Reduced Retinal Deficits in an Albino Mammal with a Cone Rich Retina: A Study of the Ganglion Cell Layer at the Area Centralis of Pigmented and Albino Grey Squirrels

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In all albino mammals studied the central retina is underdeveloped and there is a rod deficit. Central ganglion cell density is ~25% below normal. This is not seen in birds, which have a cone dominated retina. Here we examine the ganglion cell layer in a cone rich mammal, the squirrel *Sciurus carolinensis leucotis*. Central cell densities were only <5% lower in the albinos than in pigmented squirrels. Squirrels are the only known albino mammal to survive successfully in the wild, reinforcing the notion that their visual deficits are minor. The relative immunity of these albino retinae from this deficits may be related to different patterns of cell production between rod and cone dominated eyes. © 1998 Elsevier Science Ltd. All rights reserved.

Albino   Retina   Optic chiasm

INTRODUCTION

Melanin related agents regulate retinal development, because in all mammals studied albino strains have a range of visual abnormalities. The central retina is underdeveloped, chiasmatic pathways are systematically disrupted, and there is a rod deficit (Stone, Rowe & Campion, 1978; Jeffery, Darling & Whitmore, 1994; Jeffery, 1997). Central retinal deficits are particularly marked in mammals where this region is normally highly specialised and displays a steep gradient in ganglion cell density between central and peripheral regions. In hypopigmented carnivores the reduction in ganglion cell density at the area centralis is approx. 25% (Stone *et al.*, 1978; Jeffery & Kinsella, 1992). It is of a similar magnitude in albino rabbits (Oyster, Takahashi, Fry & Lam, 1987) and marsupials (Jeffery *et al.*, submitted). In primates including man, developing ganglion cells migrate away from the central retina to form the fovea. This fails to occur in human albinos and cell density in the macular region is reduced (Elschnig, 1913).

Although deficits have been identified in every hypopigmented mammal that has been studied, they have not been observed in non-mammalian species. Even though central retinal specialisations are common in birds, where there is frequently a sharp gradient in cell density across the retinal surface, the central retina of the albino bird is normal (Jeffery & Williams, 1994). It is not clear why this species difference exists.

Unlike the vast majority of mammals, bird retinae are cone dominated (Walls, 1942). Rods are specifically affected in albinism, while cone numbers and their mosaic distribution are normal (Jeffery *et al.*, 1994). Hence, bird outer retinae may be relatively immune to this deficit. One way in which it may be possible to cast light on this problem is to examine the central retina of an albino mammal that is cone rich.

Although the spatial development of the retina is very similar between birds and mammals, the pace at which maturation proceeds is considerably greater in birds (Rager & Rager, 1978). Retinal neurogenesis is biphasic. In mammalian retinae, the vast majority of cells are produced in the second phase, when rods and bipolar cells are produced. Cones are produced in the first phase (Harman & Beazley, 1989). Hence, in a cone dominated retina there is a shift in patterns of cell production, with a larger proportion of cells generated early. Could there be a relationship between deficits found in the rod population and the cell deficits found in the ganglion cell layer at the area centralis? One way to test this hypothesis is to examine the central retina of an albino mammal that is cone rich. In such animals any cell specific deficits in the outer retina may be reduced because rods would form a much smaller proportion of the photoreceptor population.

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In nearly all mammals, the overwhelming majority of photoreceptors are rods, with few cones (Walls, 1942). However, squirrel retinae are cone rich. In California, ground squirrels rods form only about 10–15% of the photoreceptor population (Long & Fisher, 1983), while in gray squirrels, rods account for around 40% of the photoreceptor population (West & Dowling, 1975). Further, squirrels are highly visual animals, depending on their visual systems more than any other rodent (Anderson & Jacobs, 1972; McCourt & Jacobs, 1984). Here we examine the density of cells in the ganglion cell layer in the central retina of pigmented and albino gray squirrels *Sciurus carolinensis leucotis* to determine whether there is a deficit in cell density in the ganglion cell layer at the albino area centralis.

METHODS

The animals used in this study were obtained during routine culling in the wild. Albino squirrels occur occasionally in the U.K. and are established in small colonies at a number of locations. Pigmented and albino animals were trapped in baited cages and terminally anaesthetised with sodium pentobarbitone. All of the animals used here were mature. One albino that was trapped was not used because it appeared to be very old, having patchy hair loss and being thin. This raised concerns regarding age related ganglion cell loss that might have resulted in biassed counts. All of the albino animals had white coats and pink eyes. The heads were removed and placed in 10% formalin. Approximately 2–3 weeks later the eyes were removed and the retinae dissected free. Because squirrels have a flat horizontally elongated optic nerve head (Walls, 1942; Long & Fisher, 1983), retinal orientation was unambiguous. Retinae were whole mounted onto gelatinised slides, briefly air dried and Nissl stained. The area centralis was identified by sample counts made in the temporal retina. Once this was located, detailed counts of cells in the ganglion cell layer were made in a square measuring 0.028mm^2. All cells in the ganglion cell layer were counted. A grid was centred on the area centralis and counts were then made in alternate squares, in a chequerboard pattern around the area centralis covering approximately 50–100 squares. Limited counts were also made in the periphery of each retinal quadrant. All counts were undertaken with a X60 oil immersion lens. Peak cell density was calculated from the square with the highest value. The data presented in this study come from four pigmented and two albino animals.

Sections of skin from the neck were cut on a cryostat at approx. 12 μm. These were mounted onto slides and processed to determine whether the animals were tyrosinase$^+$ or $^-$ (Bancroft & Stevens, 1990). Tyrosinase is the key enzyme in the production of retinal melanin. Although it is possible to be tyrosinase$^+$ and be albino, the genetic basis of such forms of albinism are poorly understood.

RESULTS AND DISCUSSION

Skin sections from all of the pigmented animals confirmed that they were tyrosinase$^+$, while those from the albinos were tyrosinase$^-$. In all animals there was a clear region of increased cell density in the ganglion cell layer running in a horizonal streak under the optic nerve head, with a peak in density in the temporal retina. There was a marked gradient in cell density in the ganglion cell layer between the area centralis and the retinal periphery in both strains (Fig. 1, photomicrographs). There were no obvious differences in retinal blood vessel patterns between the strains, as has been reported in some hypopigmented animals (Stone et al., 1978).

Peak cell densities for pigmented and albino animals are given in Table 1 and are represented graphically in Fig. 2. They ranged roughly between 8000 and 9000 cells/mm^2. Although the albinos have a lower mean than that found in the pigmented animals, the difference between the two groups is relatively minor and is not statistically significant ($|t| = 1$, df $= 3$. NS). Figure 1 shows spline isodensity plots for the region of the area centralis in a pigmented and an albino squirrel. The patterns are very similar for the two animals. No attempt has been made to filter these data or to apply functions that would smooth the representations. Small irregularities in cell density in and around the area centralis were common in the both pigmented and albino animals and are faithfully represented in the figure. There were no consistent strain variations in any of the gradients in cell density in the region of the area centralis other than that the peaks were slightly lower in the albinos.

The results of this study show that the deficits found in the ganglion cell layer at the area centralis of the albino squirrel are much less marked than those found in other albino mammals, with an average reduction in cell density of < 5%. This compares with a reduction of approximately 25% in a range of mammalian species including carnivores, lagomorphs and marsupials (Stone et al., 1978; Jeffery & Kinsella, 1992; Oyster et al., 1987; Jeffery et al., in preparation). However, it should be noted that although the deficit is small in albino squirrels, the limited number of animals available for examination means that a comprehensive analysis is not possible, nor is it possible to estimate the range of variability in measures of their peak cell density in these animals.

That albino squirrels are not badly affected by the deficits normally found at the area centralis in albino mammals is supported by the observation that they had survived and bred in the wild. To the authors' knowledge, all other examples of mammalian albinism, with the exception of man, are either confined to the laboratory, domesticated, in agricultural environments or found in zoos. Presumably, this is because their visual abnormalities bestow disadvantages that would not allow them to survive and breed successfully in the wild. In the area where the squirrels used in this study were trapped, albinos had been seen for many years, where they lived in close association with normally pigmented animals, and as noted above, an old albino was actually trapped, but
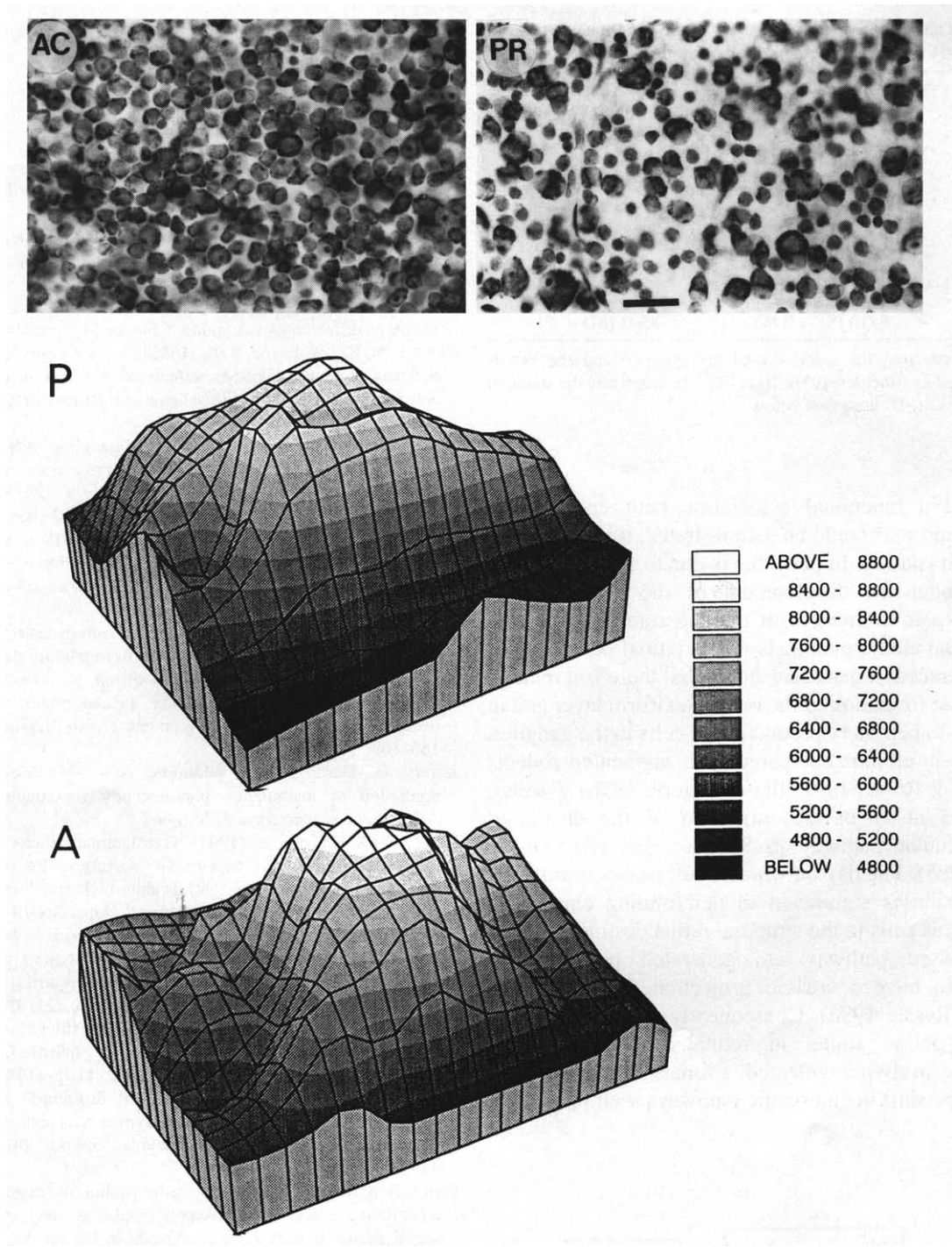
FIGURE 1. The two upper photomicrographs at the top of the figure show the relative cell densities found at the area centralis (AC) and at the peripheral retina (PR). Scale = 30 μm for both pictures. The spline plots are representations of the peaks in cell density in the ganglion cell layer at the area centralis in a pigmented (P) and an albino (A) squirrel. All cells stained in the ganglion cell layer were counted. There were variations in the patterns of the gradient around the area centralis between all of the animals, but none of these were consistent between the strains. These data have not been filtered. The length of the front axis of the spline plots is approximately 3.3 mm. In both cases the linear optic nerve head is above and slightly nasal to the plots. The long axis of each plot represents the horizontal retinal axis.

not used. Further, albino squirrels were observed for 2 days before the traps were set. During this period their behaviour was not distinguishable from that of normal animals. They were frequently observed to leap considerable distances often at tree top level, implying that their visual acuity was not significantly impaired by their albinism.

The results presented here are consistent with the notion that deficits in ganglion cell density at the area centralis in mammalian albinism are associated with the rod population. However, they do not reveal a functional association between rods and the development of the area centralis. Unfortunately, the relative rarity of albino squirrels precluded examination of their chiasmatic pathways and/or their outer retina, which may have cast further light on them.

TABLE 1. The peak number of cells/mm^2 in the ganglion cell layer of the retina in the animals used in this study

	Pigmented	Albino
	9141 (R)	8540 (R)
		8574 (L)
	9212 (R)	
		8362 (R)
	8236 (R)	8539 (L)
	8490 (L)	
	8935 (R)	
	8849 (L)	
Mean	8810 (SD = 346)	8503 (SD = 83)

Retinae taken from the same animal are grouped and the eye is identified as either left (L) or right (R). The mean and the standard deviations (SD) are given below.

Even if a functional association between the area centralis and rods could be demonstrated, it is difficult to envisage its nature. In part, this is due to the little that is known about the development of the albino retina, particularly in animals with marked retinal specialisations. In the albino mammals where retinal development has been traced, it has been shown that there is a relative delay in the formation of the outer plexiform layer and in the centre to periphery production of cells in the ganglion cell layer in albinos compared with pigmented rodents (Webster & Rowe, 1991; Ilia & Jeffery, 1996). Further, there is a delay in the formation of the uncrossed retinocollicular pathway in Siamese cats (Berman & Payne, 1985). The day on which a cell passes through its final division is significant in determining chiasmatic pathways, as cells in the temporal retina destined to have an uncrossed pathway are generated before those destined to have a crossed projection (Drager, 1985; Baker & Reese, 1993). Consequently, it is conceivable that the delays found in retinal development and outgrowth in hypopigmented animals could possibly produce the shift in chiasmatic pathways seen in albinos.
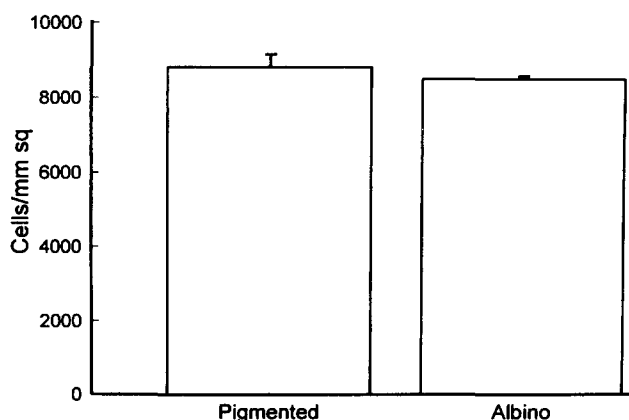

FIGURE 2. Histograms showing the mean peak cell densities at the area centralis in the ganglion cell layer in pigmented and albino squirrels. The data for these histograms come from Table 1. Although the peak is lower in the albino, the differences between the groups are not statistically significant.

However, this does not explain the other deficits found in them.

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