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Retinal projections to the subcortical visual system in congenic albino and pigmented rats

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Abstract

The primary visual pathway in albino mammals is characterized by an increased decussation of retinal ganglion cell axons at the optic chiasm and an enhanced contralateral projection to the dorsal lateral geniculate nucleus. In contrast to the primary visual pathway, little is known about the organization of retinal input to most nuclei of the subcortical visual system in albino mammals. The subcortical visual system is a large group of retinorecipient nuclei in the diencephalon and mesencephalon. These areas mediate a range of behaviors that include both circadian and acute responses to light. We used a congenic strain of albino and pigmented rats with a mutation at the *c* locus for albinism (Fischer 344-*c*/+; La Vail and Lawson, 1986) to quantitatively assess the effects of albinism on retinal projections to a number of subcortical visual nuclei including the ventral lateral hypothalamus (VLH), ventral lateral preoptic area (VLPO), olivary pretectal nucleus (OPN), posterior limitans (PLi), commissural pretectal area (CPA), intergeniculate leaflet (IGL), ventral lateral geniculate nucleus (vLGN) and superior colliculus (SC). Following eye injections of the neuroanatomical tracer cholera toxin- β , the distribution of anterogradely transported label was measured. The retinal projection to the contralateral VLH, PLi, CPA and IGL was enhanced in albino rats. No significant differences were found between albino and pigmented rats in retinal input to the VLPO, OPN and vLGN. These findings raise the possibility that enhanced retinofugal projections to subcortical visual nuclei in albinos may underlie some light-mediated behaviors that differ between albino and pigmented mammals.

Keywords

Retinal projection; Subcortical visual system; Congenic rat; Albino

Introduction

In albino mammals the primary visual pathway is characterized by an increased decussation of retinal ganglion cell axons at the optic chiasm resulting in lower percentages of ipsilateral retinofugal fibers and enhanced innervation of contralateral thalamic targets (Lund, 1965; Lund et al., 1974; Guillery, 1971, 1974, 1996; Guillery et al., 1975; Cunningham and Lund, 1971; Giolli and Creel, 1973, 1974; Hickey and Spear, 1976; Klooster et al., 1983; Guillery et al., 1984; Dreher et al., 1985). In all albino mammals that have been studied, there is a shift in the naso-temporal division of the retina such that ganglion cells in a segment of retina close to the

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vertical meridian that would normally project ipsilaterally, instead project to the contralateral side of the brain (Guillery, 1996; Jeffery, 2001). This results in an abnormal representation of the binocular map in regions that receive input from the retina including the lateral geniculate nucleus. Abnormalities are also present in the geniculocortical pathway, although the animals appear to behave normally (Montero and Guillery, 1978; Huang and Guillery, 1985; Akerman et al., 2003).

In contrast to the primary visual pathway, little is known about the organization of retinal input to many nuclei of the subcortical visual system in albino mammals. The subcortical visual system is a large group of retinorecipient nuclei in the hypothalamus, lateral geniculate complex, pretectal nuclear group, and superior colliculus (Morin and Blanchard, 1998; Marchant and Morin, 1999). These areas mediate a range of behaviors that include both circadian and acute responses to light. As with the retinogeniculate projection, there are abnormalities in retinal input to the superior colliculus in albinos, with a reduction in axons that project ipsilaterally (Lund et al., 1980; Zhang and Hoffman, 1993). However, little is known about retinal input to other subcortical retinorecipient nuclei.

There are many differences between albino and pigmented mammals in light-mediated responses. Albinos are reported to have reduced visual acuity (rat, Prusky et al., 2002), elevated visual thresholds (mouse, rat, Balkema, 1988; Balkema and Drager, 1991; Balkema et al., 2001), impaired visual discrimination (mouse, Rhoades and Henry, 1977), lower contrast sensitivity at all spatial frequencies (rat, Birch and Jacobs, 1979); different electroretinogram responses (guinea pig, Bui et al., 1998), altered pupillary light reflex thresholds (mouse, Green et al., 1994), impaired oculomotor responses (mouse, Balkema et al., 1984; rabbit, Collewijn et al., 1978; human, Collewijn et al., 1985; ferret, Telkes et al., 2001; Hoffmann et al., 2004), and shortened circadian rhythms (mouse, Possidente et al., 1982). Furthermore, rapid eye movement (REM) sleep increases dramatically following a light to dark transition in albino, but not pigmented strains of rats (Johnson et al., 1970; Tobler and Borbely, 1978; Benca et al., 1991, 1993, 1998; Leung et al., 1992; Miller et al., 1998; Obermeyer and Benca, 1999).

Whereas the differences in some light-mediated behaviors between albino and pigmented mammals may be related to retinal pigment abnormalities (Drager and Balkema, 1987; Guillery et al., 1999; Balkema et al., 2001; Jeffery, 1997; Rachel et al., 2002; Daly et al., 2004), differences in retinal projections to nuclei of the subcortical visual system may also underlie some of these differences. Furthermore, projections from retino-recipient nuclei in the subcortical visual system to their respective targets may also be aberrant, as has been shown in the primary visual pathway of albino rats (Montero and Guillery, 1978; Shatz and LeVay, 1979).

In the present study, we used a strain of rats that are congenic at the *c* locus for albinism (Fischer 344-*c*/+) to assess the effects of albinism on retinal input to nuclei of the subcortical visual system. Albino rats have two copies of the gene for albinism (*c/c*), whereas pigmented (hooded) but otherwise identical rats have a single copy of the wild-type gene (*c/+*) (La Vail and Lawson, 1986). Retinal projections to the ventral lateral hypothalamus (VLH), ventral lateral preoptic area (VLPO), olivary pretectal nucleus (OPN), posterior limitans (PLi), commissural pretectal area (CPA), intergeniculate leaflet (IGL), ventral lateral geniculate nucleus (vLGN) and superior colliculus (SC) in albino and pigmented animals were labeled with the neuroanatomical tracer cholera toxin- β , and quantified using digital image analysis software.

Experimental Procedures

All procedures were performed in compliance with the *NIH Guide for the Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committee at the

University of Wisconsin School of Veterinary Medicine. A total of 16 congenic rats (8 albino, F344c/c and 8 pigmented, F344c/+; 3–4 months of age) were obtained from a colony maintained at the University of Wisconsin. This rat strain was originally produced by transferring a wild-type (+) allele at the albino locus from the Long-Evans strain to the albino F344 strain (LaVail and Lawson, 1986). These rats have virtually identical genotypes except for a difference at the c locus for albinism. The c/+ rats are black-hooded and black-eyed, whereas the c/c rats are albino. The colony is maintained by backcrossing F344c/+ male rats to inbred F344 female rats (Charles River, Kingston, NY), which produces litters with equal numbers of c/c and c/+ genotypes.

Anterograde Tracing

Rats were anaesthetized with isoflurane and received 0.5 mg/kg buprenorphine via subcutaneous injection. A topical analgesic solution (Proparacaine HCL 0.5%; Akorn, Buffalo Grove, IL) was applied to the left eye. A small hole was made in the temporal scleral margin of the eye with a sterile 26-gauge needle, and 1.0 µl of cholera toxin-β (CT-β) dissolved in saline (List Biologicals, Campbell, CA) with 0.1% Evans Blue (Sigma) was injected into the vitreous through the previously-made hole using a 10 µl Hamilton microliter syringe with a 30-gauge needle. The needle remained in place for 20s after the injection.

Perfusion

Five days after the eye injection, rats were anaesthetized with sodium pentobarbital (120mg/kg, i.p.) and transcardially perfused with 200ml of heparinized saline (10,000 units/liter) followed by 500ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4).

Tissue Preparation

Brains were removed and stored overnight at 4°C in fixative, then cryoprotected for 24–36 h at 4°C with 20% sucrose and 5% glycerol in 0.1M phosphate buffer. Sections were cut in the coronal plane with a freezing microtome at a thickness of 50µm. Sections that were not reacted immediately were stored in 0.1M phosphate buffer containing 0.02% sodium azide at 4°C.

Immunocytochemistry (ICC)

Free-floating sections were incubated in 1% hydrogen peroxide in 0.1M phosphate buffered saline (PBS) for 30 min. Sections were washed in 0.3% Triton X-100 in 0.1 M PBS with 2% bovine serum albumin (BSA) (2×2 min, 1×15 min), and incubated for 45 min with a blocking solution of 20% normal rabbit serum, 0.3% Triton X-100, and 2% BSA in 0.1 M PBS. Sections were then reacted with antiserum to CT-β (goat; 1:20,000; List Biologicals, Campbell, CA) overnight at room temperature. Sections were washed again (2×2, 2×15 min) and incubated for 3h with biotinylated rabbit anti-goat IgG (1:300; Vector Laboratories, Burlingame, CA). Sections were washed again and incubated for 1h with ABC complex (Vectastain Standard PK4000 kit, Vector Laboratories, Burlingame, CA). Sections were washed with 0.1 M PBS (5×5 min), and reacted with 0.04% DAB in 0.1 M PB with 0.01% hydrogen peroxide.

Analysis

Consistency of uptake of CT-β tracer by the retina was determined by examining the retinal projection to the contralateral superior colliculus (SC). The superior colliculus contains a retinotopic map of the retinal projection that covers the entire rostral-caudal and medio-lateral extent of the superficial layers (Huerta and Harting, 1984). Only tissues from animals that showed an even distribution of CT-β tracer throughout the superficial layers of the contralateral SC were analyzed. Based on these criteria, five albino and six pigmented animals were selected for analysis. Analyses were performed by an individual unaware of the pigmentation status of

the animals. The following subcortical regions were analyzed: superior colliculus (SC), ventral lateral hypothalamus (VLH), ventral lateral preoptic area (VLPO), olivary pretectal nucleus (OPN), posterior limitans (PLi), commissural pretectal area (CPA), intergeniculate leaflet (IGL), and ventral lateral geniculate nucleus (vLGN).

Digital photographs (16 bit; 65,536 grey levels) of brain sections were taken using a SPOT camera (Diagnostic Instruments), and quantitative image analysis was performed with ImagePro Plus software. All images were obtained under identical bright field illumination.

Retinal label varies considerably (e.g., sparse in the VLPO, dense in the core of the OPN), and does not lend itself easily to a single quantitative method. Thus, we used several different approaches: measuring the area containing retinal terminals in camera lucida drawings, or in digital images in which nuclear boundaries were defined by an area of interest outline (AOI); measuring the optical density in the core of a densely-labeled nucleus in digital images; counting patches of label in the microscope. For many nuclei more than one quantitative method was used. For nuclei with clearly defined borders, camera lucida drawings of retinal label in sections were made at a magnification of 10X. A perimeter was then drawn around the region containing any labeled axons and terminals. These drawings were digitized and the outlined area was measured (μm^2) using ImagePro Plus software. This measurement approximates the area in a cross section through a nucleus within which retinal terminals can exert a direct effect on postsynaptic neurons. For nuclei with indistinct borders, or regions where retinal terminals were distributed irregularly with regions of dense label interspersed with regions containing only a few terminals, digital images were made and an AOI roughly corresponding to the boundary of the nucleus was drawn (see Prichard et al., 2002) (Fig. 1). Using ImagePro software, a threshold was applied to distinguish stain from background in the AOI, and the area of label was then counted and expressed as area of label in $\mu\text{m}^2/1000 \mu\text{m}^2$. For some brain regions it was necessary to use two different AOIs, reflecting differences in the shape of the nucleus in different sections. For nuclei in which stain was very dense, we measured the optical density in a defined area in the core of a nucleus. Optical density measures the relative darkness of each pixel. Optical density was determined by plotting the mean intensity level of pixels within the defined area on a standard optical density curve calibrated to incident light and dark. Thus, in a densely-stained region where individual terminals cannot be resolved (e.g., OPN core), optical density may provide some information as to the density of retinal terminals. Data were analyzed using a two-way repeated measures ANOVA (SigmaStat), with pigmentation status and section level as variables. Data were also analyzed using the non-parametric Mann Whitney rank sum test (SigmaStat). Differences were considered significant at $P < 0.05$.

SC—Five matched sections throughout the rostrocaudal extent of the superior colliculus of each animal were reacted for the presence of label and examined. Three sections were analyzed quantitatively (Bregma -5.90, -6.40, -6.90). In the contralateral SC, optical density in a rectangle ($23,686 \mu\text{m}^2$) superimposed in two locations in the superficial layers was measured in digital images. In the ipsilateral SC, patches of label were counted.

VLH—Seven matched sections throughout the rostrocaudal extent of the VLH of each animal were reacted for the presence of label and examined. Five sections were analyzed quantitatively (Bregma -0.90, -1.10, -1.35, -1.55, -1.75). In the contralateral VLH, the entire area containing retinal terminals was outlined and measured in camera lucida drawings and expressed in μm^2 . In sections through the area of densest label, an oval AOI ($16,640 \mu\text{m}^2$) was overlaid in the area of densest staining in digital images of sections, the number of stained pixels was measured and expressed as area of label in $\mu\text{m}^2/1000 \mu\text{m}^2$. Ipsilateral label was too sparse to measure.

VLPO—Nine matched sections throughout the rostrocaudal extent of the VLPO of each animal were reacted for the presence of label and examined. As label was very diffuse and sparse, only two sections were analyzed quantitatively (Bregma -0.45, -0.70). In the contralateral VLPO, an AOI (41,000 μm^2) was overlaid in the area of densest staining in digital images of sections, and the number of stained pixels was measured. Ipsilateral label was too sparse to measure.

OPN—Nine matched sections throughout the rostrocaudal extent of the OPN of each animal were reacted for the presence of label and examined. The area of densest label was used for quantitative analysis, and consisted of 3 sections, two of which were measured (Bregma -4.50, -4.60). The area containing retinal terminals was outlined and measured in camera lucida drawings. As there was a distinct “shell” and “core” in retinal label in the contralateral OPN, these were measured separately. In the ipsilateral OPN there was no clear distinction between shell and core, and they were measured together. As retinal label was very dense in the “core” of the contralateral OPN, optical density was also measured in an oval (33,716 μm^2) superimposed on the core in digital images of the nucleus.

PLi—Eight matched sections throughout the rostrocaudal extent of the PLi of each animal were reacted for the presence of label and examined. Two sections through the area of densest staining in the contralateral PLi were analyzed (Bregma -4.6, -4.8). An AOI (155,210 μm^2) was overlaid on digital images of the nucleus (Fig. 1), and the number of stained pixels was measured. Ipsilateral label was too sparse to measure.

CPA—Six matched sections throughout the rostrocaudal extent of the CPA of each animal were reacted for the presence of label and examined. Two sections through the contralateral CPA were analyzed quantitatively (Bregma -4.80 and -5.20). Two different AOIs were used: a wedge-shaped rostral AOI (312,958 μm^2), and a crescent-shaped caudal AOI (387,909 μm^2) (Fig. 1), and the number of stained pixels was measured. Ipsilateral label was too sparse to measure.

IGL—Nine matched sections throughout the rostrocaudal extent of the IGL of each animal were reacted for the presence of label and examined. Five sections were analyzed quantitatively (Bregma -4.30 and -4.80). In the contralateral and ipsilateral IGL, two different AOI's were used: rostral (117,958 μm^2) (Fig. 1), and caudal (157,454 μm^2), and the number of stained pixels measured. As retinal label was very dense in the “core” of the contralateral IGL, optical density was also measured in a rectangle (6,865 μm^2) superimposed on the core in digital images of the nucleus.

vLGN—Nine matched sections throughout the rostrocaudal extent of the IGL of each animal were reacted for the presence of label and examined. Four sections were analyzed quantitatively (Bregma -4.30 and -4.80). In the contralateral and ipsilateral vLGN, two different AOIs were used: rostral (454,788 μm^2) (Fig. 1), and caudal (326,952 μm^2), and the number of stained pixels measured.

Results

Superior Colliculus

CT β -stained retinal fibers were present in the superficial and intermediate layers of the SC contralateral and ipsilateral to the injected eye in both albino and pigmented rats. Label was densest in the superficial layers of the contralateral SC, and diminished ventrally in the intermediate layers. Ipsilateral label was concentrated in the deepest part of the superficial layers and the upper part of the intermediate layers (Fig. 2A, B). Consistent with previous

reports in both albino and pigmented rats (Albers et al., 1988; Lund, 1965; Lund et al., 1976; Dreher et al., 1985), dense label in the contralateral SC extended to a depth of 250µm-300µm, with less dense label extending ventrally to a depth of 500µm-700µm. Label in the ipsilateral SC extended from a depth of 200–250µm to about 500µm in both albino and pigmented animals (Fig. 2A, B). There was no difference in the optical density of label in the contralateral SC between albino and pigmented rats ($P=0.692$) (Table 1). Consistent with previous reports (Lund et al., 1980), the pattern of label ipsilaterally differed between albino and pigmented rats. Across the mediolateral extent of the intermediate layers of the SC, pigmented animals showed a patch-like arrangement of retinal input, whereas albino rats had a more evenly distributed projection (Fig. 2A, B). In pigmented animals, distinct dense patches of stain were observed, with up to 5 patches per section. Only two of the five albino animals had a suggestion of this patch-like arrangement, with only one patch per section; patches did not appear to be as densely stained as in pigmented animals.

Ventral Lateral Hypothalamus

In all animals, there was a dense contralateral projection to the ventral lateral hypothalamus dorsal and lateral to the optic chiasm (Fig. 2C, D). Whereas the rostrocaudal extent of the projection (~ 1.4 mm, from Bregma -0.80 to -2.2) did not differ between albino and pigmented rats, the total area of CT-β label was almost three-fold greater in albino than in pigmented rats ($P=0.046$) (Table 1). When an area of interest was drawn around the region of densest label (VLH core) in sections through albino and pigmented rats, there was also a three-fold difference in the area of label. Although this was not statistically significant with ANOVA ($P=0.276$) (Table 1), a Mann Whitney test showed significance with $P<0.001$. The VLH ipsilateral to the injected eye had only sparse, scattered labeled axons and there were no obvious differences between albino and pigmented rats, although this was not quantified.

Ventral Lateral Preoptic Area

The retinal projection to the VLPO was primarily contralateral with very few ipsilateral fibers. The contralateral projection was sparse, and most animals had a greater projection to the caudal part of the nucleus. There were no differences in the area of retinal label in the contralateral VLPO between albino and pigmented animals ($P=0.418$) (Table 1). Ipsilaterally there were no obvious differences between albino and pigmented rats, although this was not quantified.

Olivary Pretectal Nucleus

In both albino and pigmented animals, the OPN contralateral to the injected eye showed dense CT-β staining in the central core of the rostral part of the nucleus, with less dense label in the shell. The ipsilateral projection was considerably less dense than the contralateral projection, and label was more prominent in the shell than the core. No significant differences were found between albino and pigmented rats in the area of staining for the following: contralateral core ($P=0.326$), contralateral core plus shell ($P=0.567$), contralateral shell ($P=0.236$), ipsilateral core plus shell ($P=0.717$) (Table 1). There was also no difference in the optical density of the contralateral core ($P=0.390$) (Table 1). The ipsilateral core could not be clearly delineated from the shell and was therefore not analyzed separately.

Posterior Limitans

Retinal fibers in the PLi contralateral to the injected eye differed between albino and pigmented rats in both area and pattern of label (Fig. 2E, F). All five albino rats had several distinct, dense patches of label in the PLi (Fig. 2E). In contrast, patches were observed in only half of the pigmented animals, and were considerably less dense. The area of CT-β staining in the contralateral PLi was three-fold greater in albino than in pigmented rats (albino: 123.01 ± 38.1

$\mu\text{m}^2/1000 \mu\text{m}^2$; pigmented $32.99 \pm 34.77 \mu\text{m}^2/1000 \mu\text{m}^2$). Although this was not statistically significant with ANOVA ($P=0.115$) (Table 1), a Mann Whitney test showed significance ($P=0.011$).

The ipsilateral projection to the PLi was considerably less dense than the contralateral projection in all animals. No differences were observed between albino and pigmented rats in staining density ($P=0.659$) (Table 1), or the distribution of patches.

Commissural Pretectal Area

The area of CT- β staining in the area of interest that outlined the contralateral CPA was greater in albino than in pigmented rats (albino: $267.9 \pm 104.4 \mu\text{m}^2/1000 \mu\text{m}^2$; pigmented $43.8 \pm 94.4 \mu\text{m}^2/1000 \mu\text{m}^2$). Although this was not statistically significant with ANOVA ($P=0.144$) (Table 1), a Mann Whitney test showed significance ($P=0.004$).

Label was sparse in the ipsilateral CPA, and there were no obvious differences between albino and pigmented animals, although this was not quantified.

IGL

With ANOVA, there were no differences in retinal staining in the area of interest that outlined the IGL between albino and pigmented rats contralateral ($P=0.169$) or ipsilateral ($P=0.771$) to the injected eye with ANOVA (Table 1). However, a Mann Whitney test showed significant differences in retinal staining in the contralateral IGL ($P=0.003$). Optical density analysis in the area of densest CT- β label showed a significant difference in the IGL contralateral to the injected eye, with albino rats having more label than pigmented rats (ANOVA; $P=0.020$) (Table 1).

vLGN

There were no differences in retinal axon staining in the area of interest that outlined the vLGN between albino and pigmented rats contralateral ($P=0.592$) or ipsilateral ($P=0.892$) to the injected eye (Table 1).

Discussion

This study provides a comprehensive, quantitative analysis of retinal projections to nuclei of the subcortical visual system in congenic Fisher F344-c/+ albino and pigmented rats. Previous studies of albino and pigmented rats described an enhanced contralateral and a reduced ipsilateral retinal projection to thalamic targets (Lund, 1965; Lund et al., 1974; Guillery, 1971, 1974, 1986, 1996; Giolli and Creel, 1973; Klooster et al., 1983; Guillery et al., 1984; Dreher et al., 1985). This study extends previous work by showing the absence of a coherent pattern in retinal input to subcortical visual nuclei in albino vs. pigmented rats. As predicted, some nuclei of the subcortical visual system show an enhanced contralateral retinal projection in albino rats (VLH, PLi, CPA, IGL), but others do not (SC, VLPO, OPN, vLGN). With the exception of the superior colliculus, no reduction in ipsilateral retinal input was detected in any of the subcortical visual nuclei in albino rats. However, as ipsilateral label was sparse in several of these nuclei, with the methods used in this study small differences would be difficult to detect. The lack of a coherent pattern of augmented/reduced retinal input to subcortical nuclei in albino rats is intriguing. Several factors may be involved including: (1) the spatial origin of retinal ganglion cells giving rise to the aberrant projection, (2) the type of retinal ganglion cell, (3) presence or absence of visuotopic organization within the retinorecipient nucleus, (4) the degree of branching of retinal axons giving rise to the abnormal projection.

Previous studies of albino cats show that visuotopic maps within the primary visual pathway are misaligned (Guillery et al., 1974). Geniculate layers that receive the normal crossed input from the nasal retina are normal in albinos. Layers that receive input from the temporal retina have normal and abnormal segments. The abnormal segment receives a mirror-reversed input from the central part of the ipsilateral visual field, whereas the normal part receives a normal input from the peripheral part of the contralateral visual field (Guillery et al., 1979; Guillery, 1986, 1996). Geniculocortical projections are also misaligned, with the reversed pattern of input also present in the visual cortex (Montero and Guillery, 1978; Huang and Guillery, 1985; Akerman et al., 2003). Nonetheless, the behavior of these animals appears to be normal.

The superior colliculus is also retinotopically organized with a strong projection from the contralateral nasal retina, and a weak but overlapping projection from the ipsilateral temporal retina. Central visual fields are represented more rostrally and peripheral fields more caudally (Huerta and Harting, 1984). In the Siamese cat which has similarities to albinos with respect to retinal pathways, the ipsilateral projection is reduced to a small region at the caudal pole of the colliculus (Kalil et al., 1971). Albino ferrets also have a reduced ipsilateral retinocollicular projection (Zhang and Hoffmann, 1993). However, it is not clear from these studies whether the misdirected contralateral projections replace the missing ipsilateral projections in their locus of termination. We were unable to detect an enhanced contralateral projection in the present study, although this may reflect the sensitivity of the method used. Because the ipsilateral projection is patchy, it would be difficult to detect small increases in misrouted ipsilateral label embedded within the homogeneous contralateral label. Although the role of patches in the superior colliculus is poorly understood, they are thought to represent functional modules (Illing, 1996). Thus, the absence of patches in the ipsilateral SC albino rats may be a consequence of a reduced retinal contribution to shaping this compartmental organization during development.

The functional consequence of a reduced ipsilateral retinotectal projection is not clear. Previous studies in mice reported that the dark threshold of single units recorded in the superficial layers of the superior colliculus were higher in albino than in pigmented mice (Balkema and Dräger, 1991). Pigmented mice also performed better than albinos in a water maze escape paradigm under the same illuminance conditions, confirming a difference in visual sensitivity (Hayes and Balkema, 1993). A reduced ipsilateral retinocollicular projection in albino rats may impair visual function and subsequent escape behavior, especially under low illuminance conditions (Overton and Dean, 1988; Dean et al., 1989; Brandao et al., 1999). However, in addition to the SC, other retinorecipient nuclei in the subcortical visual system may be involved in the water maze escape paradigm.

Previous studies of the retinopretectal projection reported a smaller ipsilateral projection to the OPN in Sprague Dawley albino rats by comparison with Lister pigmented rats (Chan et al., 1995), and a reduced projection in albino rabbits (Klooster et al., 1983). Additionally, Green et al. (1994) reported that pupillary light responses (PLR) in congenic albino mice were “smaller and more sluggish” than in pigmented mice. OPN neurons are essential to the pupillary light response (Trejo and Cicerone, 1984; Clarke and Ikeda, 1985a,b; Young and Lund, 1994; Gamlin and Clarke, 1995; Buttner-Ennever et al., 1996). Although our quantitative analysis did not detect differences in retinal input to either the shell or the core of the OPN in albino and pigmented rats, it is possible that the albino mutation could preferentially affect the small population of melanopsin-containing retinal ganglion cells that project to the OPN (Gooley et al., 2001, 2003; Lucas et al., 2003; Melyan et al., 2005; Fu et al., 2005). Melanopsin-containing retinal ganglion cells contribute to the pupillary light response as well as being involved in circadian photoentrainment (Lucas et al., 2003; Gooley et al., 2003; Hannibal and Fahrenkrug, 2004; Freedman et al., 1999; Berson et al., 2002; Hatter et al., 2002; Morin et al.,

2003). As the OPN is retinotopically organized (Scalia and Arango, 1979), even a small change in retinal input from melanopsin-containing neurons in albinos could preferentially diminish the neuronal output of the OPN to a number of targets including those associated with the pupillary light reflex.

We found an enhanced retinal projection to the contralateral IGL in albino rats by comparison with pigmented animals, which is consistent with findings in albino Sprague Dawley vs. hooded Long Evans rats (Hickey and Spear, 1976). The IGL has extensive connections with the circadian system, and photic information can access the SCN via the IGL (Johnson et al., 1988; Zhang and Rusak, 1989). Additionally, the retina innervates the IGL *via* collaterals of the retinohypothalamic tract that project to the SCN (Pickard, 1985; Morin, 1994). Previous studies from our lab have shown an enhanced bilateral projection to the SCN in congenic albino rats as well as in albino Lewis vs. Brown Norway rats (Steininger et al., 1993; Miller et al., 1996). An enhanced retinal projection to the SCN and/or the IGL in albinos might result in altered circadian rhythms, as has been reported in albino mice which showed shorter circadian rhythms than pigmented controls (Possidente et al., 1982). Recently it has been suggested that the IGL may regulate other systems, including those related to visuomotor function and sleep/arousal, by means of its extensive anatomical connections (Morin and Blanchard, 2005). For example, an augmented retinal projection to IGL in albino rats could preferentially influence REM sleep-promoting neurons in the lateral dorsal tegmental nucleus and pedunculopontine tegmental nucleus with which it is connected (Jones, 2004).

Retinal input to the VLPO was sparse and there were no differences between albino and pigmented rats. The VLPO is a small cluster of neurons lateral to the optic chiasm at the level of the rostral SCN, rostral to the supraoptic nucleus (SON) that has a sleep-promoting function (Sherin et al., 1996; Lu et al., 2000; Gaus et al., 2002). Directly caudal to the VLPO lies the VLH, a region that receives dense retinal input in Sprague Dawley and Wistar rats (Lu et al., 1999; Hannibal and Fahrenkrug, 2004). Retinal input to the VLH in congenic albino and pigmented F344 rats forms a column (~1 mm rostrocaudal), dorsal and lateral to the optic chiasm, with maximal label at Bregma -1.50 (Paxinos and Watson, 1997). In contrast to the VLPO, there was a significantly larger retinal projection to the contralateral VLH in albino rats by comparison with pigmented rats (Table 1). Several observations suggest that the VLH and VLPO are closely associated, and the VLH may overlap with the “extended VLPO” (Lu et al., 2000, 2002) which has also been implicated in sleep regulation: 1) retinal input is continuous from the rostral tip of the VLPO to the caudal pole of the VLH; 2) following injections of a retrograde tracer into the core of the VLPO, neurons in the VLH were labeled (Chou et al., 2002), suggesting that VLH neurons project to the VLPO; 3) galanin immunoreactive neurons have been described in the VLPO (Gaus et al., 2002), and galanin-immunoreactive axons and terminals are also present in the VLH (Behan, unpublished observations). Taken together, these data suggest that the VLPO and the VLH are reciprocally connected. Neurons in the extended VLPO show increased neuronal activity during REM sleep as determined by increased expression of the immediate early gene *c-fos* (Lu et al., 2002). Thus, an enhanced retinal input to the VLH in albino rats could contribute to the REM-sleep triggering observed in response to light to dark transitions in albino rats (Chamberlin et al., 2003; Benca et al., 1991, 1993; Leung et al., 1992).

There were no differences in the retinal projection to the OPN between albino and pigmented rats. Previous studies in our lab have shown that chemical ablation of the pretectum results in the elimination of REM sleep triggering in albino rats following a light to dark transition (Miller et al., 1999). The identity of the specific pretectal nucleus/nuclei responsible for this behavior is not yet known, although one candidate is the OPN as this nucleus was extensively damaged in all of rats in which REM sleep triggering was eliminated (Miller et al., 1999).

Although we failed to find any difference in CT β label in either the shell or the core of the OPN between congenic albino and pigmented rats, this does not eliminate the OPN from a potential circuit controlling REM sleep-triggering. Rather, it suggests that other nuclei in the pretectum may also be involved in the control of sleep/wake behaviors. As there are extensive interconnections between virtually all nuclei of the subcortical visual system (Morin and Blanchard, 1998), OPN neurons may be modulated by inputs from other pretectal nuclei that show significant differences in retinal input in albino vs. pigmented rats. Although not statistically significant, we found differences in retinal input to the PLi and the CPA between albino and pigmented rats, with albino rats showing an enhanced contralateral projection (Table 1). Previous studies in our lab have shown that these two pretectal nuclei are particularly sensitive to acute increases in illuminance, as measured by *c-fos* expression (Prichard et al., 2002). Thus, the PLi and CPA could be involved in mediating REM sleep-triggering in albino, but not pigmented rats. Interestingly, Prichard et al. (2002) found a significant change in Fos immunoreactivity in the PLi of albino rats following a light to dark transition at zeitgeber time 18 (ZT18; 18 hours after lights-on), but not at ZT6, suggesting possible circadian modulation. Melanopsin-containing RGCs project to the PLi (Hannibal and Fahrenkrug, 2004), and a recent study has shown that melanopsin expression in RGCs is under circadian control (Hannibal et al., 2005). Thus retinal input to the PLi could regulate light/dark sensitivity in this nucleus at different circadian times.

Albino animals have been widely used in studies of light mediated behaviors, and numerous studies have compared pigmented and albino strains. However, phenotypically identical albino strains can vary dramatically in their sensitivity to light (LaVail and Lawson, 1986). The use of a congenic rat strain, in which albino and pigmented animals are genetically identical except for a mutation at the *c* locus for albinism, provides a powerful tool with which to investigate the structure and function of visual pathways. Albino mammals lack a functional gene for tyrosinase, a necessary enzyme in the production of melanin in the retina. Introduction of a tyrosinase gene results in a normal pigmented retina and chiasmatic pathway (Jeffery et al., 1994, 1997). By using a congenic rat strain, the present study confirms that many of the previously described differences in retinal projections in other strains of albino and pigmented rats can be attributed to a mutation at (or near) the *c* locus. In summary, differences in retinal projections to subcortical visual nuclei in albino vs. pigmented rats may underlie alterations in a range of behaviors. The present study highlights differences between albino and pigmented rats in retinal input to subcortical nuclei that are involved in visuomotor (SC), circadian (IGL, PLi) and sleep/wakefulness (VLH, PLi, CPA) functions.

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Figure 1.

Photomicrograph of cholera toxin β (CT β) label in the contralateral diencephalon. This section is representative of labeled retinal input to this region in an albino rat. The dashed lines indicate areas of interest (AOI) used for quantitation in nuclei of the subcortical visual system. OPN, olivary pretectal nucleus; CPA, commissural pretectal area; PLi, posterior limitans; IGL, intergeniculate nucleus; vLGN, ventral lateral geniculate nucleus. Scale bar = 600 μ m.

Figure 2.

Photomicrographs showing the distribution of CT β -labeled retinal axons in matched sections through albino and pigmented rats. A, B: ipsilateral superior colliculus (SC) of albino (A) and pigmented (B) rats. Sections were selected through a region that showed the greatest number of patches. Note the presence of uniform label across the mediolateral extent of the colliculus in the albino, and distinct patches of dense label (arrows) in the pigmented animal. C, D: contralateral ventral lateral hypothalamus (VLH) of albino (C) and pigmented (D) rats. Sections were selected through the region of densest label in the nucleus. E, F: contralateral posterior limitans (PLi) nucleus of albino (E) and pigmented (F) rats. Sections were selected through a region that showed the clearest patches. Note the presence of patchy dense label in the PLi (arrows) in the albino rat (E). Scale bar for A, B, E, F = 500 μ m; C, D = 250 μ m.

Table 1

Brain Region	# sections	Albino (mean $\pm$ S.E.M.)	Pigmented (mean $\pm$ S.E.M.)	P (ANOVA)
cSC (optical density)	3	0.39 $\pm$ 0.05	0.36 $\pm$ 0.05	P = 0.692
cVLH (μm^2)	5	152090 $\pm$ 30709	55862 $\pm$ 28033	P = 0.046*
cVLH core (area/1000 μm^2)	5	113.94 $\pm$ 52.75	31.17 $\pm$ 48.16	P = 0.276
cVLPO (area/1000 μm^2)	2	31.46 $\pm$ 16.10	12.95 $\pm$ 14.70	P = 0.418
cOPN core (optical density)	2	0.49 $\pm$ 0.08	0.58 $\pm$ 0.07	P = 0.390
cOPN core (μm^2)	2	148872 $\pm$ 30440	106028 $\pm$ 27789	P = 0.326
cOPN shell (μm^2)	2	121680 $\pm$ 11853	142051 $\pm$ 10820	P = 0.236
cOPN core + shell (μm^2)	2	270552 $\pm$ 27964	248079 $\pm$ 25528	P = 0.567
iOPN core + shell (μm^2)	2	256424 $\pm$ 21144	245730 $\pm$ 19302	P = 0.717
cPLi (area/1000 μm^2)	2	123.01 $\pm$ 38.09	32.99 $\pm$ 34.77	P = 0.115
iPLi (area/1000 μm^2)	2	5.60 $\pm$ 2.66	7.25 $\pm$ 2.43	P = 0.659
cCPA (area/1000 μm^2)	2	267.9 $\pm$ 104.4	43.8 $\pm$ 94.4	P = 0.144
cIGL (optical density)	5	0.60 $\pm$ 0.44	0.43 $\pm$ 0.04	P = 0.020*
cIGL (area/1000 μm^2)	5	492.10 $\pm$ 76.21	337.59 $\pm$ 69.57	P = 0.169
iIGL (area/1000 μm^2)	5	180.48 $\pm$ 82.42	147.04 $\pm$ 75.24	P = 0.771
cvLGN (area/1000 μm^2)	4	425.85 $\pm$ 101.05	349.81 $\pm$ 92.24	P = 0.592
ivLGN (area/1000 μm^2)	4	79.78 $\pm$ 31.36	85.69 $\pm$ 28.63	P = 0.892

* denotes significance.

Note: when compared with a Mann Whitney rank sum test, the cVLH core, cPLi, cCPA and cIGL (area/1000 μm^2) were statistically significantly different. c, contralateral; i, ipsilateral; SC, superior colliculus; VLH, ventral lateral hypothalamus; VLPO, ventral lateral preoptic area; OPN, olivary pretectal nucleus; PLi, posterior limitans; CPA, commissural pretectal area; IGL intergeniculate leaflet; vLGN, ventral lateral geniculate nucleus.