Abnormal Axons in the Albino Optic Tract

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PURPOSE. There have been suggestions that the misrouting of axons at the optic chiasm leading to the abnormal ratio of crossed to uncrossed axons in the optic tract of albinos is a consequence of abnormal timing of ganglion cell axon outgrowth. The sequence of genesis of ganglion cell classes and their axon outgrowth is correlated with the deep-to-superficial distribution of their axons by size in the mammalian optic tract. Optic tract axon order in albino and normally pigmented ferrets was, therefore, examined to determine whether an abnormal pattern of retinal ganglion cell genesis and axon outgrowth is evident in albinos.

METHODS. Light and electron microscopy were used to study axon diameters and myelin thickness of axons in the optic tracts of adult albino and pigmented ferrets.

RESULTS. In the optic tract, large-diameter axons are confined superficially in the normally pigmented ferret but are present throughout their depth in albinos. The abnormally located large axons and neighboring small-diameter axons in the albino have an abnormal axon diameter/myelin thickness ratio; large-diameter axons are poorly myelinated, and small-diameter axons exhibit an abnormally thick myelin sheath. These deep abnormal axons originate from the contralateral retina.

CONCLUSIONS. In addition to the known disruptions of normal organization in the visual system, albinos have an abnormal axon diameter distribution and a population of morphologically abnormal axons in the optic tract. These abnormal axons may represent the population of aberrantly crossed axons found in all albino mammals. (Invest Ophthalmol Vis Sci. 2009; 50:5516–5521) DOI:10.1167/iovs.09-3950

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Albinism is associated with a disruption of tyrosinase expression resulting in reduced melanin synthesis and, therefore, pigmentation. A consequence of this reduced melanin production in the retinal pigment epithelium of albino mammals is abnormal organization at various levels of the visual system from the retina through to the primary visual cortex. In albino primates, for example, a fovea is lacking or underdeveloped, and in other albino mammals, the retina, especially the area centralis, has an abnormally low complement of rod photoreceptors and rod bipolar cells. In the retinofugal pathway, a misrouting of axons arising from the temporal retina has been shown at the chiasm in all mammalian species studied. In all these species, an abnormally large proportion of retinal ganglion cell axons crosses the midline at the level of the chiasm and reaches the contralateral side of the brain. This axonal misrouting produces abnormal neural maps in the central target nuclei, such as the dorsal lateral geniculate nucleus and the superior colliculus, and ultimately anomalous maps in the more central targets, such as the primary visual cortex.

In normally pigmented rodents and carnivores, retinal ganglion cells in temporal retina with ipsilaterally coursing axons are more likely to be generated at earlier stages than cells with crossing projections from the same retinal region, and uncrossed axons from the temporal retina have been found to reach the optic chiasm at earlier stages than neighboring cells in the temporal retina with crossing axons. In all mammalian species examined, timing of retinal ganglion cell axon outgrowth has been linked to fiber reorganization along the retinofugal pathway. In ferret, for example, fiber order in the juxtafascicular optic nerve and optic chiasm is a product of the timing of growth of axons through the region during development.

It may not be surprising, therefore, that mistiming of axon growth through the chiasmal region has been proposed as potentially involved in the misrouting of axons from the temporal retina across the chiasmal midline. For example, some have suggested that the ipsilaterally projecting axons from temporal retina are delayed in their course to the optic chiasm in albinos. Furthermore, the timing of other features of visual system development has also been shown to be disrupted in albinos, such as a delay in cell production in the retina that is most obvious late in development.

If the abnormal chiasmal routing of axons is, even partially, a consequence of abnormal timing of outgrowth during development, an important question is where along the pathway such mistiming could be appropriately assayed. In normally pigmented mammals, the temporal sequence of genesis of retinal ganglion cell classes and the timing of their axonal growth is best seen in the order of axons in the optic tract. The axons of the first-born cells are found deepest in the tract, and those from the latest born cells are found most superficially. Given that different retinal ganglion cell classes in each species are generated at different developmental stages and each cell class has axons of different diameter ranges, the different axon diameter ranges, therefore, exhibit distinct distributions across the depth of the optic tract. The aim of this study was to examine the axon diameter distributions in the optic tracts of albino ferrets to determine whether they display abnormalities consistent with the disordered retinal ganglion cell development proposed in albinos.

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RESULTS

Abnormal Distribution of the Largest Axons in the Optic Tract of Albino Ferret

Observations of the optic tract in normally pigmented ferrets show the partial segregation of large-, medium-, and small-diameter axons. The segregation is most obvious for the large-diameter axons. This is evident in Figure 1A, in which the deep half of the tract (upper part of the micrograph) is clearly free of these large axons. Figure 1B shows, at greater magnification, that the deep margin of the tract is devoid of the large axons, which, by contrast, have a pronounced presence at the superficial border (Fig. 1C). This substantiates earlier observations of axon diameter distributions in the optic tract of normally pigmented ferrets.

The distribution of axons by size in the albino ferret optic tract is in stark contrast to that in the normally pigmented ferret. Large-diameter axons can be seen in the deep and the superficial parts of the tract of the albino. Figure 2A shows a relatively low-magnification view of an albino optic tract in which the large-diameter axons can be found across the entire depth of the optic tract. Figure 2B shows, at higher magnification, the presence of large-diameter axons that are not found at a similar location in the pigmented tract (compare this region with the equivalent region in Fig. 1B). In contrast, the superficial region of both the albino tract (Fig. 2C) and the pigmented tract (Fig. 1C) appear to have similar populations of large axons.

To quantify the apparent difference between the deep optic tracts of albino and normally pigmented ferrets, the distribution of the largest axons (with diameters greater than 2 μm) was measured at this location in both strains (Fig. 3). The number of these axons was divided by the total number of measured axons in the deep tract of each of the 10 animals to compare the prevalence of the large axons in the deep tract of both strains. It is clear that the proportion of deep large axons is always greater in albino than in pigmented animals. Those axons greater than 2 μm in diameter represent 15.5% of all the axons measured deep in the optic tract of pigmented animals, whereas this proportion is 37.1% in albino animals. This difference is significant, according to a two-tailed unpaired Student’s t-test (t_9 = -4.39; P < 0.01).

Abnormal Myelin Sheath Thicknesses in the Albino Optic Tract

Further light microscopic observation of the large axons in the deep and superficial regions of the albino optic tract suggests
that although their internal diameters are within a similar range, there appears to be a difference in the myelin sheath thickness of the large axons in the deep tract (Fig. 2B) compared with those located superficially (Fig. 2C). The similarity of the sheath thicknesses for the superficially located large axons in both strains (compare Figs. 1C and 2C) suggests that it is the deep large axons in the albino optic tract that are abnormal.

Comparison of the deep large axons in Figure 2B with the large axons found superficially in either the albino (Fig. 2C) or the pigmented (Fig. 1C) animals appears to suggest that the large axons found deep in the albino tract have an abnormally thin complement of ensheathing myelin. The ultrastructure of these large axons deep in the albino optic tract was, therefore, examined. Figures 4A and 4B show electron micrographs of axons in the deep optic tract of a pigmented and an albino ferret, respectively. The abnormally large axons are evident in the albino optic tract, and they have a thin myelin sheath relative to their internal diameters compared with their smaller diameter neighbors (see axons indicated by open arrows).

Unexpectedly, these micrographs also suggest that it is not only the large axons deep in the albino optic tract that appear to exhibit this abnormal myelin-diameter relationship; some of the small diameter axons have what appears to be a disproportionately large myelin sheath (Fig. 4B, stars).

The ratio between the internal and external diameters of axons—the g-ratio—was, therefore, compared in equivalent regions of the albino and pigmented optic tracts (Fig. 5). This g-ratio describes the coefficient of myelination in relation to the axonal diameter. The electron micrograph of the pigmented animal’s deep optic tract shows no large-diameter axons and shows the degree of myelination normally found in axons of this region. This normal organization is characterized by a relatively constant g-ratio that reflects a gradient of increasing myelin thickness with increasing axon diameter (Fig. 5, filled diamonds). In contrast, axons of widely varying diameters in the deep optic tract of the albino appear to have a less evident relationship to their myelin thickness (Fig. 5, open circles). There is instead a trend toward an increasing g-ratio with increasing internal diameter compared with the relatively consistent relationship in the pigmented ferret.

For axon diameters smaller than 1 μm, the g-ratio for the deep axons in the albino tract (mean, 0.556 ± 0.093 μm) was >20% smaller than that for the pigmented animals (mean, 0.703 ± 0.075 μm). That is, for small axons, the myelin sheath is thicker in the albino than in the normally pigmented ferret. However, for axons of diameters between 1 and 2 μm, the difference between the different phenotypes is much less marked (albino mean, 0.658 ± 0.067 μm; pigmented mean, 0.681 ± 0.034 μm). Further, for the largest diameter groups (>2-μm diameter), a reversal of the tendency observed in the smallest diameter axons is found. In other words, the g-ratio for the deep axons in the albino tract (mean, 0.785 ± 0.070 μm) was almost 8% larger than for those of the pigmented animals (mean, 0.728 ± 0.030 μm). It should be noted that the g-ratio values for the largest axons in the optic tract of pigmented animals were collected from superficially located large axons examined. Figures 4A and 4B show electron micrographs of axons in the deep optic tract of a pigmented and an albino ferret, respectively. The abnormally large axons are evident in the albino optic tract, and they have a thin myelin sheath relative to their internal diameters compared with their smaller diameter neighbors (see axons indicated by open arrows).
given their absence from the deep part of the optic tract in these animals. Hence, as expected from visual observations, these data reveal a poor degree of myelination relative to axon diameter for the largest axons in the deep half of the albino optic tract.

Although many of the axons deep in the albino optic tract have an abnormal complement of myelin, the myelin sheaths appear morphologically normal. Electron microscopy comparison of the myelin sheaths of axons deep in the pigmented and albino optic tracts reveals no evident differences, and the distances between major dense lines and intraperiod lines within the compacted myelin lamellae are comparable in transverse sections (Fig. 6.).

**Ocular Origin of Abnormal Axons**

To determine whether the abnormally distributed axons have a retinal origin, both optic tracts were examined in an albino ferret in which one eye was removed 10 days before perfusion, allowing the discrimination of axons according to their eye of origin. Figure 7 shows both optic tracts of this animal. The micrograph on the left indicates the prevalence of the abnormally distributed large axons in the deep part of the optic tract contralateral to the remaining eye (Fig. 7A). Close examination of this deep region revealed no degenerate (i.e., ipsilaterally coursing) axons. Although few in number, as might be expected because of the chiasmatic misrouting of axons in albinos, such degenerate darkly staining profiles were predominately located in the more superficial half of the tract. In contrast, the abnormal axons were not evident in the optic tract ipsilateral to the remaining eye (Fig. 7B). Here, very few intact axons could be observed because the contralateral axons have degenerated after enucleation.

These data provide evidence that the abnormal axons deep in the albino optic tract are not a population of axons arising aberrantly from within the brain or displaced axons of the supraoptic commissures; rather, they belong to the retinofugal pathway. Furthermore, these observations clearly indicate that the abnormal axons take a contralateral course at the optic chiasm.

**Figure 5.** Albinism affects the myelination of optic axons. This plot demonstrates the different distributions of g-ratios for axons in the deep region of the optic tract in albino (*open circles*) and pigmented (*filled diamonds*) ferrets relative to their internal diameter. It can be seen that there is a virtually constant g-ratio for axons of different diameters in the pigmented ferret optic tract. In contrast, the albino g-ratio increases in parallel with internal diameter, suggesting a distinct relationship between myelin thickness and internal diameter in this phenotype. In addition, these measurements confirm our light microscopy study: abnormally large axons can be found deep in the albino ferret optic tract. Note that large-diameter axons that are found predominantly in the superficial part of the pigmented ferret optic tract (*filled triangles*) are included for comparison.

**Figure 6.** Compacted myelin lamellae of abnormal axons appear morphologically normal. These electron micrographs show the organization of the myelin sheath of a deep optic tract axon in a normally pigmented ferret (A) and of two adjacent abnormal axons deep in the albino optic tract (B). Ultrastructurally, the compacted myelin lamellae appear to exhibit no significant differences. Scale bar, 0.2 μm.

**Figure 7.** Transverse sections of both optic tracts of a monocularly enucleated albino ferret. This figure shows the optic tracts contralateral (A) and ipsilateral (B) to the remaining eye of an albino ferret in which one eye was removed 10 days before perfusion. The deep border of each optic tract is uppermost in both photomicrographs. (A) Note that the large axons are particularly visible in the deep part of the tract. (B) In contrast, no large-diameter axons are visible in the optic tract contralateral to the remaining eye. This indicates that the abnormal large axons are retinal ganglion cell axons that have crossed the chiasmatic midline. Scale bar, 50 μm.
DISCUSSION

The albino retina and visual pathways have been shown previously to be abnormally organized.1,2 The observations in this present study show a previously unreported additional abnormality, one that affects the morphology of retinal ganglion cell axons in the optic tract.

In albino ferrets, the segregation of retinal ganglion cell axons according to diameter is less pronounced than it is in pigmented ferrets. The deep part of the albino optic tract contains many large axonal profiles, whereas the equivalent location in the tract of pigmented animals is devoid of these large axons. Furthermore, the proportional relationship between myelin thickness and axonal diameter, the g-ratio, is inverted in these abnormal large axons and in the smallest diameter axons of the deep half of the albino optic tract.

The order of axons of different diameters in the mammalian optic tract has previously been shown to reflect the addition of axons of different classes during development, forming a deep-to-superficial, first born-to-last born, organization in the optic tract.19–21,28,31 The altered axon order in the albino optic tract might, therefore, indicate an abnormal pattern of retinal ganglion cell axon outgrowth in this phenotype because of a hypothesized temporal disruption of retinal maturation and the time of arrival of axons in the developing chiasm in albino.23,24 This could be confirmed or not using the ganglion cell birth dating techniques previously used for normally pigmented mammals, which have indicated a correlation between axon diameter distribution in the optic tract and the sequence of ganglion cell class genesis.28–30

The abnormality found in the albino ferret tract could, however, have resulted from an abnormal morphology of some cells rather than an abnormal sequence of axon outgrowth. Some abnormal “giant” cells have been found in the Siamese cat retina, for example.54,55 Our observations of the optic nerves of albino ferrets have not revealed any abnormal profiles, and though this suggests that the abnormality may only occur postchiasmatically, axons are smaller in the nerve36 and are less segregated,21 and an abnormal population may be less likely to be identified.

In general, the larger an axon’s diameter, the thicker its myelin sheath. This truism is challenged, however, by the observation of an abnormal ratio between axon diameter and myelin thickness for axons deep in the albino optic tract. Our results show that the g-ratio is abnormal for small and large axons in albino ferrets, but that myelin thickness is roughly similar to the thickness found in medium-diameter axons normally found in this tract location in pigmented ferrets. In other words, the myelin thickness is relatively uniform regardless of axon size; therefore, the processes that govern the degree of myelination may be uncoupled from those mechanisms regulating axon diameter in the albino ferret’s deep optic tract.

In all mammalian albinos studied, there is a decrease in the number of ganglion cell axons coursing ipsilaterally at the optic chiasm with a corresponding increase in the number of contralaterally projecting axons.8–13 This study shows that the abnormal axons in the albino optic tract have a retinal origin and are contralaterally projecting axons. Furthermore, the abnormal axons in the albino optic tract are located deep in the optic tract, whereas the majority of ipsilaterally projecting axons are found in normally pigmented ferrets.22,37 It seems possible, therefore, that the axons with abnormal morphology are those axons that are misrouted at the optic chiasm of albinos.

This study was driven by the initial hypothesis that axonal misrouting at the optic chiasm in albinos may reflect abnormal timing of retinal ganglion cell axon outgrowth. However, if the abnormal axons in the optic tract do represent axons that were misrouted, it could be argued that the axons are at the expected deep location but that the disrupted axon diameter/myelin thickness relationship is a consequence of their location in the wrong optic tract. One further implication of our results may be that the mechanisms of myelination in the optic tract have specificity for particular subsets of axons, in this case for axons with distinct retinal origins.

Finally, it seems likely that the abnormal diameter and the abnormal myelination may result in functional changes in these retinal ganglion cell axons, especially with regard to the conduction velocity of the neural signal along their length. Such axonal abnormalities may explain observations in albino humans in whom the latency of visual evoked potentials has been found to differ from that seen in subjects with normal pigmentation.38–40

References


