The Journal of Experimental Biology 206, 2665-2673

doi:10.1242/jeb.00470

Regeneration of ultraviolet-sensitive cones in the retinal cone mosaic of thyroxin-challenged post-juvenile rainbow trout (*Oncorhynchus mykiss*)

Craig W. Hawryshyn*, Garnet Martens†, W. Ted Allison and Bradley R. Anholt

Department of Biology, University of Victoria, PO Box 3020 Stn. CSC, Victoria, British Columbia, Canada V8W 3N5

*Author for correspondence (e-mail: chawrysh@uvic.ca)

†Present address: BioImaging Facility, University of British Columbia, 6770 University Blvd, Vancouver, British Columbia, Canada V6T 1ZA

Accepted 29 April 2003

Summary

Previous studies in our laboratory have examined the loss of ultraviolet-sensitive (UVS) cones and UV sensitivity. This study looks at the question of regeneration of UVS cones and its topographic distribution, along with several other measures of the cone mosaic. Topography of the cone mosaic in rainbow trout smolts (post-metamorphic juveniles) was examined under normal growth conditions and during an exogenous thyroid hormone (TH) challenge. Growth of trout retina was studied over six weeks. Retinas sampled at 0, 3 and 6 weeks were embedded in EPON resin, and thick (1 μm) tangential sections were stained with Richardson's stain. Sites representing central ventral, ventral, temporal, dorsal and nasal retina were sampled. Variables measured were cone densities, mean double cone diameter and mean spacing between cones of the same type. These same variables were compared with those of fish that were challenged with L-thyroxin (T4), and regeneration of UVS cones was assessed. Principal components of the correlation matrix of all photoreceptor measurements were analysed using analysis of variance. Here, we show several interesting effects of thyroxin exposure on post-metamorphic rainbow trout: (1) controls at week 0 have a high density of UVS cones in the temporal and dorsal sampling regions and a high density of blue (short-wavelength)-sensitive (SWS) and double cones across all regions sampled; (2) both control and TH-treated fish had less abundant, larger and less tightly packed SWS and double cones and a lower density of UVS cones in the temporal and dorsal sampling regions three and six weeks into the experiment compared with the starting condition at week 0; (3) fish treated with TH had a higher UVS cone density in the nasal and ventral sampling regions and there were higher densities of SWS and double cones in the central ventral, temporal and ventral regions, but lower densities in the nasal sampling regions, relative to the controls. The regeneration of UVS cones into the ventral retinal hemisphere in post-juvenile salmonids has important implications for visually guided behavior.

Key words: retina, teleost, salmonid, fish, ultraviolet-sensitive cones, ontogeny, topographic mapping, principal component analysis, neuroregeneration.

Introduction

Salmonid fishes undergoing the process of smoltification have elevated levels of thyroid hormones (Barron, 1986; Hoar, 1988; Datta et al., 1999; Eales, 1990). Thyroid hormones play a central role in controlling many of the alterations in physiology, morphology and behavior noted during this metamorphic transition in juvenile salmon (Hoar, 1988). Thyroid hormones have also been identified in altering the visual system during smoltification. During migrations from freshwater to marine environments, the visual pigment composition of the retina changes from vitamin A_2 to A_1, and changes in this ratio can also be induced by exposure to thyroxin (T4; Alexander et al., 1998; Allen, 1977; Cristy, 1974). The use of a deiodinase inhibitor, methimazole, blocks activation of T4 and inhibits these pigment alterations (Alexander et al., 1998).

The loss of corner single cones from the square cone mosaic occurs during smoltification (Lyall, 1957b; Bowmaker and Kunz, 1987; Hawryshyn et al., 1989; Kunz, 1987; Novales-Flamarique and Hawryshyn, 1996). Corner single cones have been identified as the ultraviolet-sensitive (UVS) cone through a combination of spectral sensitivity experiments (loss of UV sensitivity), histology (decrease in number of corner single cones; Beaudet et al., 1993; Bowmaker and Kunz, 1987; Loew and Wahl, 1991) and *in situ* hybridization against opsin mRNAs (Allison et al., 2003). Ultrastructural evidence has shown that the UVS cones disappear as a result of apoptosis (Kunz et al., 1994). Browman and Hawryshyn (1992, 1994) mimicked smoltification by treating parr rainbow trout with T4 and described a similar loss of both UV sensitivity and UVS cones, which was thought to be a uniform loss across the retina. However, a recent study (Deutschlander et al., 2001), using functional mapping techniques, demonstrated that a T4

challenge on parr resulted in ventral loss of UV sensitivity but had no effect on the dorsal UV sensitivity.

Sexually mature Pacific salmon return to their natal streams to spawn and eventually die. Analysis of sexually mature salmon retinas revealed the presence of cones in the position of UVS cones (Beaudet et al., 1997). A recent study (Novales-Flamarique, 2000) has shown that sexually mature sockeye salmon (*Oncorhynchus nerka*) have UV sensitivity and the retina exhibits a similar UVS cone distribution to that of a parr. Together, these studies suggest that a natural pattern of neural plasticity exists in the salmonid retina and that UV sensitivity and UVS cones regenerate at or near the time of sexual maturity. Challenging rainbow trout (*Oncorhynchus mykiss*) smolts with T4 has been shown to result in a return of UV sensitivity (Browman and Hawryshyn, 1994). UVS cones also reappeared during this treatment, although spatial distribution of the UVS cones was not analysed. Because UVS cones have subsequently been shown to be present in some locations of smolt retinas (initial analysis in this paper, see Fig. 1; Allison et al., 2003), the histology may have been confounded by sampling location. Thus, it is important to re-examine this regenerative event employing a topographic analysis. The connection between the timing of natural reappearance of UVS cones and the elevation in T4 in sexually mature salmonids has not been examined but it is proposed that increased serum T4 leads to the regeneration of UVS cones (Beaudet et al., 1997).

The first objective of the current study was to compare T4-challenged and control rainbow trout smolt retinas to determine UVS cone distribution and to quantify T4-induced UVS cone regeneration. The second objective consisted of two parts: the first was to determine whether significant growth of the control retina occurred in a short time span of six weeks, and the second compared T4-challenged fish with the controls to determine whether the normal retinal growth was altered with T4 challenge and, in particular, whether these fish exhibited regionally specific UVS cone regeneration.

Materials and methods

Fish holding and care

Rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) weighing 60–140 g were obtained from the Fraser Valley Fish Hatchery (Provincial Government of British Columbia, Abbottsford, BC, Canada), maintained in a freshwater flow-through system (University of Victoria, Aquatic Holding Facility) and kept on a 12 h:12 h light:dark cycle for at least eight weeks prior to experimentation (fluorescent lighting; model F32T8/735; Luxline; Atlanta Light Bulbs Inc., Tucker, GA, USA; colour temperature 3500°K, 40 W lamps). Water temperature was maintained at 15±1°C. Fish were fed a daily ration of BioDiet Grower pellets (Nelson and Sons Inc., Murray, UT, USA). Procedures described herein were in accordance with the guidelines established by the Canadian Council on Animal Care. The University of Victoria Animal Care Committee approved all experimental protocols.

Experimental design

All treatments were performed between December 1999 and February 2000. Fish were placed in 80-litre tanks in groups of 6–8, where they remained for the duration of the experiment. Fish were allowed to accommodate to their new surroundings for two days on aerated flow-through water. Fish were fed twice a week to satiation in order to minimize mass gain.

Experimental fish (12 in total) were exposed to an exogenous bath of L-thyroxin (T4; Sigma T2501) with a final concentration of 300 μg l^{-1} T4 dissolved in 0.1 mol l^{-1} NaOH. Half the volume of the water was changed daily and fresh T4 was added to maintain concentration. The method of T4 delivery has been previously used in salmonids to elevate the serum levels of thyroid hormone (Eales, 1965; Browman and Hawryshyn, 1992). Control fish (12 in total) were treated identically except that only 0.1 mol l^{-1} NaOH was added to the water. An additional six fish were sacrificed at the initiation of the study to establish starting conditions. Control and experimental fish were sacrificed at week 3 (T4-treated, N=6; control, N=6) and at week 6 (T4-treated, N=6; control, N=6).

Dissections and fixation

All dissections were performed between 12.00 h and 15.00 h. Light-adapted fish were euthanized by deeply anaesthetising them in tricaine methanesulphonate (0.3 mg ml^{-1}; Cresent Chemicals, Phoenix, AZ, USA) for at least 10 min. The eyeballs were marked for orientation by cutting the cornea at the dorsal edge prior to removal from the socket. When possible, the right eye of each fish was used for all analyses. Retinas were dissected in 0.1 mol l^{-1} phosphate buffer (PBS), pH 7.4. Retinas were fixed overnight in 4% formaldehyde, secondarily fixed in 1% glutaraldehyde in PBS for a minimum of two hours at room temperature, with a final fixation in 4% osmium tetroxide in PBS overnight at 4°C (except the week 0 control group, which were not fixed in osmium tetroxide).

Retinas were flattened and secured to Sylgard in a petri dish. An overhead projector was used to allow clear notation of sampling location on an expanded view of the retina projected onto a wall. Retinal pieces were embedded in EPON according to Luft (1961). To facilitate the dehydration of many pieces of retina (80 pieces, two retinas at a time), a strainer for the retinal pieces was constructed from a 96-well culture dish [bottom surface removed and 100 μm Nitran mesh (Argent Labs, Redmond, WA, USA) attached]. Thick sections (1 μm tangential sections) were cut using glass knives and treated with Richardson's stain.

The retinas from each group were sampled at five standard sites (central ventral, ventral, temporal, dorsal and nasal) per retina for histological examination, counts and measurements. One retina from each group had at least 15 other sites chosen to generate a retinal map of UVS cone and mosaic type distributions (Fig. 1).

Photomicrographs were taken using a 10-bit digital black-and-white camera (Q-Imaging, Burnaby, BC, Canada) through a 100× oil immersion lens on a Zeiss Axioskop-2 microscope.

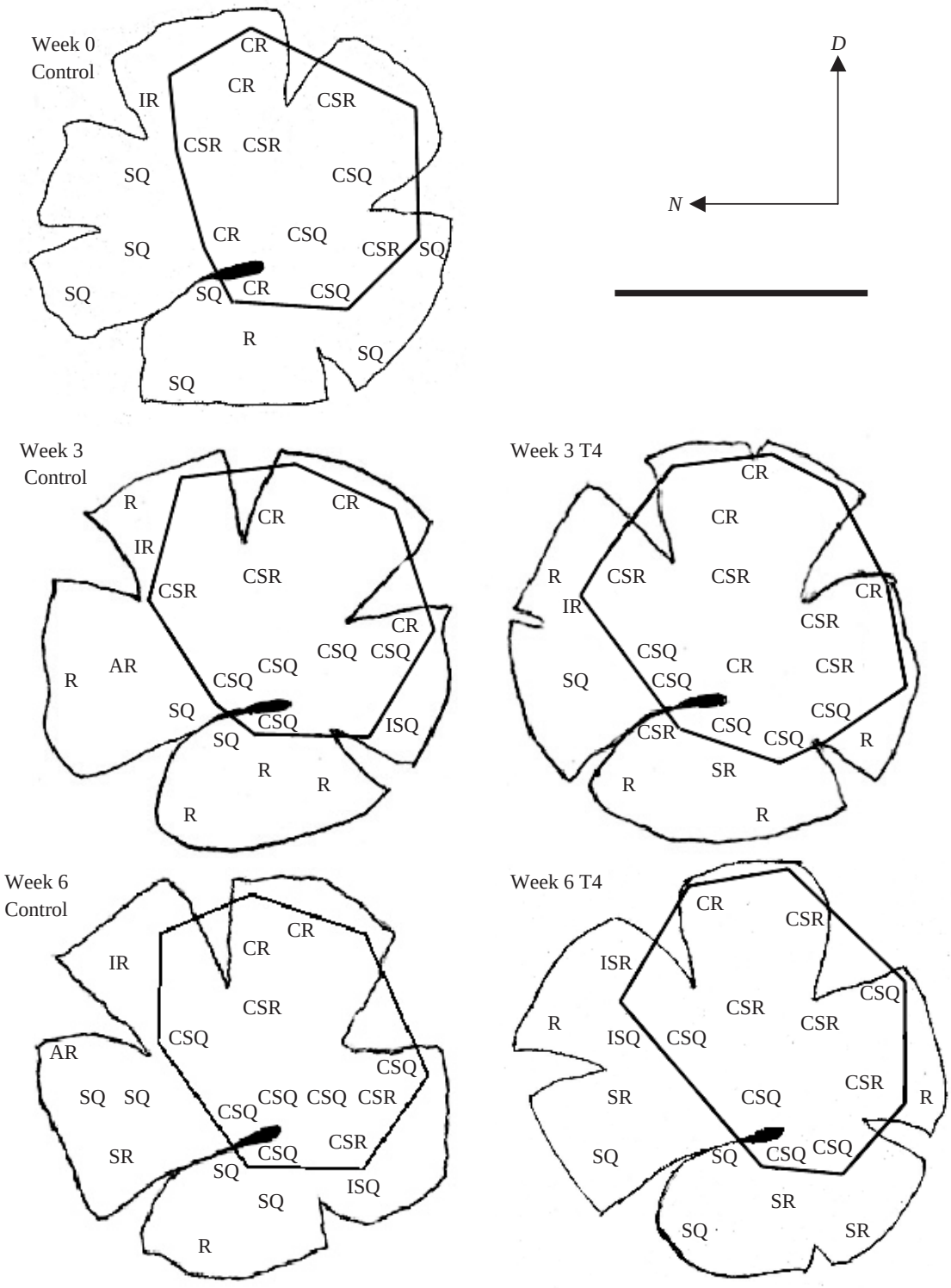

Fig. 1. Retinal maps showing the distribution of mosaic types for each group. Retinas are oriented with the dorsal pole (D) at the top and the nasal pole (N) at the left. SQ, square mosaic [ultraviolet-sensitive (UVS) cones completely missing]; CSQ, complete square mosaic; R, row mosaic (UVS cones completely missing); IR, incomplete row mosaic (most UVS cones missing); CR, complete row mosaic; SR, square-row mosaic; CSR, complete square-row mosaic; AR, alternating row mosaic. Scale bar, 10 mm.

Digital photomicrographs were captured using Q-Capture V1.01 software (Q-imaging). Image quality was adjusted using Adobe Photoshop 4.0.1. To standardize measurements within and between fish retinal samples, images were taken at a focal plane where the blue (short-wavelength)-sensitive (SWS) cone outer segments were equal. At this focal plane, all cone types were clearly identifiable. This procedure was used for all retinas and sections examined.

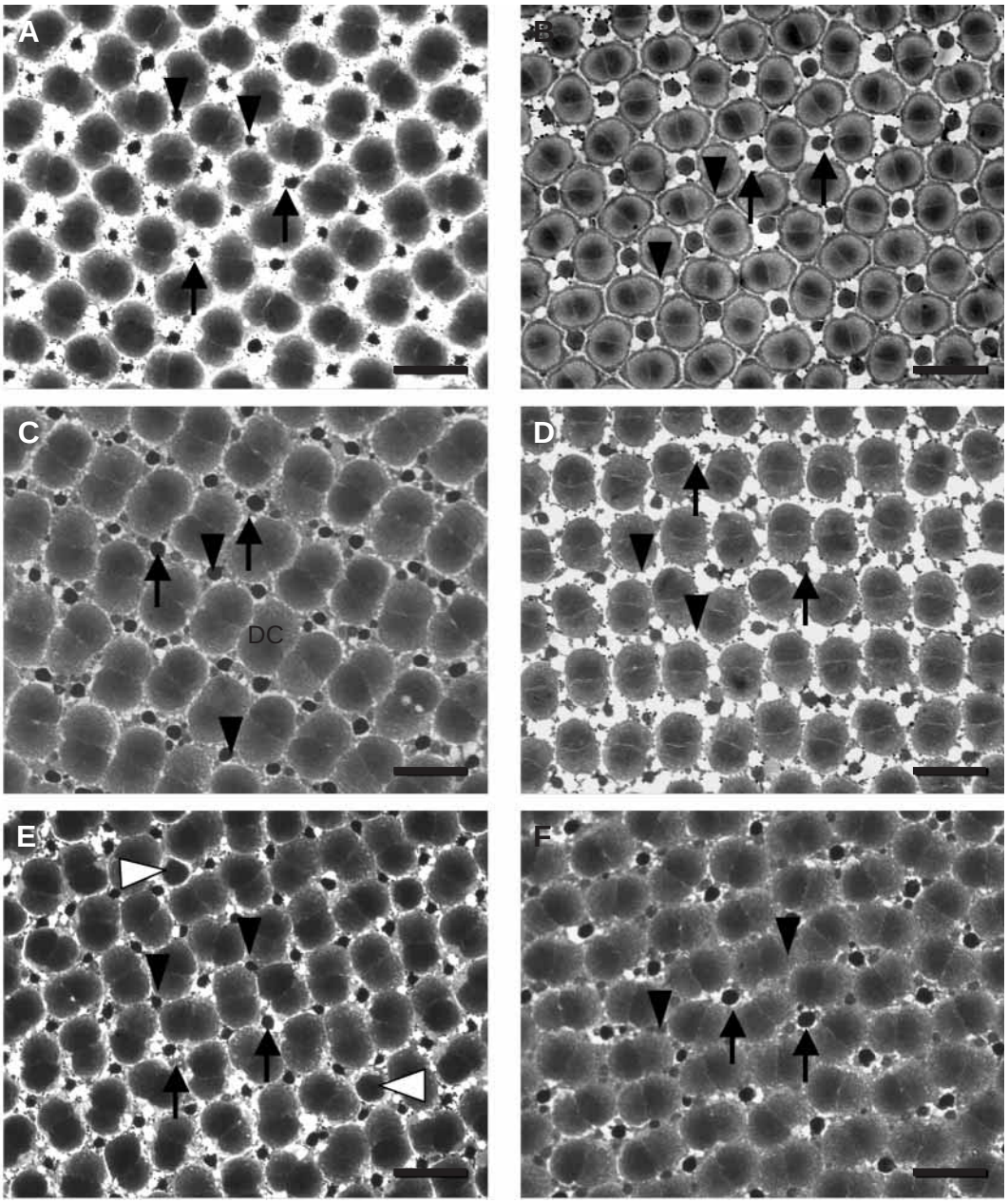

Fig. 2. Mosaic types present in the rainbow trout smolt retina. (A) Complete square mosaic. (B) Incomplete square mosaic. (C) Complete row mosaic. (D) Incomplete row mosaic. (E) Complete square-row mosaic. Note the large single cones identified by the white arrowheads. (F) Incomplete square-row mosaic. For all mosaics, arrows point to short-wavelength-sensitive (SWS) cones, while arrowheads point to ultraviolet-sensitive (UVS) cones (complete mosaics) or where UVS cones are missing (incomplete mosaics). Scale bars, 20 μm for all mosaics.

Five regions of the retina were examined (as listed above) and six measurements were made in each region for a total of 30 variables per fish. The six measurements were: (1) UVS cone density; (2) SWS cone density; (3) double cone density; (4) double cone diameter; (5) distance between double cones and (6) distance between SWS cones.

Measurements consisted of cone densities within square blocks of double cones (multiples of 11 by 11 double cones), double cone diameter perpendicular to the partitioning membrane, and cone spacing, the distance between neighbouring cones of the same type. Optimas™ computer software (Mediacybernetics, Carlsbad, CA, USA) was used for the physical measurements. The mean of double cone diameter and spacing between cones was used in the analysis because there were many samples from each animal pooled into one large sample, whereas with cone density only one measurement per animal was obtained.

Statistical analysis

The 30 variables are likely to be highly inter-correlated.

Within any region, increases in the density of one type of cone will have to come at the expense of the density of other cones or their size. Increasing the density of cones will reduce the distance between them. In addition, developmental cues almost certainly affect more than one region of the retina at a time. Analyzing each of these variables separately can easily lead to false rejections of the null hypothesis of no treatment effect because of the repeated tests.

An alternative to analyzing each measurement separately is to extract principal components of the correlation matrix (Quinn and Keough, 2002) and analyze the principal components. Principal components are interpreted by the contribution of individual variables to the component scores. Sequential components are, by definition, uncorrelated with each other. This method thus summarises many variables into fewer uncorrelated variables while retaining most of the variance in the original data. Because many of the variables are alternative measurements of the same underlying process (e.g. density of double cones and distance between double cones), the principal components can be more accurate than the original measurement.

Six individuals were missing up to six measurements because one region of the retina was damaged during preparation. Principal components can only be calculated for complete measurement sets so we imputed the missing data with the mean for all other measurements of that variable regardless of their treatment or time of measurement (Quinn and Keough, 2002). This is conservative because it increases the variance within treatments without increasing the variance among treatments, thereby not biasing the principal component scores towards rejection of the null hypothesis. It also allowed us to make full use of all the animals in the experiment.

We analyzed the first two principal components using general linear models with Type III sums of squares. We excluded the week 0 controls in the analysis, which collapsed into a simple two-way analysis of variance with equal sample sizes.

In situ *hybridization*

In situ hybridization of opsin probes onto trout retina was carried out using standard protocols, as described previously (Allison et al., 2003). Briefly, retina was cryosectioned (10 μm thickness), treated with proteinase, acetylated and hybridized with dioxigenin labelled antisense riboprobe corresponding to the rainbow trout SWS1 (UVS cones; 596 bp in length; GenBank accession number AF425074) or SWS2 (SWS cones; 734 bp in length; AF425075) opsin genes. Riboprobe was detected using anti-dioxigenin antibody conjugated to alkaline phosphatase (Roche Biochemicals, Basel, Switzerland), and a dark precipitate was produced using BCIP/NBT [5′-5-bromo-4-chloro-3-indolyl-phosphate 4-toluidine salt (Gibco-BRL, Gaithersburg, MD, USA)], plus nitroblue tetrazolium (Gibco-BRL), with levamisole (Sigma, St Louis, MO, USA). Whole-mount *in situ* hybridization was carried out in a similar manner on pieces of neural retina dissected from dark-adapted trout, however no acetylation was required. For whole-mount retina, the development of signal utilized the alkaline phosphatase substrate FastRed (Roche Biochemicals) to produce a fluorescent signal. This signal was detected using epifluorescence and the above microscope with a standard rhodamine filter set.

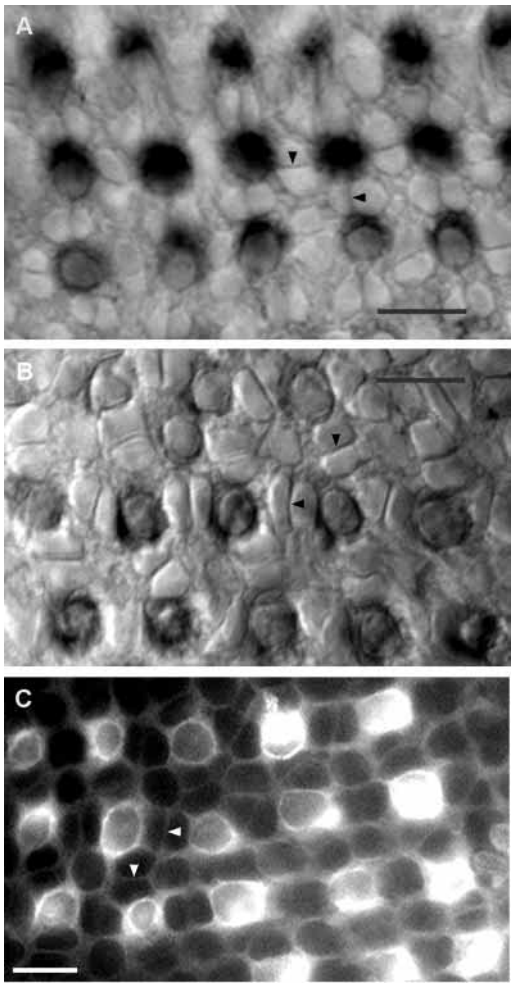

Fig. 3. Confirmation of cone identity by *in situ* hybridization using riboprobes against ultraviolet-sensitive (UVS) and short-wavelength-sensitive (SWS) opsins. (A) SWS opsin mRNA appears only in the central single cones. (B,C) UVS opsin mRNA appears only in the corner single cones. Arrowheads point to the partitioning membrane of the double cones. The partitioning membrane points to the central single (SWS) cone. A and B are 10 μm cryosections of retina in a tangential plane, and *in situ* labelling is represented by a dark precipitate. C is whole-mount retina in a tangential view, with fluorescent labelling of riboprobe. Scale bars, 10 μm.

Results

Cone mosaics in rainbow trout smolts – confirmation of cone class identity

All groups studied exhibited examples of complete square mosaic (with UVS cones) and square mosaic without corner cones (Fig. 2A,B), along with complete row mosaic and row

Table 1. *Principal component analysis table with loading values for pertinent variables and retinal locations*

	UVS density	SWS density	Double density	Double diameter	Distance double	Distance SWS
PC1						
Central ventral	–0.027	0.175	0.169	–0.201	–0.209	–0.215
Dorsal	0.228	0.238	0.235	–0.164	–0.189	–0.214
Nasal	0.014	0.171	0.179	–0.108	–0.194	–0.185
Temporal	0.167	0.215	0.213	–0.163	–0.183	–0.198
Ventral	0.074	0.184	0.162	–0.188	–0.186	–0.199
PC2						
Central ventral	0.072	0.182	0.178	–0.002	0.031	0.106
Dorsal	0.050	0.001	–0.017	0.236	0.0777	0.095
Nasal	0.177	–0.279	–0.272	0.356	0.280	0.304
Temporal	–0.127	0.176	0.201	0.016	–0.111	–0.149
Ventral	0.230	0.253	0.305	–0.085	–0.096	–0.121

Note that only the first two principal components were used in our analysis as they account for most of the variance in the original data.
UVS, ultraviolet-sensitive; SWS, blue (short-wavelength)-sensitive.

mosaic without UVS cones (Fig. 2C,D). There were select regions that contained a mosaic pattern that appeared to be intermediate between a square mosaic and row mosaic (Fig. 2E,F).

In situ hybridization for the UVS and SWS opsin mRNA, performed on tangential cryosections, was used to conclusively demonstrate cone identities (Fig. 3). As defined in our histological examination of plastic-embedded sections (e.g. Fig. 2A), the partitioning membranes of double cones pointed to the central single cones, which clearly express the SWS opsin mRNA as indicated by the dark precipitate (Fig. 3A). However, corner single cones expressed only UVS mRNA (Fig. 3B). Because the mRNA for the UVS opsin is vitread relative to the other cone opsins in teleosts (Allison et al., 2003; Hisatomi et al., 1996, 1997; Raymond et al., 1993), it was not possible to acquire a large area of tangential section where the UVS opsin mRNA and the double cone partitioning membrane can both be visualized. Thus, we also completed *in situ* hybridization labelling of UVS mRNA in whole-mount retina, indicated by the fluorescent label, which clearly validated the conclusion that the corner single cone is the UVS cone (Fig. 3C). We have previously established the specificity of these labelling protocols through double labelling experiments that show that the UVS and SWS opsin localize to separate single cone photoreceptors and that the SWS opsin mRNA co-localizes within cells labelled by our antibody against goldfish SWS opsin (see Allison et al., 2003 for details on *in situ* hybridization methodology and analysis). Finally, we have used these labels to demonstrate that a dramatic decrease in cells expressing UVS opsin mRNA, but no decrease in cells expressing SWS opsin mRNA, was associated with a decrease in sensitivity to UV light measured with electroretinograms (Allison et al., 2003). Photoreceptor labelling as a methodology to obtain morphometric data can be an effective strategy, although labelling techniques require tissue processing that obscures much of the histological detail. Because the current study was directed at questions regarding the exact spacing and morphology of cone types and we are now able to conclude that the corner single cone is the UVS cone, plastic sections were the most appropriate methodology.

Anomalies in the cone mosaic

The cone mosaic was not consistent between all sample sites. In some examples, mosaics exhibited extensive branching. This extensive branching was usually associated with the optic nerve head. Many cases of branching were associated with the presence of a large single cone (Fig. 2E) that was present in all areas of the retina but seems to be in higher numbers in the temporal retina. In many cases, the large single cone replaced a double cone in the mosaic. Sometimes, however, these large single cones were not in the position of a double cone and these situations usually exhibited branching in the mosaic. Triple cones sometimes appeared in the position of double cones in the mosaic and also caused a slight disturbance, similar to the large single cones, in the mosaic.

Principal component analysis and the effects of thyroxin exposure

The first principal component (PC1) accounted for 48.3% of the variance in the full set of measurements. PC2 accounted for an additional 10.0% and PC3 an additional 7.7%. These three derived variables accounted for almost two-thirds of the total variation in the initial data set of 30 variables. For the purposes of this paper, we will describe the results for PC1 and PC2.

The density of SWS and double cones in all regions of the retina loaded strongly on PC1, as did the densities of UVS cones in the temporal and dorsal regions. The densities of UVS cones in other regions loaded weakly on PC1. The diameter and spacing of all cone types in all regions of the retina loaded strongly and negatively on PC1 (Table 1). Thus, high positive values of PC1 in subsequent discussion (e.g. Fig. 4) reflect

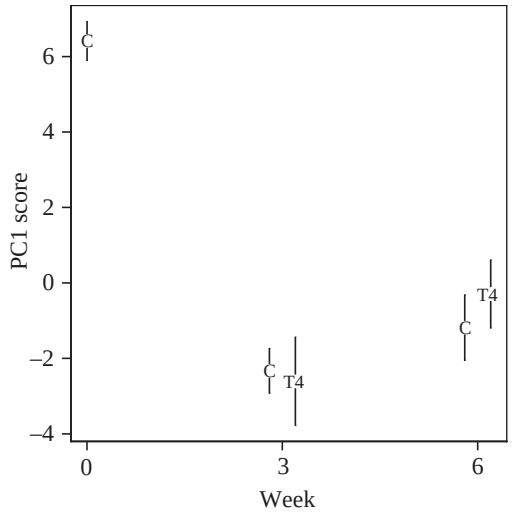

Fig. 4. First principal component scores (overall density and size of blue and double cones; see Table 1 for loadings) ± S.E.M. of control (C) and thyroxin (T4)-treated rainbow trout smolts from the initiation of the experiment (week 0) until termination (week 6).

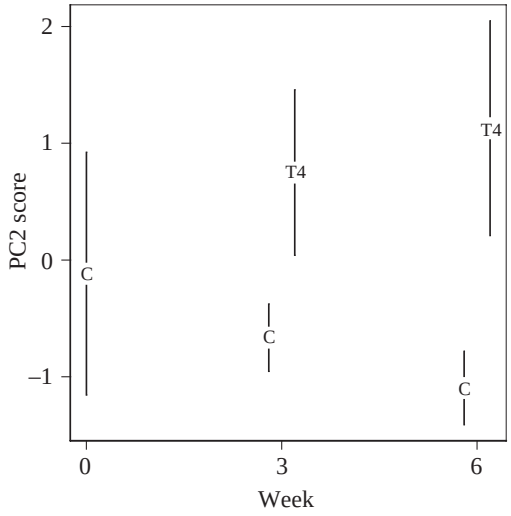

Fig. 5. Second principal component scores (relative distribution of cones; see Table 1 for loadings) ± S.E.M. of control (C) and thyroxin (T4)-treated rainbow trout smolts from the initiation of the experiment (week 0) until termination (week 6). The value of PC2 is significantly different between C and T4 (see Table 2).

densely packed SWS and double cones, and high negative values of PC1 reflect small cone diameters and small inter-cone distances.

High values of PC2 represent individuals that, for a given value of PC1, had high densities of SWS and double cones in the central ventral, temporal and ventral regions and low densities in the nasal region of the retina. The density of UVS cones loaded strongly and positively onto PC2 in the nasal and ventral regions but negatively in the temporal region. At the same time, the diameter of the double cones loaded strongly in the dorsal and nasal regions, and the distance between double and between SWS cones loaded strongly in the nasal region but weakly in the temporal regions (Table 1). Thus, for any given value of PC1, high positive values of PC2 in subsequent discussion (e.g. Fig. 5) reflect, amongst other changes, higher densities of UVS cones in the nasal and ventral regions of the retina.

Treatment effects

The PC1 score was high for the controls at week 0 (Fig. 4). This reflects a high density of UVS cones in the temporal and dorsal sampling regions and a high density of SWS and double cones across all regions sampled. Both control and T4-treated fish had less abundant, larger and less tightly packed SWS and double cones and a lower density of UVS cones in the temporal and dorsal sampling regions three and six weeks into the experiment compared with the starting condition at week 0 (Fig. 4; Table 2). A significant interaction term reflected the higher values of the control fish at week 0.

Fish treated with T4 had much higher PC2 scores than control fish both three and six weeks into the experiment. For a given value of PC1, T4-treated fish had a higher UVS cone density in the nasal and ventral sampling regions and lower UVS cone densities in the temporal retina; there were higher densities of SWS and double cones in the central ventral, temporal and ventral regions but lower densities in the nasal sampling regions relative to the controls (Fig. 5; Table 2).

Discussion

Synopsis and significance of principal component analysis

The use of principal component analysis in this study has revealed important changes in the cone mosaic of post-metamorphic rainbow trout treated with thyroxin. The results of this multivariate analysis can be summarized into several main points. (1) Both controls and T4-treated fish exhibit a decrease of UVS cone density in the temporal and dorsal sampling regions and a decrease of SWS and double cones across all sampling regions in week 3 and week 6 relative to the controls in week 0. These changes do not appear to be linked with T4 treatment but

Table 2. *Two-way analysis of variance of PC1 and PC2 values excluding week 0 controls*

	Degrees of freedom	Sum of squares	*F* value	Probability (*F*)
PC1				
Treatment	1	0.56242	0.114810	0.74
Week	1	17.91527	3.657177	0.07
Treatment by week	1	2.06369	0.421275	0.52
Residuals	20	97.97322		
PC2				
Week	1	0.00400	0.001744	0.97
Treatment	1	19.85749	8.668442	0.01
Week by treatment	1	0.98728	0.430980	0.52
Residuals	20	45.81559		

rather reflect time-related changes in all experimental fish. (2) T4-treated fish exhibited a higher density of UVS cones in the nasal and ventral sampling locations relative to the controls. (3) Furthermore, T4-treated fish had a higher density of SWS and double cones in the central ventral, temporal and ventral regions but a lower density in the nasal area.

Topographical distribution of UVS cones

The identity of cone types is central to the interpretation of our histological data. Several lines of evidence allowed us to conclude that the single corner cone is the UVS cone, while the central single cone is the SWS cone. Firstly, our previous microspectrophotometry analysis indicated that single cones, and only single cones, contain UVS and SWS opsin (Hawryshyn and Harosi, 1994; Hawryshyn et al., 2001). Single cones containing UVS opsin (as determined by microspectrophotometry) are shorter than cones containing SWS opsin, consistent with the observation that the corner single cone is shorter (in radial section of the retina) than the central single cone (Lyall, 1957a). Secondly, a loss of the single corner cone coincident with a loss of visual sensitivity to UV light has been demonstrated in several scenarios (Bowmaker and Kunz, 1987; Beaudet et al., 1993; Browman and Hawryshyn, 1992, 1994). Finally, *in situ* hybridization for the UVS and SWS opsin mRNA, performed on tangential cryosections, conclusively demonstrated cone identities (Fig. 3).

Fig. 1 shows representative maps of the retina from each group. Row mosaic was primarily found in the ventral-nasal area but sometimes extended into the periphery of the temporal retina. Examples of square mosaic were found in all regions, and mosaics showing both square and row mosaics were primarily found in the dorsal-temporal region. Complete mosaics were only found in the dorsal-temporal region, as indicated by the polygons on the retinal maps.

The distribution of UVS cones in an individual from each group was mapped and the presence of UVS cones is noted by the polygons in Fig. 1. The UVS cones were found to be present in the dorsotemporal quadrant of the retina, very similar to our recent topographical mapping of UVS cones in smolts using *in situ* hybridization (Allison et al., 2003). It is reasonable to assume that the single corner cones in T4-treated smolts express UVS opsin, as we have clearly demonstrated in untreated fish (Fig. 3), because T4 treatment is known to increase the fish's sensitivity to UV light (Browman and Hawryshyn, 1994).

T4 induction of neuroplasticity in the UVS cone photoreceptor population

Thyroxin has been linked to several changes in migratory fishes (Barron, 1986; Youngson, 1989; Youngson and Webb, 1993) and/or in metamorphic transitions exhibited by fishes (Evans and Fernald, 1990; Inui and Miwa, 1985; Miwa and Inui, 1987; Tasaki et al., 1986). In the retina, thyroxin has been shown to alter retinal structure and function (Alexander et al., 1998; Browman and Hawryshyn, 1992, 1994; Deutschlander et al., 2001; Marsh-Armstrong et al., 1999) in addition to a suite of other changes. It has been demonstrated that thyroid hormone receptor-β controls asymmetric development of cone identity in mouse retina; knockout of this receptor led to a loss of dorsoventral asymmetry of UVS cones (Ng et al., 2001). Recently, asymmetric growth of the *Xenopus* retina has been shown to be under control of thyroxin and deiodinase enzymes (Marsh-Armstrong et al., 1999). Dorsal proliferative cells expressed a deiodinase enzyme that deactivates thyroid hormones, preventing cell proliferation. Ventral retinal cells did not express this enzyme, allowing for cell proliferation and leading to asymmetric growth. Whether this asymmetric enzyme expression occurs in the teleost retina is unknown. A recent study has shown the presence of both T4 outer ring deiodinase, which converts T4 to active T3 (3,5,3′-triiodothyronine), and T4 inner ring deiodinase, which converts T4 to inactive reverse T3, in the rainbow trout retina. During a T4 challenge, the T4 inner ring deiodinase activity is significantly increased, lowering T4 level and most likely inhibiting an increase in active T3 level. This may result in the differential growth between the control and T4 groups (Plate et al., 2002). Deiodinases and thyroxin have also been shown to play an important role in regulating the retinal distribution of A_1- and A_2-based visual pigments (Alexander et al., 1998; Allen, 1977; Cristy, 1974), where A_1 and A_2 visual pigments are segregated into the dorsal and ventral regions of the retina (Wheeler, 1978). Thus, thyroxin, thyroxin receptors, and genes responsible for producing deiodinase appear to work in concert to alter gene expression for a suite of structural and functional entities with distinct intra-hemispheric differences.

In the current study, the remodelling of the smolt cone mosaic in response to T4 treatment is marked by localized proliferation or neuroregeneration of UVS cones in the ventral and nasal retinal areas. This observation corroborates and extends previous studies that found T4 treatment of parr rainbow trout leads to the loss of UV sensitivity in the ventral retinal hemisphere (Browman and Hawryshyn, 1994; Deutschlander et al., 2001) and T4 treatment of fish in the later stages of development results in a return of UV sensitivity in the ventral retina (Browman and Hawryshyn, 1994). It should be emphasised that the current study is the first attempt to examine the retinal locus for UVS cone regeneration.

We would like to thank Nicole Tunstall and Martin Brummel for technical assistance. This research was supported by Operating and Equipments grants from the Natural Sciences and Engineering Research Council of Canada (C.W.H.). W.T.A. was supported by an Alzheimers Society of Canada/CIHR Predoctoral Fellowship.

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