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Photoreceptor Degeneration in Aging and Age-Related Maculopathy

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Introduction to ARM

Age–related maculopathy (ARM) is the major cause of new, untreatable vision loss in the elderly of the industrialized world. In the U.S. late ARM accounts for 22% of monocular blindness and 75% of legal blindness in adults over age 50 [81]. As the population ages, ARM will become the largest cause for vision loss among adults [19].

ARM is a heterogeneous disorder affecting the retinal pigment epithelium (RPE), Bruch's membrane, and choriocapillaris (the RPE/Bruch's membrane complex) [46, 123] (Figure 1A) and secondarily, the photoreceptors. Early ARM is characterized by minor to moderate acuity loss associated with characteristic extracellular lesions and changes in RPE pigmentation. Lesions between the RPE basal lamina and Bruch's membrane (Figure 1B) can be either focal (drusen) or diffuse (basal linear deposits). A diffuse lesion between the RPE and its basal lamina is basal laminar deposit (Figure 1C). Together, basal laminar and basal linear deposits constitute basal deposits. Late ARM is characterized by severe vision loss associated with extensive RPE atrophy with or without the sequelae of choroidal neovascularization, that is, in-growth of choroidal vessels through Bruch's membrane and under the RPE in the plane of drusen and basal linear deposits (see [27] for references).

ARM is a multi-factorial process, involving a complex interplay of genetic and environmental factors. Recent progress has been made in understanding demographics and natural history of ARM [82, 83], identifying smoking and hypertension as major preventable risk factors [62, 127], determining the biochemical composition of drusen [20, 92], and excluding genetic mutations causing some early-onset macular degenerations as risk factors [131]. Recent studies suggest that statin usage [99] and maintaining a healthy body mass index [2, 79, 126] may reduce the risk of the incidence or progression of ARM. Substantial progress has been made in developing mechanisms, animal models, and treatments for choroidal neovascularization [4, 10, 14].

The current standards of care include laser photocoagulation of the aberrant vessels or photodynamic therapy, treatments for which only a subset of patients with existing neovascularization qualify [10, 47]. Another potential treatment approach is to prevent or delay late ARM, which causes the vast majority of legal blindness. Because lesions in Bruch's membrane associated with early

ARM [129] precede neovascularization, a successful treatment approach may be to arrest the progression of the disease before the onset of late ARM and maintain visual function rather than manage the neovascularization and rescue visual function. The Age-related Eye Disease Study (AREDS) indicated that intake of several antioxidant compounds was beneficial in preventing neovascularization in ARM patients with bilateral drusen [3]. However, evaluation of the nutritional intervention was limited for the normal old adults and early ARM groups, because very few of these patients progressed to advanced (late) ARM during the course of the trial. Because of the low rate of progression for patients with mild disease, the effectiveness of the treatment for these groups was inconclusive. To increase the feasibility of clinical trials, better methods are needed to select early ARM patients with high risk of progressing to late ARM. A central idea in this review is that development of better diagnostic tests and treatments should be facilitated by and based upon improved understanding of the pathobiology of the earliest disease stages [17].

Although the most prominent clinical and histopathologic lesions of ARM involve the RPE and Bruch's membrane, it is the degeneration, dysfunction, and death of photoreceptors, through an atrophic process or a neovascular event and its consequences, that accounts for the vision loss associated with ARM. Because the most common clinical endpoint is a loss of acuity, impairment of visual functions mediated by foveal cones has been well characterized in ARM [8, 13, 36, 56, 60, 71, 97, 101, 112, 117, 133, 136]. Many of the observed visual function changes in ARM could be explained by photoreceptor loss associated with visible lesions. However, alterations in photoreceptor function during the earliest stages of the disease may provide valuable information on clinically invisible changes. Important constituents in the RPE/ Bruch's membrane complex such as basal linear deposit may not be revealed by standard imaging techniques such as fundus photography and fluorescein angiography until late in ARM or not at all [9, 27]. Therefore, the functional status of photoreceptors may serve as a bioassay of the significance of these changes.

It is important to determine which photoreceptors are most affected by aging and ARM, not only to target potential interventions to the most affected cells, but also to target mechanistic studies towards investigating the earliest disease-related changes. The rate of rod and cone degeneration is a

fundamental characteristic of any disorder affecting photoreceptors [67, 88]. Because rods and cones have distinctly different biology, the rates at which they die provide important clues to the events initiating their demise. In order to determine the relative rate of degeneration, however, one must obtain comparable information for both rods and cones at matched locations in the same well-characterized study eyes rather than try to calibrate findings between studies using fundus appearance. Ideally, outcome measurements should be quantifiable and attributable to a specific cell class. Examples of suitable endpoints are cell numbers and cone–mediated and rod–mediated visual sensitivity.

Photoreceptor Loss

Based on this approach, we characterized the topography of macular photoreceptors in retinal aging and ARM. The macula, defined anatomically as the area with one or more layers of retinal ganglion cells [113] and epidemiologically as the area within the Wisconsin Age-related Maculopathy Grading System grid [80], is approximately 6 mm in diameter and centered on the fovea. The macula contains two sub-regions with distinctly different photoreceptor content: a small cone-dominated fovea, 0.8 mm (2.75° of visual angle) in diameter, and a surrounding rod-dominated parafovea. Figure 2A-D shows small cone inner segments in the fovea and large cones with numerous small rods in the parafovea. Figure 2E shows that the peak density of cones in the foveal center is high (mean, 200,000/ mm^2) and declines precipitously (10-fold) within 1 mm (3.5°). Rods are absent in the foveal center and rise sharply to a maximum at 2-4 mm eccentricity. In young adults, rods outnumber cones in the macula by 9:1, so the macula is cone-enriched, compared to the eye as a whole (20:1), but it is not cone-dominated [24, 106].

In flat-mounted retinas from eyes with maculas lacking grossly visible drusen and pigmentary change the *total* number of cones within the 0.8 mm-diameter cone-dominated fovea was remarkably stable throughout adulthood at about 32,000 [25]. Other studies did not detect an age-related change in *peak* foveal cone density [42], but the possibility of foveal cone loss at very advanced ages remains open [40]. In contrast, the number of rods in the parafovea of the same eyes decreased by 30% [25], consistent with other results[109]. Within the macula, age-related rod loss was not spatially uniform.

Comparison of rod topography in younger and older eyes using difference maps suggests that rod loss is deepest near the fovea and widens towards the periphery over time (Figure 3D,E,F). In contrast, cone topography changed little with age (Figure 3C,D,E). The location of age-related rod loss differs from the site of maximal rod density (2-4 mm from the fovea) [24, 106] and from the site of cell loss associated with typical retinitis pigmentosa (8-10 mm from the fovea) [53]. The relative rate of rod and cone loss in extramacular retina is uncertain [25, 42, 109].

Analysis of photoreceptor topography in eyes from 12 ARM donors provided evidence that rods are preferentially affected in ARM as well [26, 100](for review [29]). Despite the presence of drusen and thick deposits, the foveal cone mosaic of non-exudative ARM eyes appeared remarkably normal, and the total number of foveal cones fell within the normal range. In contrast, the parafovea was distinctly abnormal, with few rods, broadened cone inner segments, and gaps in the mosaic of inner segments. Figure 4 shows pre-mortem fundus appearance, fluorescein angiography, and topography of cone and rod loss in an eye with pigment clumping superior to the fovea (case 2 from [26]). In this eye, photoreceptor loss occurs in relation to funduscopically visible pigment change, the area of loss extends beyond what is visible in the fundus, loss affects both cones and rods, and rod loss is deeper and more extensive in area than cone loss. The fellow eye of this patient had progressed to neovascular ARM, so the eye in Figure 4 would be considered high risk [1]. In eyes with disciform degeneration and geographic RPE atrophy (for review [29]), many histochemically-verified cones survived in pockets of subretinal space enclosed externally by fibrovascular scar. Furthermore, peripheral to the geographic RPE atrophy associated with disciform scars was a transitional zone of thick deposits, degenerating RPE, and a marked decrease in the number of rods. Rod loss was greater than cone loss at comparable locations in 3/4 of ARM eyes examined. In summary, although the macula is cone-enriched, rods show the earliest signs of degeneration in most ARM eyes, and the last surviving photoreceptors appear to be cones. Photoreceptor loss in aging and ARM occurs by apoptosis, and most apoptotic photoreceptors in eyes with geographic atrophy are rods [35, 86, 146]. In addition to cell loss, photoreceptors suffer sub-lethal metabolic insults. Outer segments overlying

drusen are shortened and misoriented, and opsin translocates from the outer segment to the plasma membrane in rod photoreceptors, typical of other degenerations [69].

Photoreceptor Dysfunction

Functional studies have supported the histological evidence for preferential vulnerability of rods in aging and ARM [66, 107]. These functional studies measured photopic and scotopic sensitivity at matched retinal locations in the same cohort of well-characterized eyes. The studies had large samples, involving 106 normal subjects from 7 decades of adulthood and 80 early ARM patients. Significantly, macular health was ascertained objectively in all subjects by grading fundus photographs, and the effect of lens density, which reduces retinal illuminance in older persons, was accounted for on an individual basis in interpreting thresholds. These studies demonstrated reduced rod-mediated light sensitivity in older adults in good retinal health, the magnitude of which was similar throughout the parafovea[63, 66]. Scotopic impairment was greater than photopic impairment in 80% of older adults evaluated, and, furthermore, scotopic sensitivity declined throughout adulthood faster than photopic sensitivity declined (Figure 5). With respect to ARM patients, mean scotopic sensitivity within 18° of fixation was significantly lower in early ARM patients as a group than in age-matched controls without ARM (Figure 6). The pattern of scotopic versus photopic sensitivity loss in the central 36° of the visual field varied considerably among individual patients with early ARM. Of the patients with reduced light sensitivity in this region, 59% showed reduced scotopic sensitivity, 27% showed both reduced scotopic and photopic sensitivity, and 14% had reduced photopic sensitivity only. In almost all (87%) of these patients, the magnitude of mean scotopic sensitivity loss exceeded the magnitude of mean photopic sensitivity loss.

It is important to note that age-related changes in night vision are not confined to laboratory settings but also impact on daily activities of older adults. One of the most pervasive visual observations reported by older adults, even those free of retinal disease, is problems seeing under dim illumination [84], especially problems with night driving [5]. It is interesting that patients in the earliest phases of ARM report difficulty with night driving problems, which are associated with their impaired scotopic sensitivity as measured in the laboratory [125].

Topography of loss and dysfunction

The topography of photoreceptor loss and dysfunction, which is spatially heterogeneous across the macula, may provide important information about the signals leading to photoreceptor demise. Figure 7A,B show that the scotopic sensitivity loss and the loss of rod photoreceptors in early ARM patients is greatest near the foveal center and declines markedly to the edge of the macula, reminiscent of the deepening and widening of photoreceptor loss around the fovea shown in Figure 2 [26, 100, 107]. In contrast, photopic sensitivity loss and the loss of cone photoreceptors are roughly constant over the same distance (Figure 7A). This heterogeneity can be used to assess the plausibility of potential mechanisms underlying photoreceptor loss and dysfunction, under the assumption that causally related events should exhibit a similar topography. This approach is valuable, because the close proximity and physiological interdependence of photoreceptors, RPE, and Bruch's membrane make it difficult to disentangle the relative contributions of these distinctive layers to the pathogenesis of ARM. In the absence of multi-parametric data from many individual eyes, we addressed this question by comparing the best topographic data available from patient-oriented and laboratory studies.

In Figure 7C,D we examine the topography of two features of the normal macula that are frequently mentioned in the context of ARM pathobiology. Lipofuscin is a prominent autofluorescent age-pigment in the RPE that is thought to represent irreducible end-products of outer segment breakdown. The striking prominence of lipofuscin in adult human macula [38], its enhanced autofluorescence in areas of incipient RPE atrophy in ARM patients, [57], its decreased autofluorescence associated with drusen [33], and the identification and isolation of a major constituent (A2E)[110] has lead to several proposed roles in the pathogenesis of ARM. These include inhibition of lysosomal function [58], promotion of apoptosis [134], enhancement of photo-oxidative injury [124], and detergent-like disruption of the plasma membrane [37]. Figure 6B shows that funduscopically visible autofluorescence due to lipofuscin follows closely the normal distribution of rods [32, 140], that is, low in the foveal center and high at 2-4 mm.

Macular pigment, comprised of lutein and zeaxanthin, is thought to protect the retina against ARM because it can act as an antioxidant [7]. Modulation of macular pigment has attracted attention as a therapeutic route, because factors associated with low macular pigment are also associated with increased risk for ARM, retinal content can be enhanced by dietary supplementation, and pigment is detectable non-invasively in patients [43, 87, 128]. Figure 7D shows that the macular pigment is highly concentrated at the foveal center sharply declining within 2 mm. Figures 7A-C together indicate that the distinctive spatial profiles of ARM-related rod dysfunction/ loss, lipofuscin accumulation, and macular pigment are not related in a straightforward manner. A reasonable expectation is that dysfunction and loss should be greater where deleterious factors (lipofuscin) are abundant and/ or beneficial factors (macular pigment) are scarce. In fact, the opposite is the case. If lipofuscin and macular pigment are theorized to have a primary role in ARM pathogenesis, the theory must account for the unexpected distribution of these factors in relation to other markers of the disease. The roles of lipofuscin and macular pigment in specific aspects of ARM pathobiology (e.g., RPE cell death) are not excluded by this analysis. However, it should be noted that net RPE cell loss in the aging macula has been difficult to establish [31, 50, 132, 137, 143], possibly due to methodologic issues, but also possibly because the RPE cell population is conserved *in vivo* despite prominent age-related lipofuscin accumulation.

Next we examined the topography of Bruch's membrane pathology. Characteristic debris accumulates within Bruch's membrane throughout adulthood [39], accompanied by reduced collagen solubility [72], accumulation of advanced glycation end-products [49] , and deposition of neutral lipids including cholesterol [30, 48, 111]. A layer just external to the RPE basal lamina is almost completely occupied by esterified cholesterol-rich droplets [30, 120]. Additional material (basal laminar deposit) accumulates between the RPE and Bruch's membrane in older adults and in ARM patients [27, 46, 123, 130]. Because Bruch's membrane and basal deposits are not directly visible in the fundus [9, 27], we used the distribution of soft drusen and RPE changes visible in the fundus of ARM patients as a surrogate for the invisible pathology, with the caveat that the lateral extent of pathology is likely to be underestimated. Figure 7D shows the location of soft drusen and RPE in participants with early ARM

in the population-based Beaver Dam Eye Study [142]. Lesions were localized to defined macular sub-fields using a validated, semi-quantitative grading system of clinical fundus photographs. Soft drusen and RPE changes cluster within the central 1 mm of the macula. Although rod function over focal deposits (drusen) remains to be directly demonstrated, the overall topographic correspondence of RPE/ Bruch's membrane pathology and rod dysfunction is striking.

Photoreceptor function as a bioassay of RPE and Bruch's membrane health

The photoreceptor layer consists of two intermixed cell types that share a common support system (RPE, Bruch's membrane, and choroid) and environmental exposure to light. Here we consider mechanisms pertaining to the support system, because its age- and disease-related changes are remarkable, and because the role of chronic light exposure in aging and ARM has been difficult to establish. In addition to investigating rod- or cone-specific mechanisms of cell death [116], we propose that developing a hypothesis to account for the differential effect on rod and cone survival in the setting of an altered RPE/Bruch's membrane environment may be instructive. A major function of the choroidal vascular system, of which Bruch's membrane is the inner border, is supply of essential nutrients and oxygen to the photoreceptors. The idea that age- and ARM-related changes in the RPE/Bruch's membrane complex ultimately impact the integrity of the photoreceptor re-supply route, resulting in degeneration and death, therefore has intuitive appeal. Changes at multiple locations along this route -- poor vascular perfusion secondary to choriocapillaris atrophy [16, 115], impaired translocation of plasma nutrients due to Bruch's membrane thickening [103], or reduced uptake from plasma or delivery to photoreceptors due to RPE senescence – could impact photoreceptor health. Because photoreceptor function is understood in exquisite detail [118] it may be eventually possible to deduce the specific essentials that photoreceptors lack by careful attention to functional deficits.

Dark adaptation is a good candidate for a test of visual function, because dark adaptation relies on retinoid cycle components contained within the same layers where ARM-associated lesions are located [85, 89, 98]. The classic dark adaptation function describes the recovery of sensitivity following a bright flash of light and consists of an early portion exclusively mediated by cones, a transition to rod function (rod-cone break), and a later portion exclusively mediated by rods [6]. The

retinoid cycle provides 11-*cis*-retinal, a metabolite of vitamin A, to the photoreceptors for photopigment regeneration [52, 70, 141]. Aging and ARM-related changes may retard dark adaptation by a variety of mechanisms working either independently or in concert to reduce the pool of 11-*cis*-retinal available to the photoreceptors. Debris in Bruch's membrane may slow the passage of vitamin A from the choroid to the RPE, the RPE may process retinoids less efficiently due to age- or disease-related change, transfer of 11-*cis*-retinal from the RPE to the photoreceptor's outer segment could be due to slowed diffusion or impaired interphotoreceptor retinoid binding protein function, and re-uptake of all-*trans*-retinol to the RPE for recycling into 11-*cis*-retinal could be compromised. Impairment of any or all of these processes could slow dark adaptation.

In older adults with good macular health, as assessed by grading of fundus appearance, the rod-mediated portion of dark adaptation is significantly slower than in younger adults [64] (Figure 8). During adulthood, the time constant of the rod-mediated component of dark adaptation increases by about 8 seconds per decade [64]. Rod-mediated dark adaptation is not correlated with scotopic sensitivity in these patients, indicating that the mechanisms underlying these two aspects of rod vision are not identical [66]. In early ARM patients, even in those with normal acuity, rod-mediated dark adaptation is much slower (13 minutes on average) than in normal age-matched controls [108] (Figure 9). Consistent with the pattern of scotopic sensitivity loss described above, delays in rod-mediated dark adaptation are greater than those for cone-mediated dark adaptation in ARM [64]. Delayed rod-mediated dark adaptation occurs in ARM patients with normal scotopic sensitivity, whereas the opposite pattern, normal dark adaptation with poor scotopic sensitivity, is rare. Rod-mediated dark adaptation may be more sensitive to the effects of early ARM than cone-mediated dark adaptation because of differences in the retinoid cycle of the rods and cones [96]. Cone photopigment regeneration can occur in the absence of the RPE, whereas rhodopsin regeneration (rod photopigment) is reliant on the RPE/ Bruch's membrane complex [44, 59, 70]. Recently, new enzymes for retinoid processing, possibly within Müller cells, have been identified in all-cone retinas [96], raising the possibility that fovea, which has a high concentration of Müller cells as well as cones[147], may have additional sources of retinoids. Thus, if cone-mediated dark adaptation is less dependent on the RPE

than rod-mediated dark adaptation, impaired rod mediated dark adaptation may be a better marker for the significant changes in early ARM than measures of cone function.

Impairment of Transport Between RPE and Photoreceptors

It is reasonable to suggest that the extensive changes found in Bruch's membrane may affect photoreceptor health and function. The accumulation of debris, particularly lipids (see above), are hypothesized to slow the transfer of fluids, essential nutrients, and large proteins across Bruch's membrane. Hydraulic resistivity increases and diffusion of selected compounds decreases with donor age in Bruch's membrane explants [61, 94, 103, 104]. Thus, lesions in Bruch's membrane may act as a barrier to nutrients moving from the choroidal blood supply to the RPE and ultimately, photoreceptors. The hypothesis that rod–mediated dark adaptation is impaired because of slowed translocation of retinoids through Bruch's membrane and ARM-associated lesions is supported by the fact that rod dysfunction and degeneration occur in various late-onset conditions with diffuse sub-RPE deposits [28, 51, 68, 78]. It is possible that sub-RPE deposits, which differ ultrastructurally and probably biochemically among these disorders, act as non-specific barriers to the re-supply of molecules preferentially essential to rods. The hypothesis is also supported by evidence that dark adaptation improves following dietary vitamin A supplementation in patients with Sorsby's fundus dystrophy, a disorder characterized by thick sub-RPE deposits [68]. Presumably, the translocation deficit was overcome via mass action in this case. Mutations in genes coding for key visual cycle components also lead to poor night vision [98] (see review [135]). In some animal models, these deficits can be bypassed by orally administered retinoid compounds [138]. In mice, vitamin A taken up by RPE is delivered in a complex with retinol binding protein [139]. However, the question of whether perfusion of retinoids through Bruch's membrane is required for the recovery of visual sensitivity remains to be answered. Further, there are currently insufficient data to evaluate the RPE as the site of impaired retinoid translocation, because little is known about age-related changes in retinoid processing. The content of retinyl esters (the storage form) increases with age in monkey macula but not in periphery or in whole human eye [11, 21]. If more retinyl esters are stored in aging human

macula, it is possible that impaired transport between the RPE and photoreceptor outer segments is responsible for aging-related impairment in dark adaptation.

Although photoreceptor dysfunction and death appear to be related topographically to the lesions in the RPE/ Bruch's membrane complex, rod susceptibility to aging and ARM and the mechanism of photoreceptor death are unknown. The results of dark adaptation studies suggest a deficiency of retinoids available to the photoreceptors [12, 108]. Vitamin A deprivation leads to outer segment degeneration and photoreceptor death *in vivo* [34, 73, 74] and accelerated degeneration of photoreceptors with mutant rhodopsins *in vitro* [90]. Lack of vitamin A affects primarily rods but eventually impacts cones as well [15, 76, 77]. It should be noted that vitamin A is necessary not only as the precursor to 11-*cis*-retinal but also as the precursor to other compounds potentially important for RPE and photoreceptor health. Within tissues, retinol is activated to retinoic acid, which binds to nuclear receptors to regulate transcription of more than 300 diverse target genes [93, 121] whose exploration in RPE is only beginning [122]. Studies to determine the non-visual effect of retinoids on photoreceptor health would be informative.

It is important to emphasize that the slowing of the retinoid cycle may be simply a marker for another process, such as generalized RPE ill-health, RPE senescence, or Bruch's membrane change, which results in local scarcity of other molecules essential to photoreceptors. For example, lack of oxygen has been implicated in photoreceptor death subsequent to retinal detachment, as mitochondria-rich inner segments are displaced from the high oxygen tension in the choroid [91]. Cone inner segments are larger and contain more mitochondria than rods [54] but the relative oxygen requirements of rod and cones are unknown. Determining the impact of thickened deposits and shortened outer segments associated with ARM to oxygen levels at the level of the inner segment, the relative usage of oxygen by cones and rods, and the effects of modulating oxygen availability on cone and rod survival would be useful. Another nutrient important for photoreceptor survival is vitamin E (tocopherol). A constituent of the AREDS anti-oxidant formulation [2], vitamin E is normally delivered to tissues by plasma low density lipoproteins, [75] and it protects unsaturated lipids in

membranes and in vitamin A. Of the four naturally occurring vitamin E isomers, α-tocopherol is abundant in the RPE/ choroid, and within neural retina, it is highest in the fovea [21-23]. Because macular α-tocopherol concentrations are independent of plasma levels, it is thought that the retina regulates its vitamin E content closely. Vitamin E increases with age in RPE-choroid in macaque and humans [41, 105]. Systemic vitamin E deficiency in rat leads to photoreceptor degeneration and loss but the relative rates of cone and rod loss are not known [119]. Interestingly, the retina may be capable of generating its own vitamin E. Mutations of the gene coding for α-tocopherol transfer protein are associated with pigmentary retinopathy in humans and mice, and this gene is expressed in retina and brain [18, 45, 148, 149]. Thus, it is possible that the potential advantage afforded to cones via an intra-retinal retinoid delivery pathway (see above) may represent a class of local mechanisms that nourish and protect photoreceptors.

Summary

In summary, anatomical and functional studies have converged to demonstrate that photoreceptor degeneration and loss occurs before disease in the RPE/Bruch's membrane complex progresses to late ARM. Furthermore, macular rods are affected earlier and more severely than cones in aging and ARM. These findings are significant for both clinical and basic research. In many patients tests of rod function may permit detection of ARM at earlier stages than do standard tests of cone function such as visual acuity. The preferential vulnerability of rods in aging and ARM is a phenomenon which should be accounted for by mechanistic theories. These findings provide a standard against which the relevance of emerging animal models [95, 114, 144] and other potentially pathogenic phenomena in the macula should be assessed. Since rods secrete factors that enhance cone survival [102], early interventions that target rod photoreceptors may have an indirect salutatory effect on cones as well.

The link between photoreceptor dysfunction, assessed by vision function studies, and risk for neovascularization in Bruch's membrane, alluded to at the beginning of this review, is most plausibly attributable to the common cause of poor RPE health, long postulated as central to ARM pathogenesis [55]. Because the RPE is polarized, problems pertaining to the re-supply of photoreceptors on the

apical aspect of the RPE (leading to photoreceptor death) should be conceptually separated from problems pertaining to waste removal on the basal aspect of the RPE (leading to Bruch's membrane damage and neovascularization), at least for the purposes of designing mechanistic experiments. These processes are governed by different proteins and pathways at the cellular level and will be reflected by different risk factors and genetic predispositions at the population level. Rigorous test of a nutrient deficiency hypothesis of ARM-associated photoreceptor death, awaits more information about normal nutrient delivery mechanisms across the RPE/Bruch's membrane complex, intra-retinal contributions to photoreceptor nutrition, changes in these mechanisms with age and pathology, and differential effects on rods and cones

Figure Captions

Figure 1. RPE/ Bruch's membrane complex and ARM-associated lesions, from [27] **A.** Normal eye, 63 year old donor. OS = outer segments of photoreceptors; RPE = retinal pigment epithelium, arrowheads delimit Bruch's membrane, ChC = choriocapillaris. **B.** Drusen (d) and basal linear deposit (between arrowheads), 60 year old donor. **C.** Basal laminar deposits (between arrows), 69 year old donor.

Figure 2. Photoreceptor mosaic and distribution, from [24]. **A.** Foveal cone inner and outer segments, longitudinal section. **B.** Foveal cone inner segments in a flat mounted retina of a 34 year old donor, Nomarski differential interference contrast optics and video. **C.** Non-foveal cone and rod inner segments, longitudinal section. **D.** Cone inner segments (large) and rod inner segments (small) in the same eye. **E.** Number of cones and rods per mm^2 of retinal surface in nasal and temporal retina, as a function of distance from the foveal center in degrees of visual angle (bottom) and mm (top). Hatched bar, optic disc. Dashed lines show macular boundaries.

Figure 3. Topography of cones and rods in aging human retina, from [25], shown as a fundus of a left eye. Black oval is the optic disc, and the ring delimits the 6 mm diameter macula. In C and F, warm colors mean that older group has higher mean density than young group and cool colors mean that older group has lower mean density than young group. A yellow-green map means that differences between groups are small. **A.** Cones, 27-36 year old donors. **B.** Cones, 82-90 year old donors. **C.** Log mean difference in cone density between younger adults and older adults is small and inconsistent. **D.** Rods, 27-36 year old donors. **E.** Rods, 82-90 year old donors. **F.** Log mean difference in rod density between younger adults and older adults is greatest at 0.5 mm to 3 mm from fovea. Purple signifies that the log mean difference (aged-young) was < -0.16 log units, i.e., that aged eyes had 31% fewer cells than young eyes.

Figure 4. Clinical imaging and topography of cone and rod loss in an eye at risk for late ARM, data from [26], 81 year old donor. Clinical images and topographic maps are not at the same spatial scale. In the difference maps (C, D), warm colors mean that ARM eye has higher mean density than age-matched controls and cool colors mean that the ARM eye has lower mean density than age-matched controls (see Figure 3B, E). Purple signifies that the log mean difference (ARM-control) was < -0.20 log units, i.e., that the ARM eye had 37% fewer cells than age-matched control eyes. **A.** Red-free fundus showing pigment hypertrophy with surrounding pigment atrophy in an arc superior to the fovea. **B.** Fluorescein angiogram showing late hyperfluorescence at 775 sec. **C.** Difference in number of cones/mm^2, relative to age-matched controls. **D.** Difference in the number of rods/mm^2, relative to age-matched controls.

Figure 5. For patients in good retinal health, mean scotopic sensitivity impairment is plotted as a function of mean photopic sensitivity impairment. Impairment for each individual was defined as the subject's average sensitivity across the test field (central 18° radius visual field) subtracted from the average of adults in their twenties. Data are corrected for pre-retinal absorption. The dashed diagonal line represents equal impairment in photopic and scotopic sensitivity under our test conditions. Numbers represent the age of each subject: 2=20s, 3=30s, 4=40s, 5=50s, 6=60s, 7=70s, 8=80s. Used with permission, reference [66].

Figure 6. For ARM patients, mean scotopic sensitivity impairment is plotted as a function of mean photopic sensitivity impairment. Impairment for each individual was defined as the subject's average sensitivity across the test field (central 18˚ radius visual field) subtracted from the average of old normal adults. Data are corrected for pre-retinal absorption. The dashed diagonal line represents equal impairment in photopic and scotopic sensitivity under our test conditions.

Figure 7. Sensitivity loss in ARM eyes (A), photoreceptor loss in ARM eyes (B), funduscopically visible autofluorescence and macular pigment in normal eyes (C), and lesions in ARM eyes (D),

plotted as a function of distance from the foveal center. A. Dark-adapted (scotopic) and light-adapted (photopic) sensitivity loss for patients with early ARM, with adjustment for pre-retinal absorption [107]; averaged across test loci on the horizontal, nasal and oblique meridians. Sensitivity loss is referenced against old adults in good retinal health with no signs of ARM. B. Loss of rods and cones is averaged across 4 meridians. ARM-related loss is the log of the mean pair-wise differences between early ARM eyes and age-matched controls [100]. C. Mean autofluorescence due to lipofuscin along the horizontal temporal meridian is normalized to the maximum in 3 observers [32, 140]. The optical density of macular pigment was measured in a normal adult, 23 years old [145]. D. The prevalence of right eyes with soft indistinct drusen and/or RPE hypo- and hyperpigmentation in specific macular regions. Bars indicate the area-weighted prevalence for 247 participants with early ARM in the Beaver Dam Eye Study [142] for lesions in regions 0-0.5 mm, 0.5-1.5 mm, and 1.5-3.0 mm, respectively, from the foveal center. These regions correspond to the central sub-field, the ring of four inner sub-fields, and the ring of four outer sub-fields, respectively, of the Wisconsin Age-related Maculopathy Grading System grading grid [80]. Arrowhead indicates outer limit of macula.

Figure 8. Dark adaptation as a function of decade in persons with normal retinal health. Individual subject's data were grouped by decade and fitted with a four-linear component model. Data are corrected for pre-retinal absorption. The resulting equations from the nonlinear regression analysis were plotted for illustration purposes. Arrows label the portion of the function representing the rod-cone break and the second and third components of rod-mediated dark adaptation. Note that the functions shift to the right with increasing decade, indicating a slowing of the rate of dark adaptation during aging. Used with permission, reference [65].

Figure 9. The dark adaptation functions for three patients with ARM and one older adult in good eye health. All patients had 20/25 visual acuity or better. Used with permission, reference [108].

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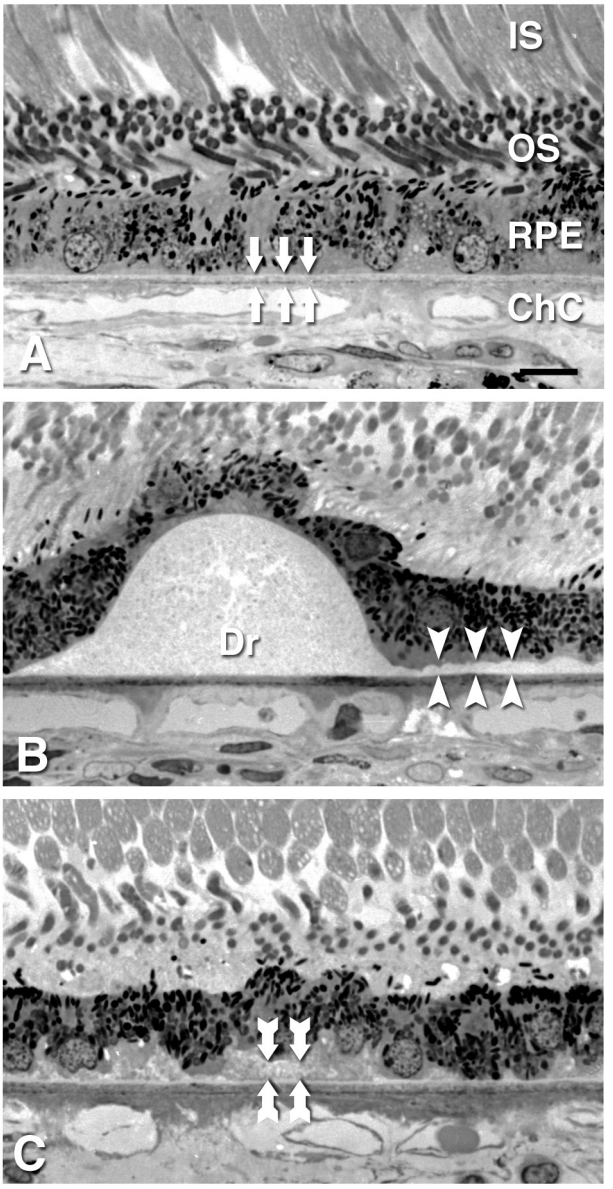
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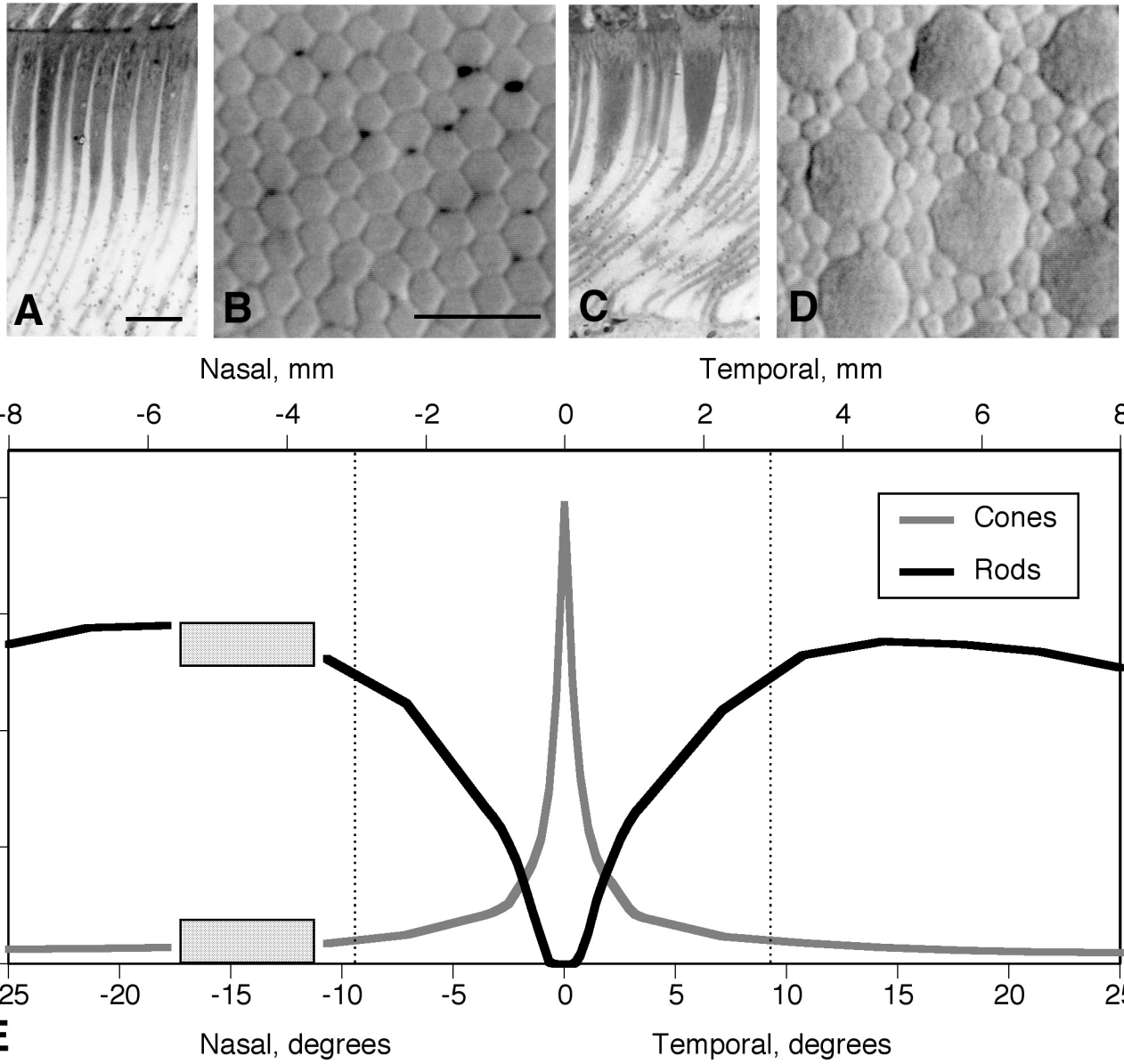
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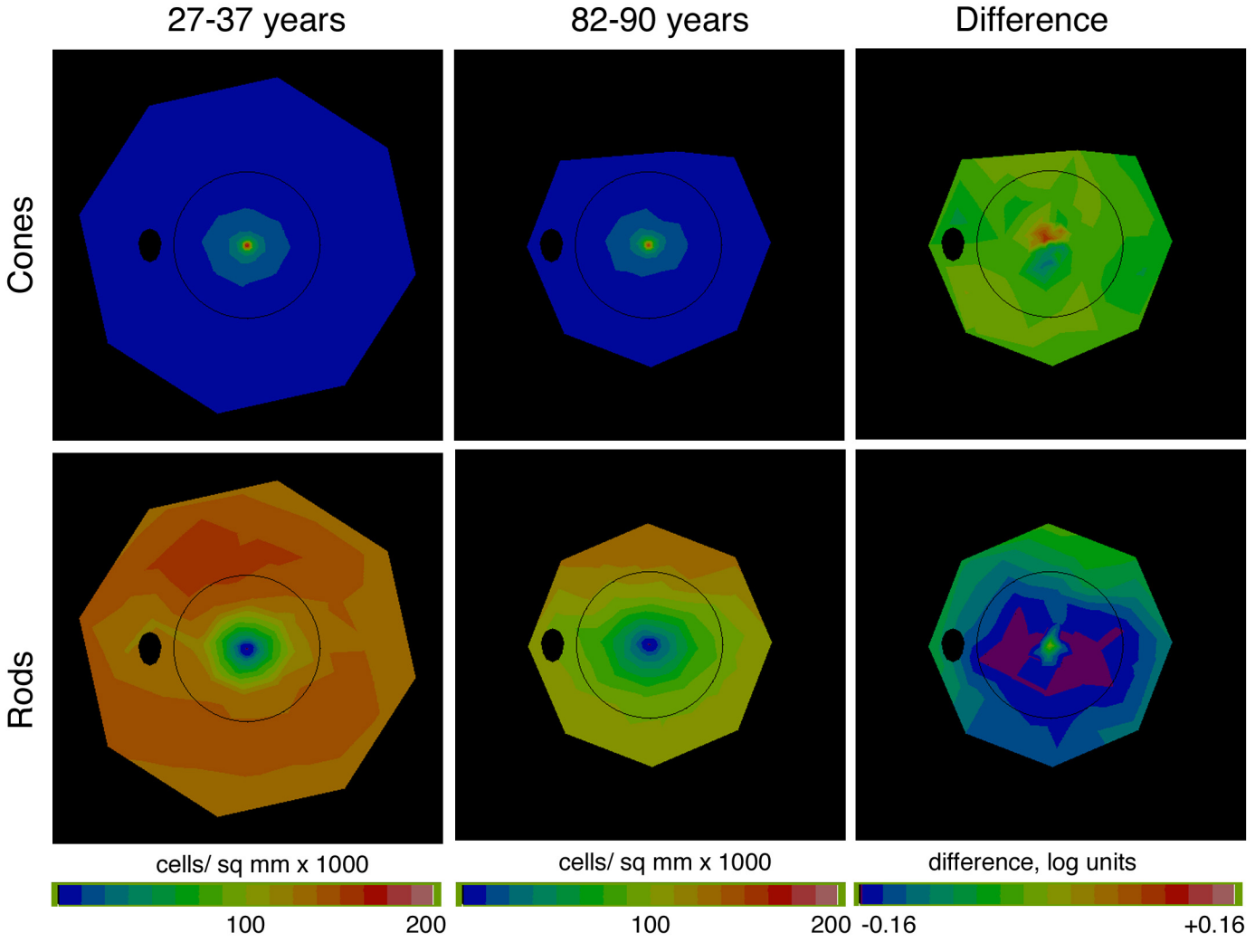
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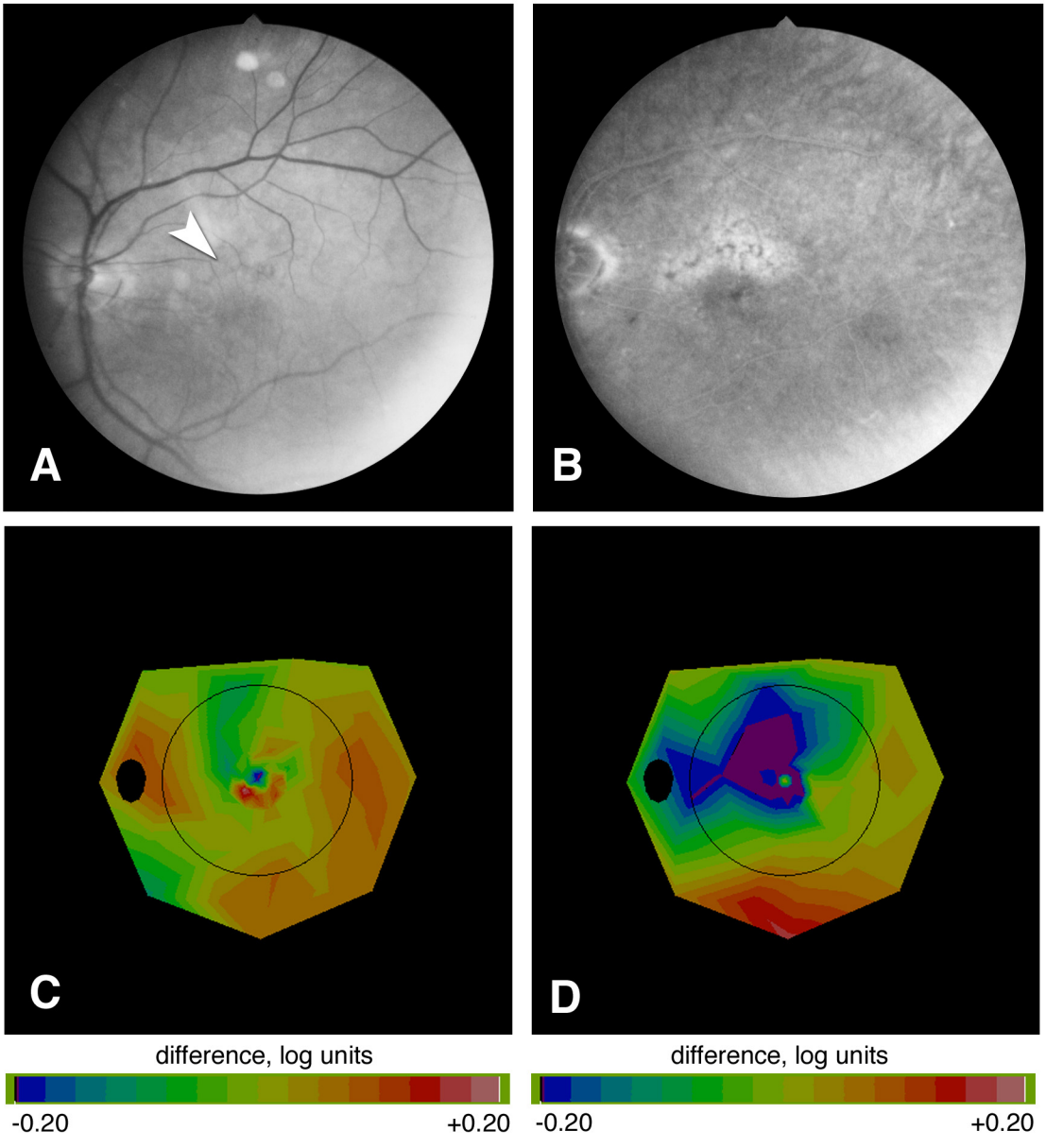
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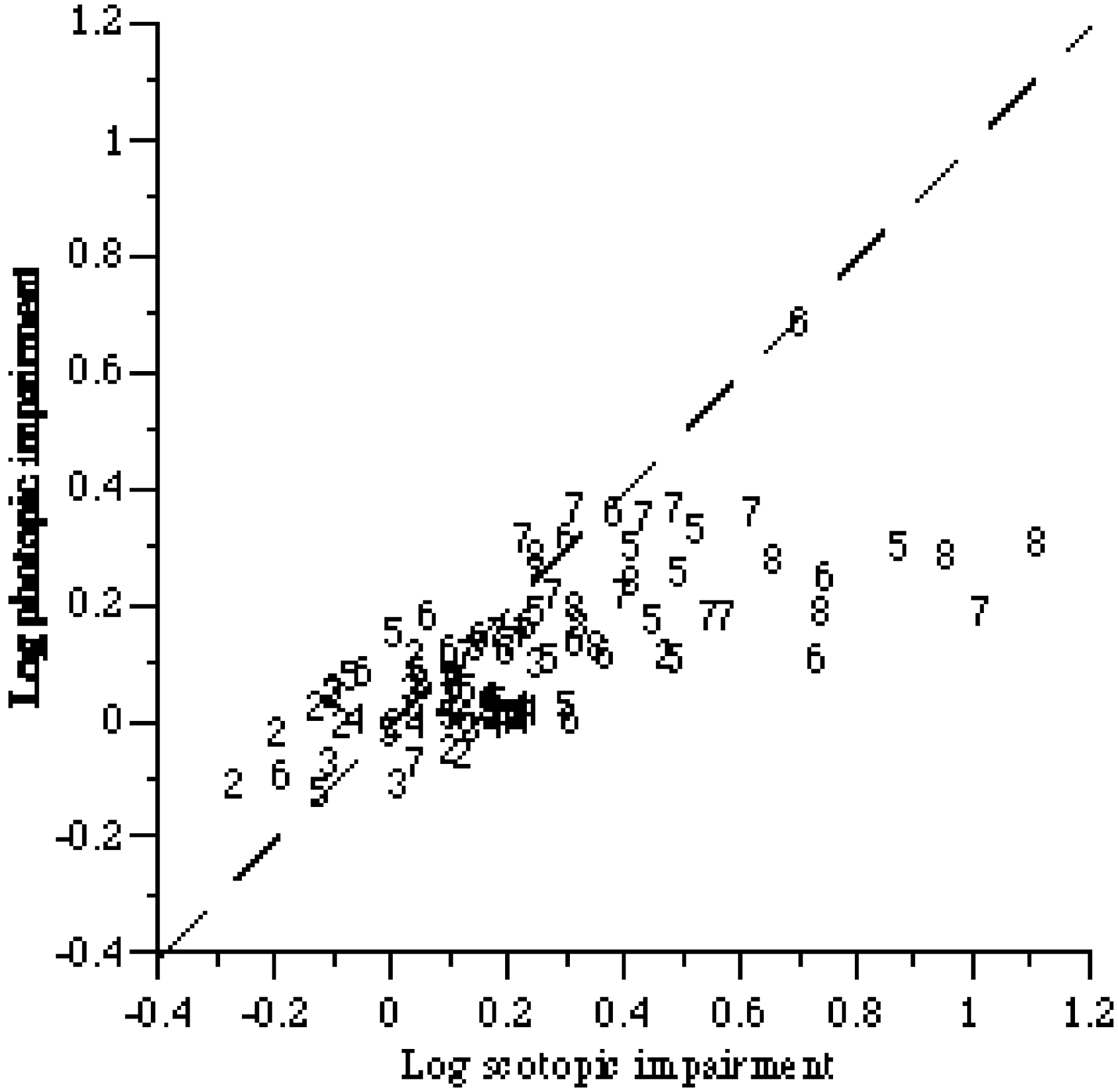
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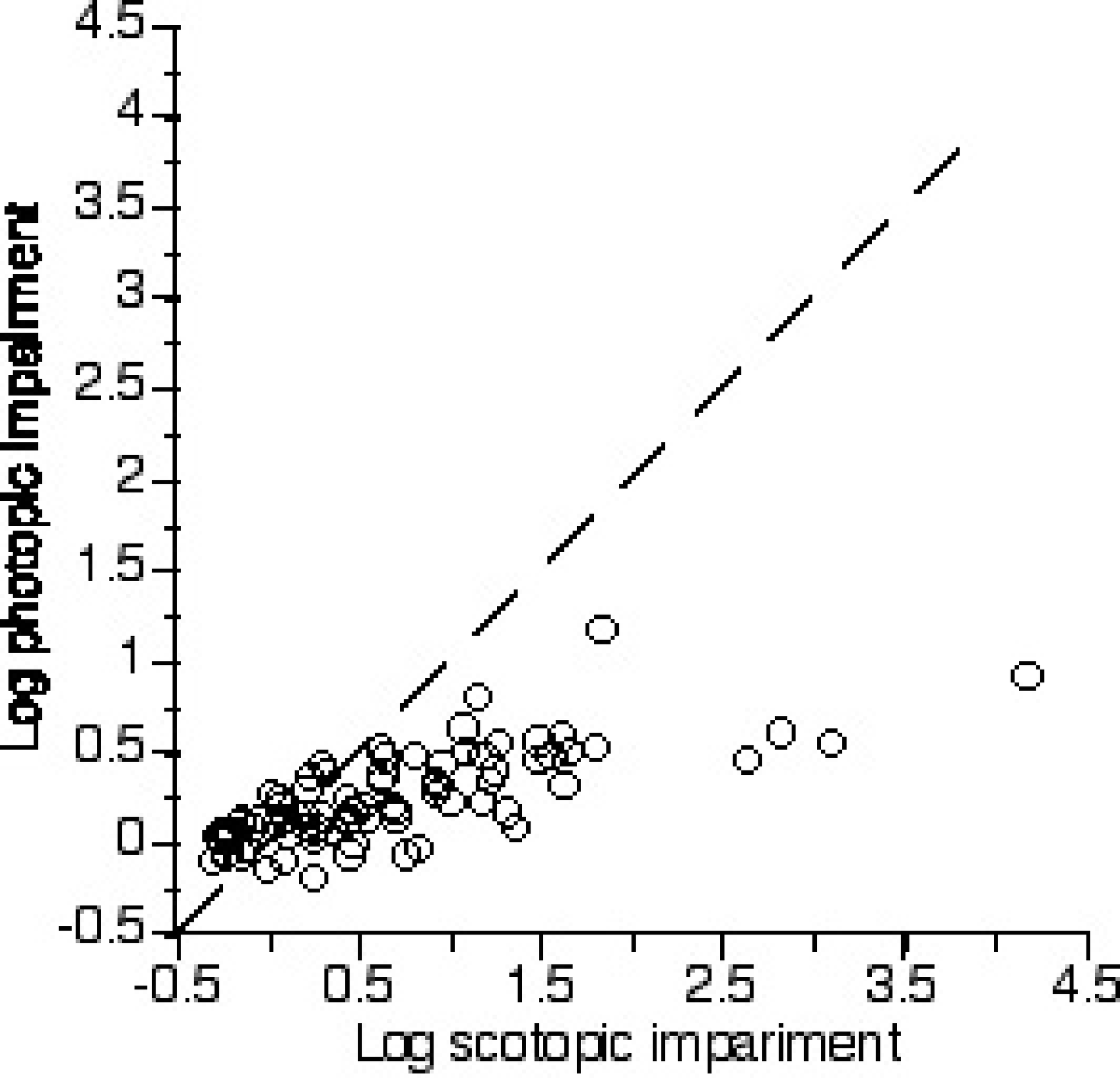
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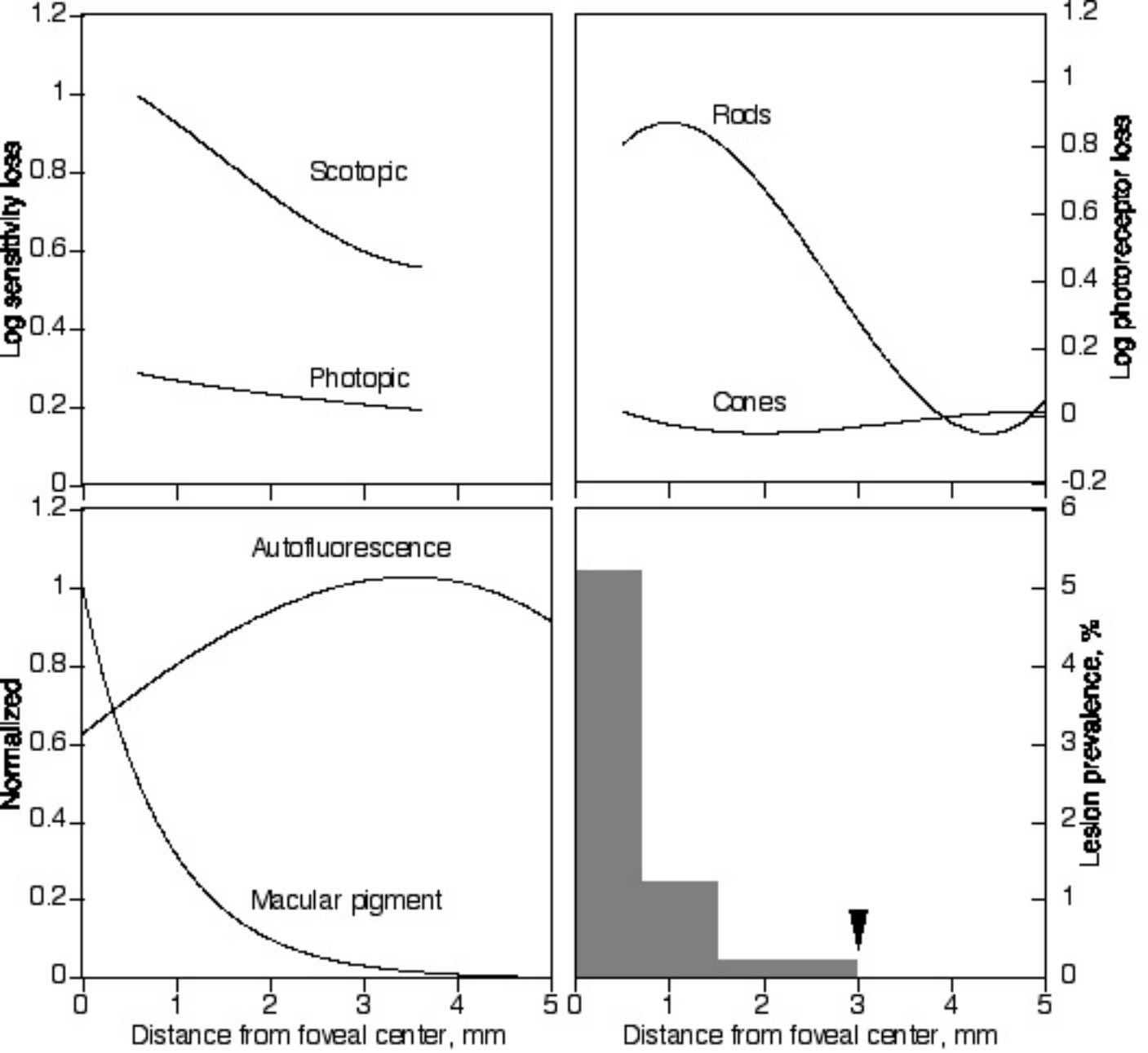
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Photopic
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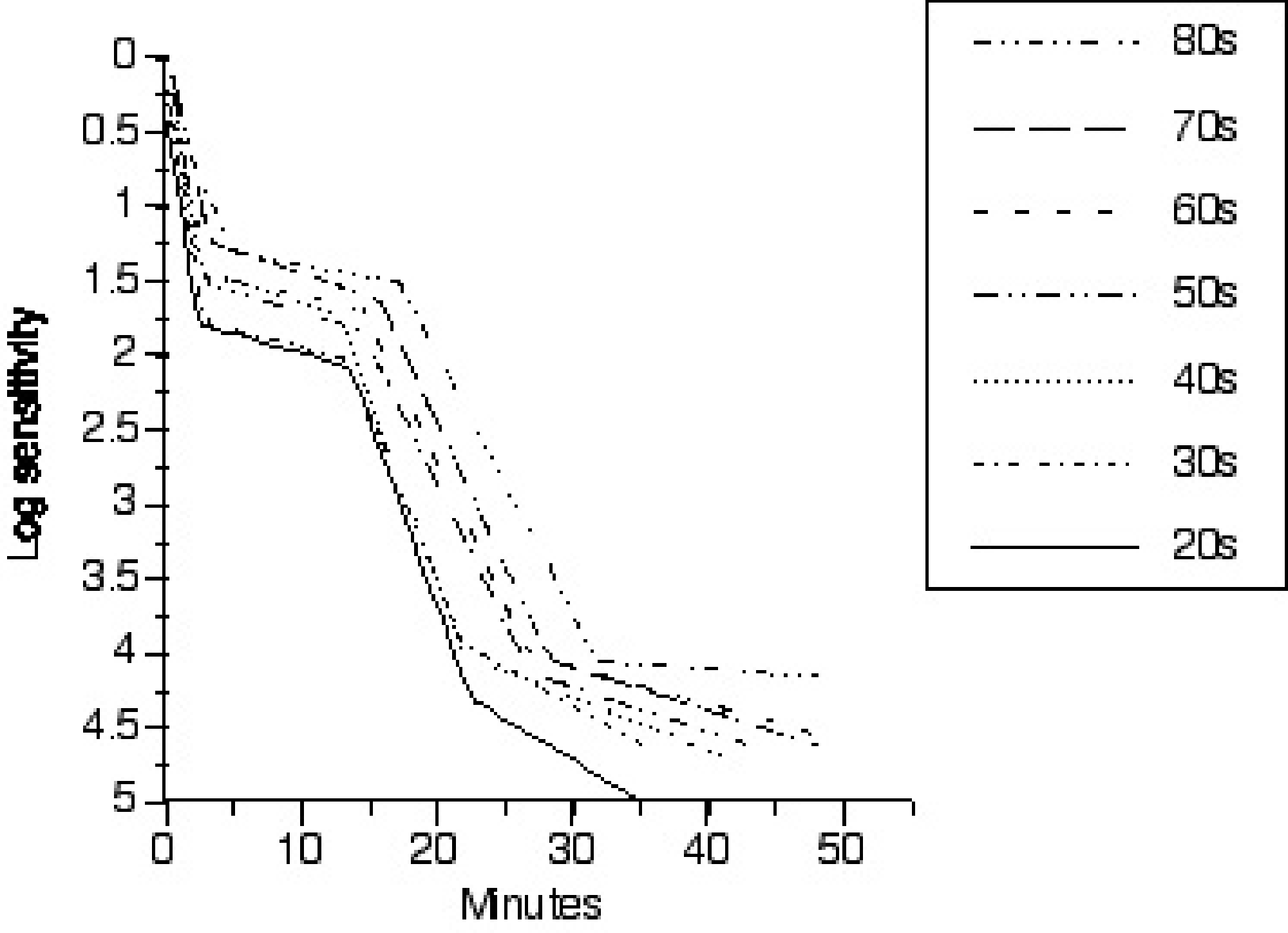
Log photoreceptor loss
Normalized
Autofluorescence
Macular pigment
Lesion prevalence, %
Distance from foveal center, mm
Distance from foveal center, mm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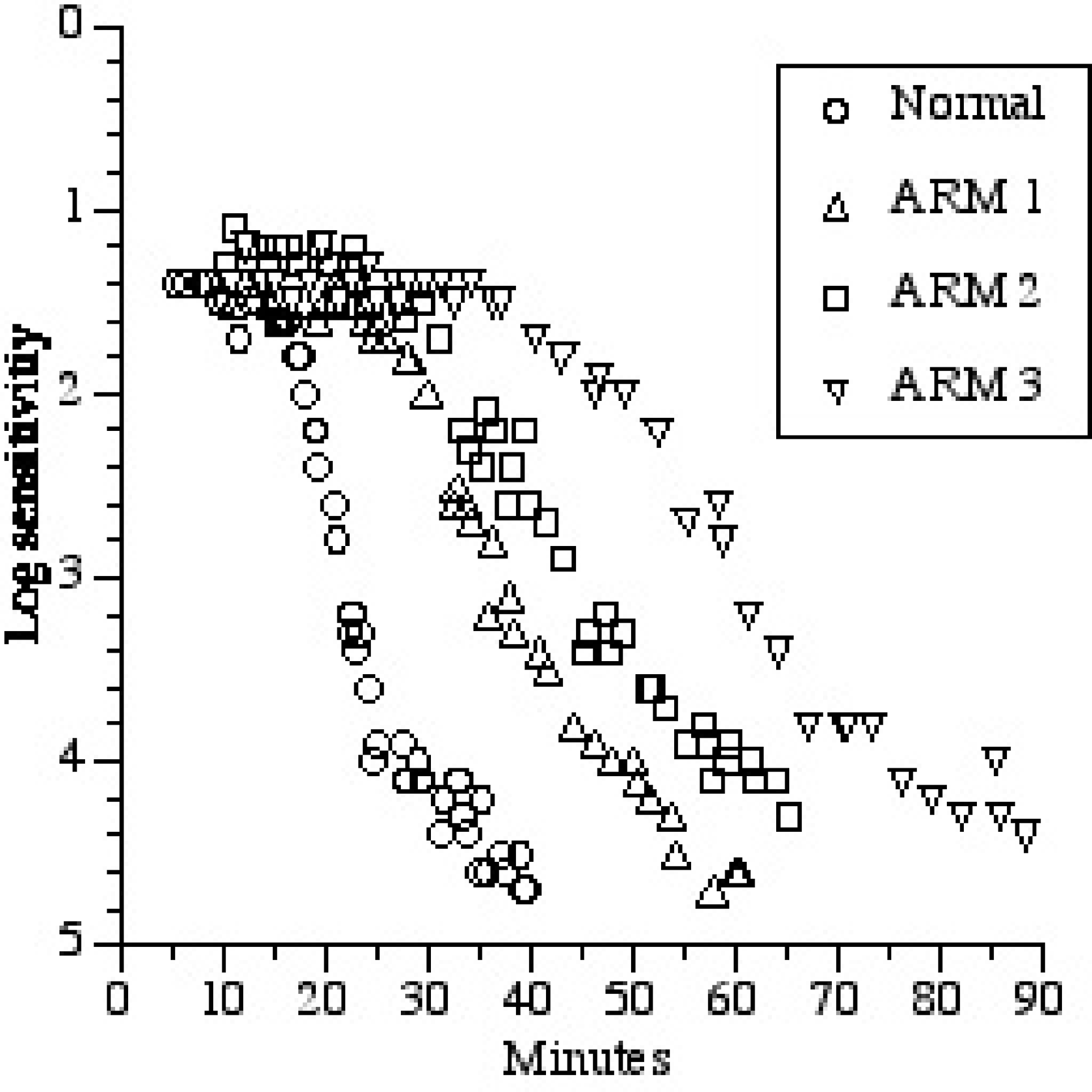
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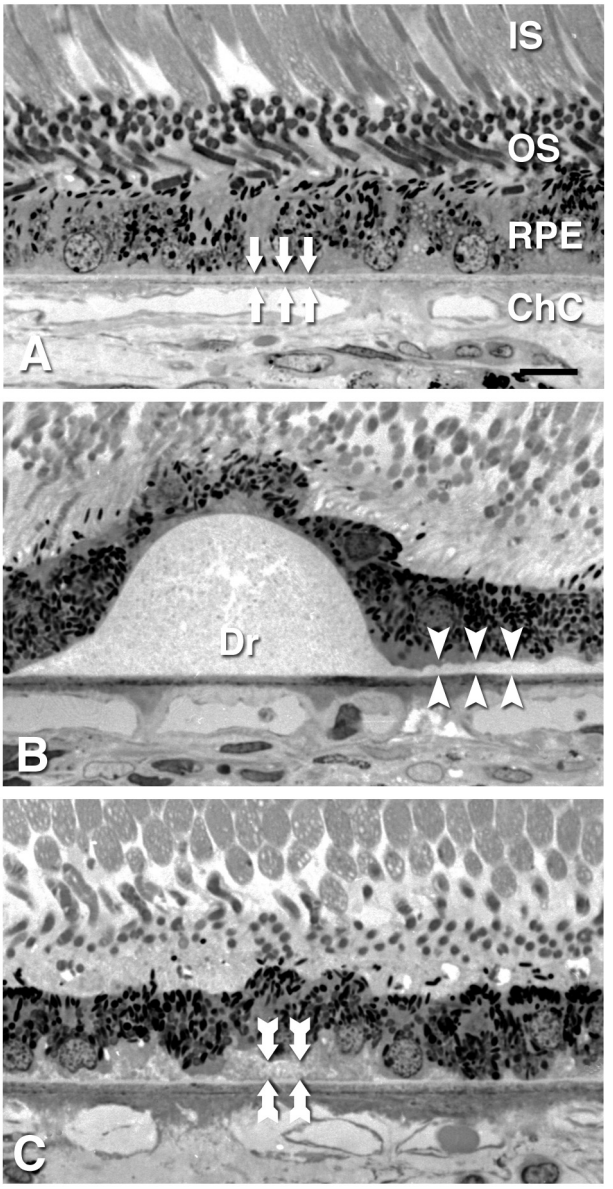
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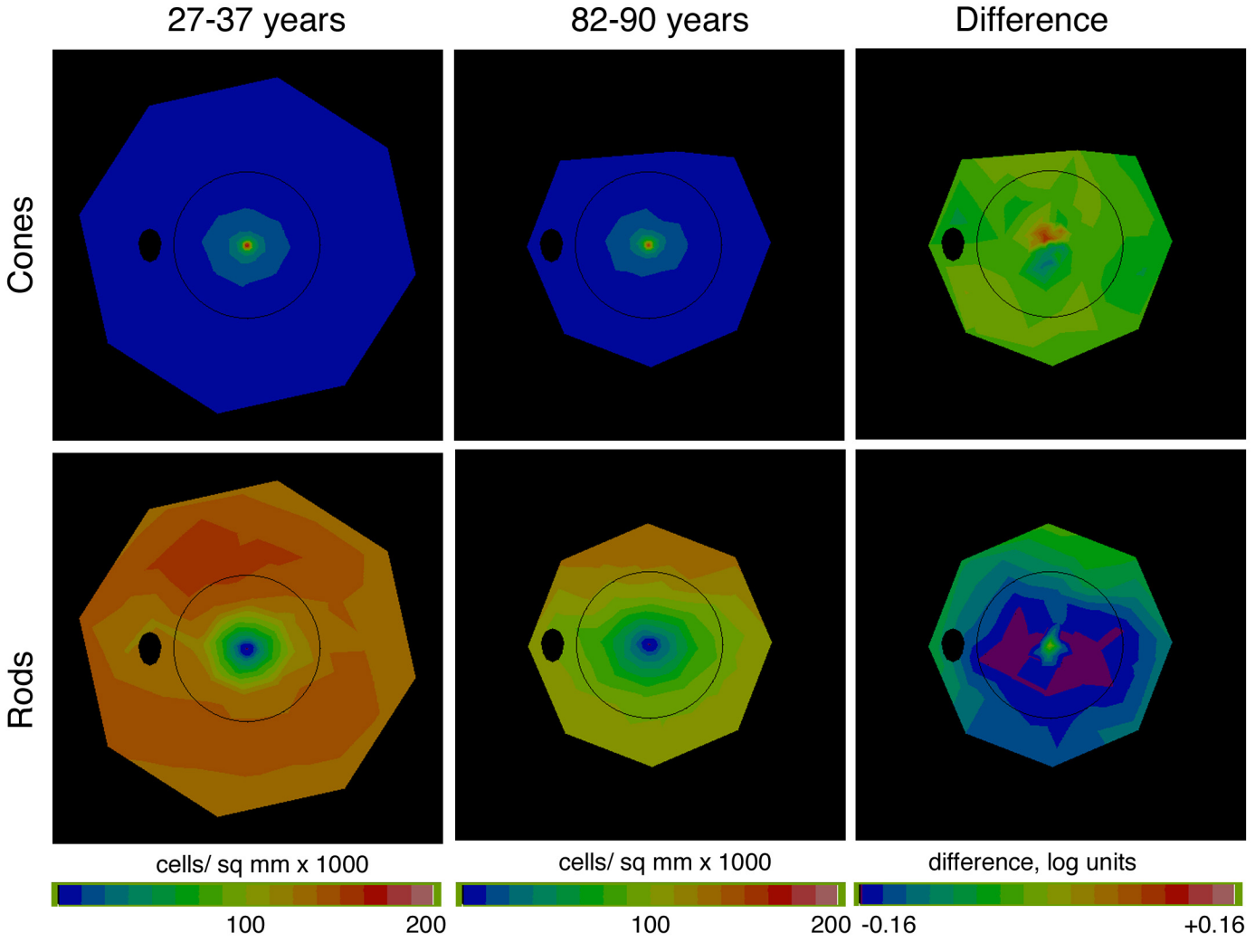
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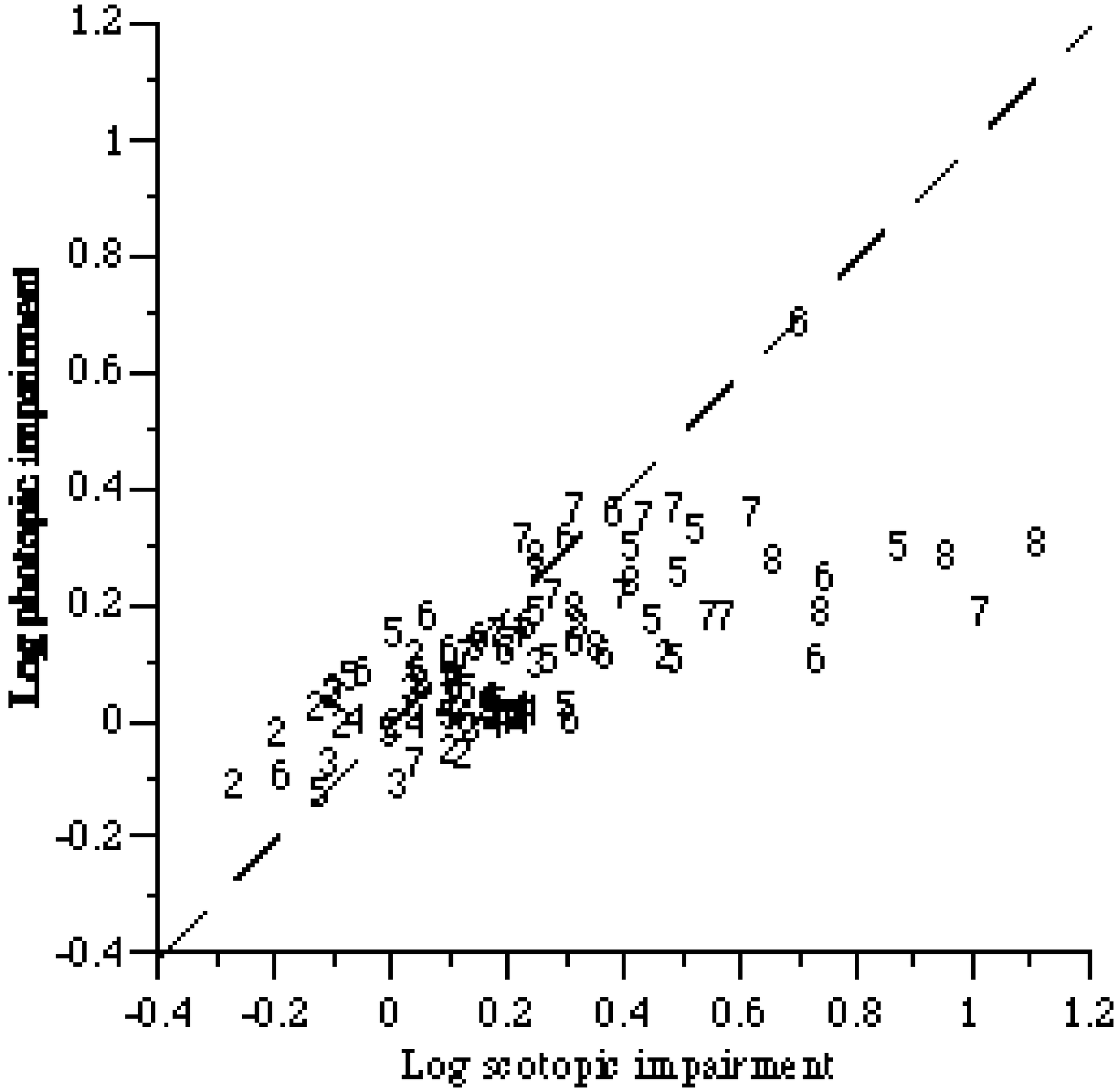
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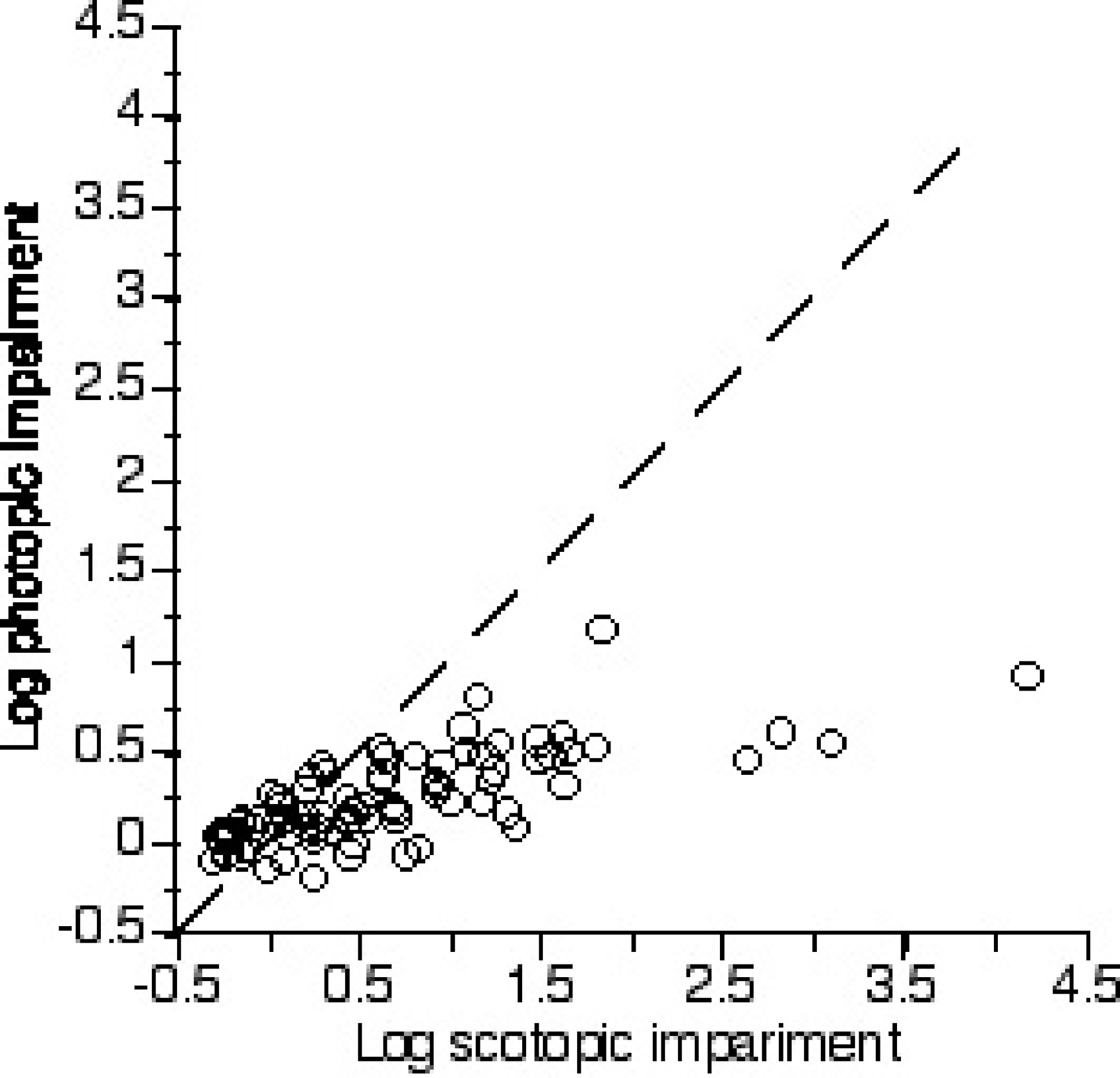
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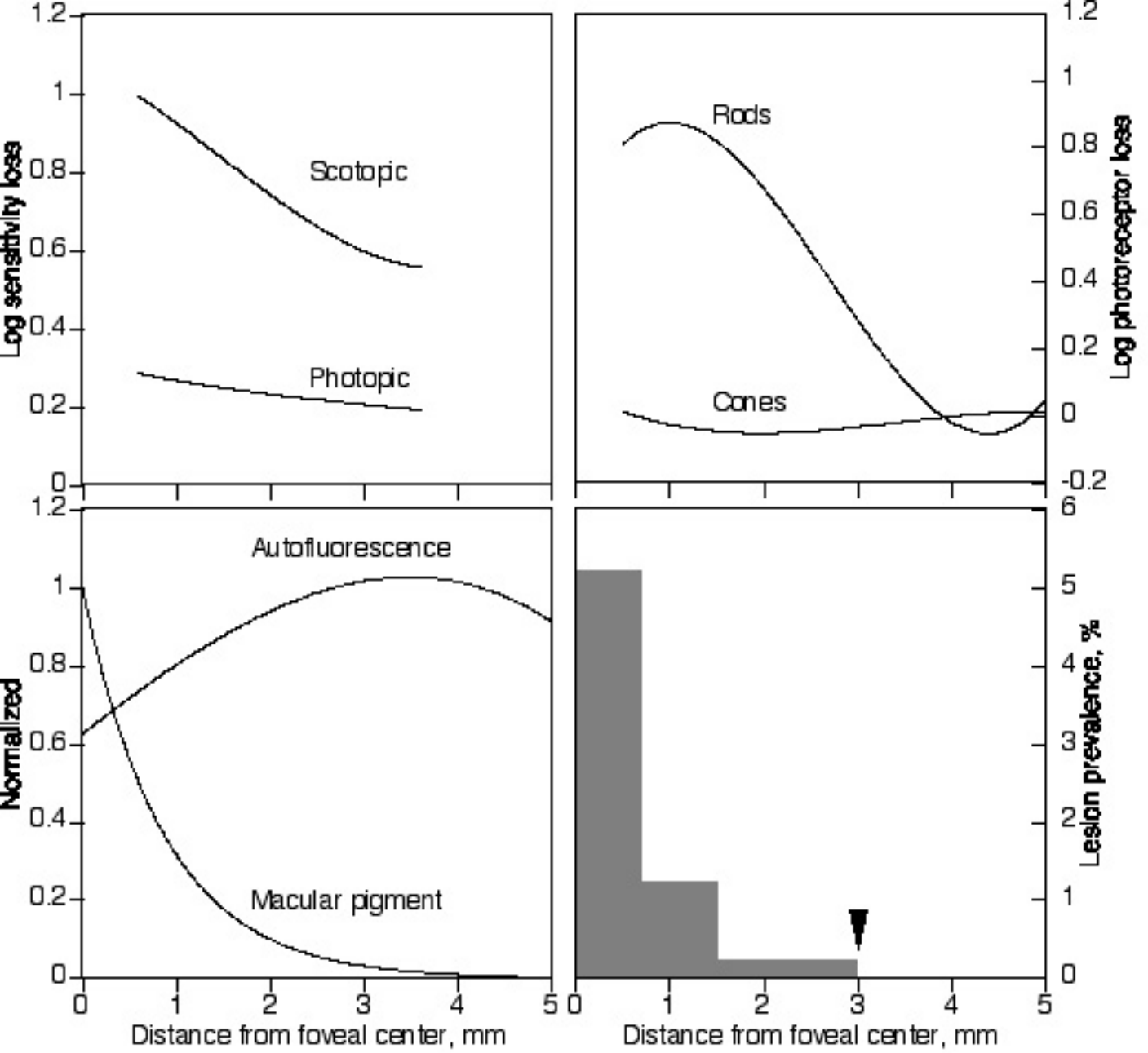
Log sensitivity loss
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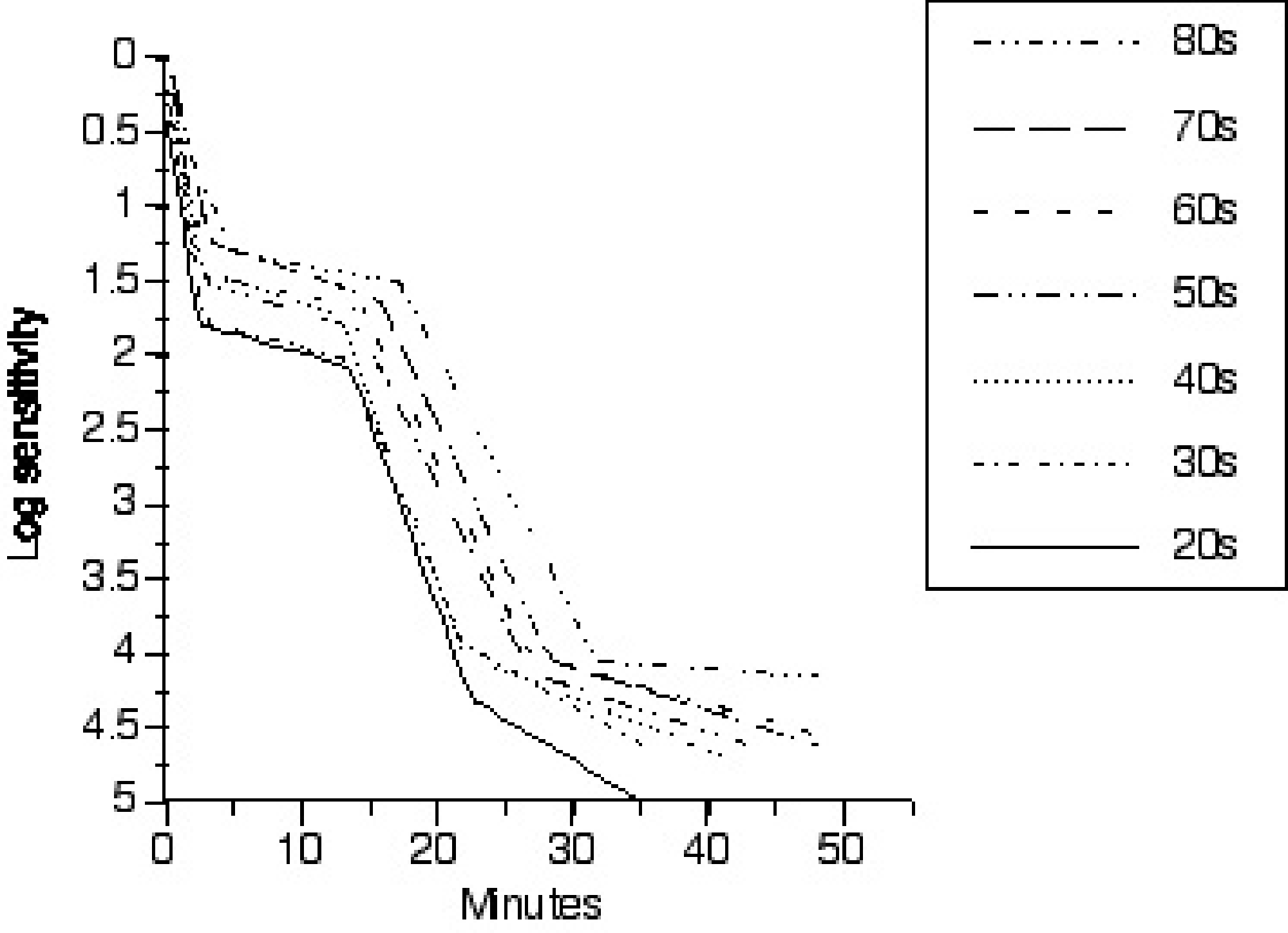
Autofluorescence
Macular pigment
Lesion prevalence, %
Distance from foveal center, mm
Distance from foveal center, mm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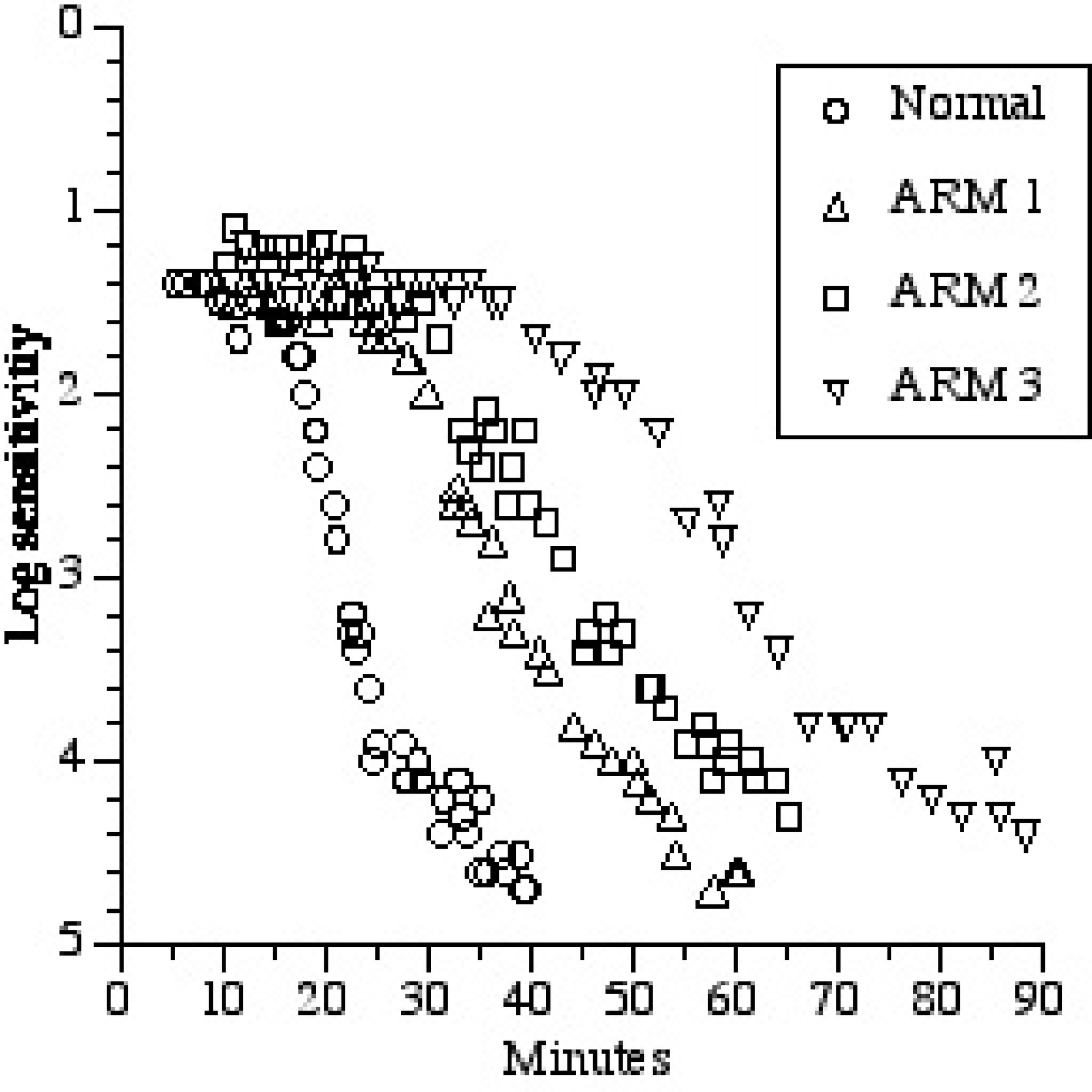
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