliquely on the part of the retina to be examined."[1] These "Gunn's dots" are commonly attributed to Müller cell's footplates.[2–4] However, the latter assumption is solely based on clinical intuition. Despite their easy observation, the description, physiologic basis, and medical interest of Gunn's dots had received little attention. Indeed, to the best of our knowledge, there has been no in-depth description of Gunn's dots in the era of modern fundus imaging. This is rather surprising because, according to the above-mentioned conception, they would be the only clinically detectable glial cells and as such are likely a biomarker of retinal diseases. Therefore, we underwent this study as an attempt to clarify the anatomical significance of Gunn's dots using high-resolution multimodal imaging.

Methods

This institutional clinical study was performed according to the principles outlined in the Declaration of Helsinki. Approval of the Ethics Committee of the Saint-Antoine hospital (Paris, France) was obtained.

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Table 1. Demographics and Gunn's Dots Density in the Study Population

Subject	Sex	Age (years)	Density (mm^{-2})
1	F	29	95.4
2	M	43	90.1
3	M	28	56
4	F	28	53
5	F	24	79.5
6	M	32	68.9
7	M	30	58.3
8	M	34	31.8
9	M	45	15.9
10	F	23	121.9
11	F	40	38
12	M	36	68.9
13	F	58	26.5
14	M	21	90.1
15	M	30	53
16	F	23	84.8
17	F	28	79.5
18	M	42	47.7

Subjects older than 18 years and without media opacities were recruited. Each subject received full oral and written information and gave written consent before inclusion.

Retinal imaging was performed at the Clinical Investigation Center of the Quinze-Vingts Hospital. Blue (488 nm) laser reflectance images were obtained using a scanning laser ophthalmoscope (Spectralis; Heidelberg Engineering, Heidelberg, Germany). En-face adaptive optics (AO) infrared fundus images were obtained using a commercially available flood imaging AO camera (rtx1 camera; Imagine Eyes, Orsay, France) using a previously described protocol[5] with slight modifications. In particular, for image acquisition, the gaze of the left eye was oriented with an external target, which allowed the exploration of the fundus of the right eye up to ~20° from the disk.

To detect directional reflection variability, AO and scanning laser ophthalmoscope images were acquired at different points of entry of the light in the pupil, without modifying the fixation point. This allowed modifying the angle of illumination relative to the retinal plane. Peak density measures were performed along the superotemporal vessels within a 0.5 mm^2 area. Multimodal image registration was performed by rotation and size adjustment using Adobe Photoshop 7.0 (Adobe Corporation, Mountain View, CA).

Results

~~Fifteen~~ eyes of 18 subjects (8 women and 10 men; age range, 18–58 years) were examined (Table 1). T1
Overall, blue reflectance image and AO yielded comparable amounts of Gunn's dots (Figure 1), which F1
were more easily detected along the temporal vascular arcades. These were also observed by color photography, yet with lower resolution than the above-mentioned modalities (not shown). In 3 young subjects, Gunn's dots were also seen in the macula, with a density similar to those seen elsewhere (Figure 2). F2
Gunn's dots thus appeared to be ubiquitous, at least in younger subjects. Magnifying the AO images showed that they were either oval or polygonal in shape (Figure 3). Their mean (±SD) diameter was F3
13.3 μm (±3.5). Their density peaked at 120 per square millimeter.

By scanning laser ophthalmoscope imaging and by AO, virtually all Gunn's dots showed a strong variability of reflectance when light incidence was modified (Figure 4). Interestingly, by blue reflectance imaging, F4
the reflection from Gunn's dots was restricted to areas in which reflectance from the inner limiting membrane (ILM) was present.

To document the change over time of the distribution or shape of Gunn's dots, 3 subjects underwent a second imaging session within 7 months to 17 months. In the 3 cases, the distribution and shape of Gunn's dots were overall unchanged (Figure 5). This F5
indicates that the Gunn's dots array remain stable

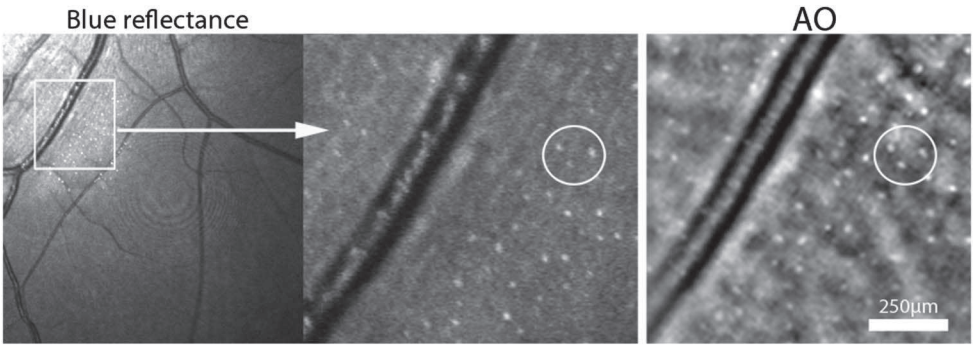

Fig. 1. Representative example of Gunn's dots seen by blue reflectance (left and middle panel) and AO infrared (right panel) imaging (Case 1, a 29-year-old woman). The circles show two corresponding areas.

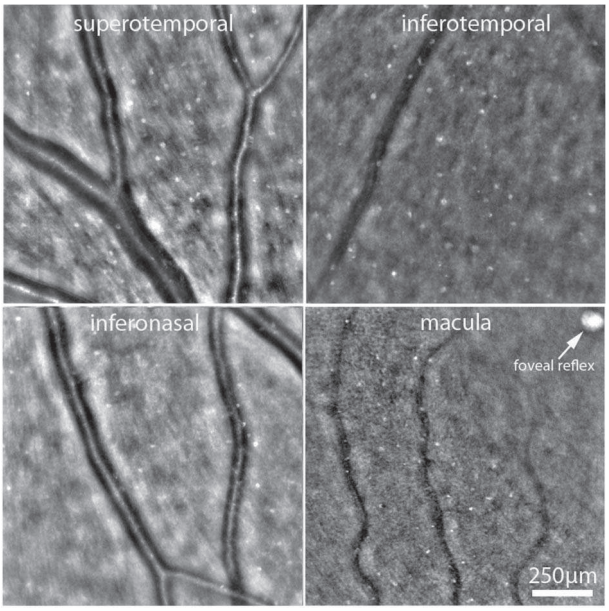

Fig. 2. Representative cases of AO imaging of Gunn's dots of 4 young subjects (Cases 2, 3, 4, and 5) illustrating their ubiquitous and uniform distribution. There is no evidence of vascular tropism.

over months; however, when the density of Gunn's dots of each patient was plotted against age, the density of Gunn's dots showed a linear decrease over years.

Discussion

Although recognized as a classical feature of the fundus and as such cited in most textbooks about fundus examination, to the best of our knowledge, this is the first investigation of Gunn's dots with modern imaging technologies. On the basis of our findings, Gunn's dots in healthy subjects can be ophthalmoscopically defined by several characteristics. They are reflective anisotropic structures, oval or polygonal, with a diameter inferior to 20 μm, either isolated or distributed in a rather regular pattern, 30 μm to 100 μm one from the other, with a peak density of $\sim$120 per square millimeter. They are more easily found along temporal vessels, although they may be detected in the macula or nasal to the disk, and therefore are probably ubiquitous. Gunn's dots are better seen within areas showing reflectance from the ILM. Their disposition and shape are stable over several months; however, the number of detectable Gunn's dots decreases over decades and can be limited to a few per square millimeter past 50 years of age.

Some anatomical characteristics of Gunn's dots may be deduced from their optical properties. As alluded to in the original article from Gunn, all Gunn's dots show a strong directional variability of their reflectance, which therefore can be considered as a clinical criterion for their identification. They thus behave optically as small mirrors more or less parallel one to the other; the most likely explanation for this property is that the surface of Gunn's dots is flat. Also, as the reflectance of the ILM paralleled that of Gunn's dots, both structures are in the same anatomical plane, and hence Gunn's dots are probably embedded in or very close to the ILM.

Among the cell types that may account for Gunn's dots, the most likely candidates are Müller cells and hyalocytes. Although it is commonly assumed that Gunn's dots are Müller cells endfeet, a clear demonstration of this is currently lacking. Müller cells endfeet are flattened and parallel to the vitreoretinal interface,[6] and hence likely to produce a light reflex when the incident light is perpendicular to the

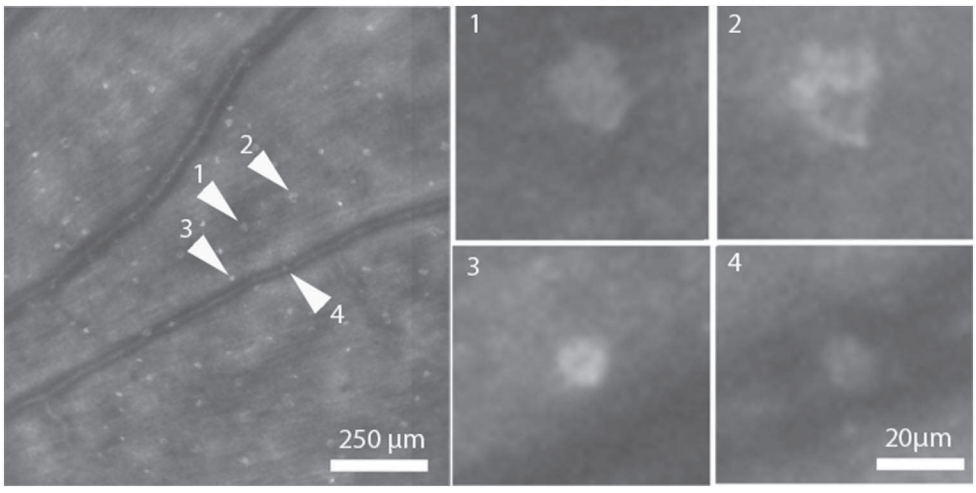

Fig. 3. High-power view of AO images of several Gunn's dots in a 32-year-old subject (Case 6) showing the variability of their shape.

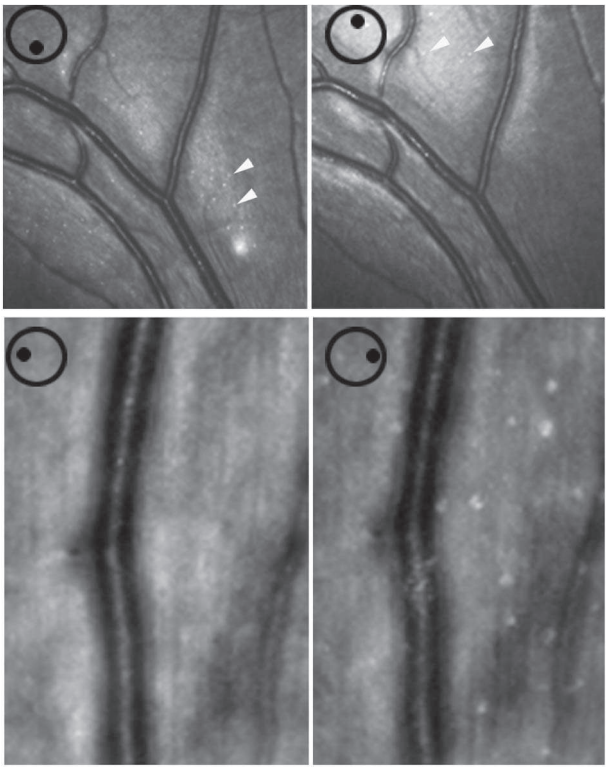

Fig. 4. Representative example of the directional variability of the reflectance of Gunn's dots. Scanning laser ophthalmoscope blue reflectance (top row: Case 7) and adaptive imaging (Case 8) have been taken at different points of entry in the pupil (indicated in the top of the images). In the blue reflectance images, note that Gunn's dots are visible (arrowheads) only in areas where the reflection of the ILM is also observed.

vitreoretinal interface. However, the density of Müller cells is in the range of several thousand per square millimeter, that is, several orders of magnitude greater than that of clinically detectable Gunn's dots.[7] Moreover, the width of their endfeet is roughly 1 μm, much less than the apparent size of Gunn's dots found here. However, a possible explanation for this discrepancy would be that some endfeet may be larger and/or coalesce, which may make them accessible to in vivo imaging.

Along the vitreal side of the ILM are also hyalocytes scattered in the vitreous cortex. Little is known about the three-dimensional features of human hyalocytes and their relationship with the ILM. In several species, the size and distribution of hyalocytes in the posterior cortex roughly matches that of Gunn's dots.[8–10] Scanning electron microscopy studies have shown that hyalocytes in contact with the ILM may be flattened.[8] In humans, hyalocytes have been observed attached to the surgical samples of ILM.[11] Finally, age-related decrease in the number of observable Gunn's dots mirrors the age-related incidence of posterior vitreous detachment.[12] Therefore, at this stage, it cannot be excluded that hyalocytes are indeed the structure accounting for Gunn's dots.

Whatever their origin, Gunn's dots may be biomarkers of a new population of clinically observable retinal cells, and as such, deserves further investigations to determine their origin and subsequently their medical interest. In a separate experimentation, using similar imaging techniques, we analyzed the fundus images of rodents and nonhuman primates to document the presence of Gunn's dots (Paques, unpublished data). Intriguingly, we failed to identify them, which suggested that the size and/or optical characteristics of Gunn's dots show notable interspecies variability. Little is known about interspecies variations of the morphology of Müller cells footplates, whereas investigations of rodent hyalocytes consistently showed that they have a star-like appearance,[13] which may theoretically make them less likely to show optical characteristics similar to those of Gunn's dots. To further progress in this field, histological analysis of the human retina and in vivo analysis of variations of Gunn's dots during vitreoretinal diseases and/or after ILM peeling may be of interest. Indeed, both Müller cells and hyalocytes are known to be highly responsive to a number of pathologic situations.[13,14] Also, additional microscopic investigations of surgical samples of the human ILM and/or posterior hyaloid would be of interest.

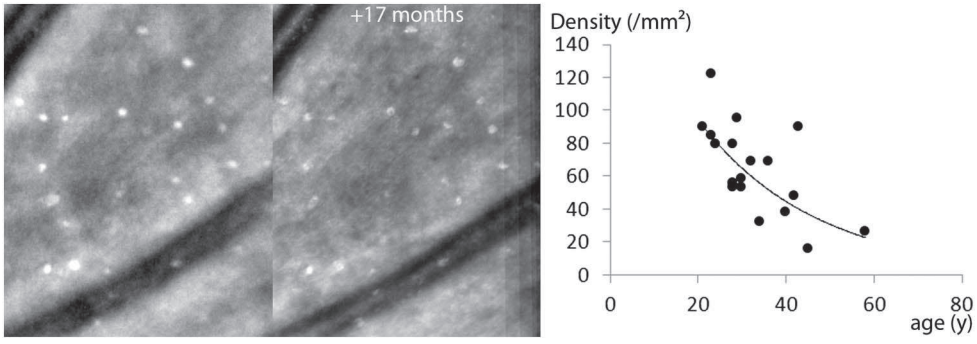

Fig. 5. Adaptive optics images of Gunn's dots taken at 2 successive time points in Case 6. There is no consistent evidence of change in the distribution pattern. Right panel, peak density of Gunn's dots plotted against age in the study population.

Key words: retina, Gunn's dots, adaptive optics, scanning laser ophthalmoscope.

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