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RESEARCH ARTICLE

Retinal projections to the accessory optic system in pigmented and albino ferrets *(Mustela putorius furo)*

C. Distler · H. Korbmacher · K.-P. Hoffmann

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Abstract We investigated if a reduced specificity of the retinal projection to the accessory optic system might be responsible for the loss of direction selectivity in the nucleus of the optic tract and dorsal terminal nucleus (NOT-DTN) and, in consequence of this, the optokinetic deficits in albino ferrets. Under electrophysiological control we performed dual tracer injections into the NOT-DTN and the medial terminal nucleus (MTN). Retrogradely labelled ganglion cells were found in the visual streak, the dorsal, and the ventral retina both after injections into the NOT-DTN and the MTN indicating that both nuclei receive input from the same retinal regions. The distribution and spacing of labelled ganglion cells did not differ between pigmented and albino ferrets. However, retinal ganglion cells projecting simultaneously to both the NOT-DTN and the MTN occurred only in albino ferrets. These results suggest that a reduced specificity of the projection pattern of direction specific ganglion cells may contribute to the loss of direction selectivity in the NOT-DTN in albino ferrets.

Keywords Retinal ganglion cells · Optokinetic system · Direction selectivity · MTN · NOT-DTN · Albinism

Introduction

Albino mammals are characterized by various anatomical and physiological abnormalities caused directly or indirectly by a depletion of DOPA in the retina due to a mutation in the tyrosine metabolism (e.g. Jeffery 1997; Blaszczyk et al. 2005, 2007; Lopez et al. 2008). Behaviourally, these animals have reduced monocular visual fields (Elekessy et al. 1973; Simoni and Sprague 1976; Garipis and Hoffmann 2003), reduced visual acuity (Birch and Jacobs 1976; Blake and Antoinetti 1976; Girelli et al. 1995; but see Hupfeld et al. 2006), and a reduced ability to perceive motion in their visual environment (Hupfeld and Hoffmann 2006; Hupfeld et al. 2006). The probably most obvious effect, however, concerns the optokinetic system: the optokinetic reaction (OKR) in albino mammals is highly variable at best but can also be completely absent, e.g. in ferrets (rat: Precht and Cazin 1979; rabbit: Hahnenberger 1977; Collewijn et al. 1978; ferret: Hoffmann et al. 2004; man: St John et al. 1984; Collewijn et al. 1985). This optokinetic deficit can be directly linked to a loss (or change e.g. in rabbit) of the direction selectivity of retinal slip neurons in the nucleus of the optic tract und dorsal terminal nucleus of the accessory optic tract (NOT-DTN), the visuomotor interface in the neuronal circuitry underlying the horizontal OKR (rat: Lannou et al. 1982; rabbit: Winterson and Collewijn 1981; ferret: Hoffmann et al. 2004).

To date, the reason for this loss of direction selectivity in the NOT-DTN is unknown. Anatomical and physiological studies have shown that also in mammals the retinal input to the accessory optic system originates from direction selective on-center ganglion cells (Oyster et al. 1972, 1980; Hoffmann and Stone 1985; Buhl and Peichl 1986; Dann and Buhl 1987; Knapp et al. 1988; Grasse et al. 1990).

It is well known that the NOT-DTN in ferret receives both retinal and cortical input (Klauer et al. 1990). Based on this knowledge we formed three hypotheses to explain the loss of direction selectivity in the NOT-DTN of albino ferrets. (1) Direction selectivity in ganglion cells projecting to the accessory optic system in albino mammals is lost. (2) The cortical input to the accessory optic system of albinos is

C. Distler (✉) · H. Korbmacher · K.-P. Hoffmann
Allgemeine Zoologie and Neurobiologie,
Ruhr-Universität Bochum, Postfach 102148,
44780 Bochum, Germany
e-mail: distler@neurobiologie.rub.de

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lost or originates from non-direction selective neurons. (3) There are direction selective retinal ganglion cells in albinos. However, their projection pattern is not specific anymore. This means that ganglion cells coding for horizontal image displacement not only project to the NOT-DTN but also to the vertical motion coding lateral and medial terminal nuclei (LTN, MTN), and vice versa. The net effect would then be a loss of direction selectivity in the target nuclei due to the unspecific sum of originally specific inputs.

In the present investigation we tested this third hypothesis by means of dual retrograde tracer injections into the NOT-DTN and the MTN of pigmented and albino ferrets. If the hypothesis of unspecific projections of direction selective ganglion cells would be true we would expect significant amounts of double labelled retinal ganglion cells in albinos, whereas in pigmented animals the NOT-DTN and the MTN projecting subpopulation of retinal ganglion cells would be separate. In addition, we analyzed the distribution and spacing of ganglion cells projecting to the NOT-DTN or the MTN in albino and pigmented ferrets.

Methods

All experiments were approved by the local authorities (Regierungspräsidium Arnsberg) and were carried out in accordance with the Deutsche Tierschutzgesetz of 12 April 2001, the European Communities Council Directive of 24 November 1986 (S6 609 EEC) and NIH guidelines for care and use of animals for experimental procedures.

Tracer injections were performed in seven pigmented and ten albino ferrets of both sexes ranging in age from 9 to 75 months. In general, tetramethylrhodamine dextrane (RD, MW 3,000, lysine fixable, Molecular Probes; 15% in 0.1 M citrate–NaOH, pH 3.0, 0.7 µl) was injected into the NOT-DTN. This proved to be a very efficient tracer in the ferret subcortical system that is transported both retrogradely labelling retinal ganglion cells and anterogradely labelling axon terminals in the inferior olive. The latter well documented projection was used as an additional control of the location of the injection site. As second tracer, Granular Blue (GB, Dr. Illing GMBH, Groß-Umstadt, 2% in a. dest., 0.7 µl) was injected into the MTN.

For the injections, ferrets were premedicated with 0.05 mg/kg atropine sulfate, initially anaesthetized with 20 mg/kg ketamine and 2 mg/kg thiazinhydrochloride, and intubated through the mouth. An intravenous catheter was introduced into the cephalic vein. Then they were placed into the stereotaxic frame and ventilated artificially with air containing 0.3–1% halothane as needed. Heart rate and body temperature were monitored and kept at physiological levels throughout the experiment. Analgesia was ensured by bolus application and subsequent infusion of fentanyl (3 mg/kg/h). After additional local anaesthesia with bupivacain hydrochloride the skin overlying the skull was cut, the temporalis muscle deflected, and a craniotomy was performed on both sides to allow access to the superior colliculus (SC) and pretectum. Pressure injections were made under electrophysiological control. The NOT-DTN was located based on its direction selective neuronal responses (in pigmented ferrets) and on its position just lateral of the representation of the ventral ipsilateral visual field in the SC (albinos) in an orthogonal approach. For MTN injections the electrode was angled 20° from the contralateral side in the frontal plane. The MTN was located with the help of the representation of the dorsal ipsilateral visual field representation in the contralateral SC. After localizing the targets with tungsten in glass microelectrodes, the electrodes were replaced with Hamilton microsyringes fitted with tracer-filled glass pipettes containing a tungsten wire for electrophysiological recordings. At the end of the experiments, bone flaps were replaced, the temporalis muscles reflected, the wound closed in appropriate layers, and covered with antibiotic ointment (Nebacetin®). After full recovery the animals were returned to their home enclosure and treated with analgetics (Carprofen, Rimadyl®) for 2 days and broadband antibiotics (Enrofloxacin, Baytril®) for 5 days.

After a survival time of 6 days the animals were sacrificed with an overdose of pentobarbital, and perfused through the heart with 0.9% saline containing 0.1% procainhydrochloride, followed by paraformaldehyde–lysine–periodate (PLP) with 4% paraformaldehyde. After postfixation over night the retinae were dissected as wholemounts, mounted on gelatinized slides, and coverslipped with glycerol: phosphate buffer as 2:1. After appropriate cryoprotection the brains were shockfrozen in isopentane at −70°C. Frontal sections through the brainstem and pretectum were cut at 50 µm at a cryostat for reconstruction of the injection sites.

Retrogradely labelled ganglion cells in the retinal wholemounts were plotted on a fluorescence microscope (Zeiss Axioskop) equipped with a computerized tracing system (Neurolucida, Microbrightfields). The data were analyzed with NeuroExplorer (Microbrightfields), the plots were exported to CorelDRAW X3, and finally edited using Adobe Photoshop 5.5. The injection sites and labelling in the inferior olive were reconstructed on a fluorescence microscope (Zeiss, Axioskop) with camera lucida, scanned, and edited using Adobe Photoshop 5.5.

Results

The data base of the current study is summarized in Tables 1 and 2 for pigmented and albino ferrets, respectively. The injection sites, labelling in the ipsilateral inferior olive (IO), as well as the distribution and amount of

Table 1 Data summary pigmented ferrets; percentage of double labelled cells refers to the smaller population of labelled cells

Animal	NOT	IO-label	Retinal label co	# Cells co retina	# Cells ipsi retina	Co:ip ratio	MTN	Retinal label co	# Cells co retina	# Cells ipsi retina	Co:ip ratio	# Double labellled co	# Double labelled ip
195912p	Tiny, superfic	–	Streak + dorsal	103 + 1α	6	17.2:1	–	–	–	–	–	–	–
191899p	Good, superfic	Sparse mediodors	Streak + dorsal + ventral	1,310 + 43α	67	19.6:1	–	–	–	–	–	–	–
212816p	Good, deep	Mediodors	Streak + dorsal + ventral	6,666 + 324α	524 + 2α	12.7:1	–	–	–	–	–	–	–
355275p	Tiny, superfic	–	Streak + dorsal	472 + 3α	11	42.9:1	Large medial co Sc corr	Streak + do + vent	132	122	1.08:1	0	0
207676p	Good	Mediodors	Streak + dorsal	201 + 2α	6	33.5:1	Large medial ip SC corr	Streak + do + vent	50	13	3.8:1	0	0
225708p	Small, lsuperfic	–	Streak (+ do + vent)	104 + 11α	1 + 1α	104:1	Large medial and anterior	Streak (+ do + vent)	52	9	5.8:1	0	0
355575p	Good, superfic	Mediodors + laterovent	Streak + dorsal + ventral	338 + 36α	67 + 1α	5:1	Large anterior	Streak + do + vent	1181	95	12.4:1	0	1 1.47%

labelled ganglion cells in the contra- (co) and ipsilateral (ip) retina are briefly characterized to allow direct comparison. It is evident that the injections and the resulting labelling vary considerably both in pigmented and albino ferrets.

Injections

In pigmented ferrets, the NOT-DTN could be localized by its distinctive preference for horizontal ipsiversive stimulus movement, i.e. neurons in the left NOT-DTN prefer stimulus movement to the left and vice versa. The NOT-DTN was found lateral to the representation of the ipsilateral and ventral visual field in the superior colliculus. Retinal slip neurons within the NOT-DTN which project to the inferior olive lie within and below the brachium of the superior colliculus (BSC) (Zhang and Hoffmann 1993; Telkes et al. 2001). In albino ferrets, retinal slip cells are not direction selective (Hoffmann et al. 2004). Thus, the relative position to collicular landmarks was used for identification of the NOT-DTN. The injections varied in anterior–posterior extent between 400 and 1,400 µm (pigmented 500–1,400 µm, albino 400–1,000 µm) and in their depth below the pretectal surface between 210 µm and 2 mm (pigmented 210–1,000 µm, albino 210–2,000 µm). Most injections both in pigmented and albino animals were superficial and included the BSC. Anterograde labelling after NOT-DTN injection was present mainly in the mediodorsal part of the inferior olive but was missing in three of seven cases in pigmented and three of ten cases in albino ferrets probably due to the very small and superficial location of these injections (see Tables 1, 2; Figs. 1a, 2b).

As a rule, effective GB injections into the MTN were very large, with an anterior–posterior extent between 1,200 and 3,600 µm (pigmented 1,200–2,500 µm, albino 1,200–3,600 µm). All MTN injections included the region around the emersion point of the oculomotor nerve. By contrast, smaller injections (three out of seven cases in pigmented, three out of ten cases in albino ferrets) were not effective.

Figure 1 presents two examples of NOT-DTN and MTN injections in pigmented ferrets. To demonstrate the variability, Fig. 1a shows the smallest NOT-DTN injection (a–p extent 400 µm) and a mid-range MTN injection (a–p extent 1,800 µm), Fig. 1b shows a mid-range NOT-DTN injection (a–p extent 800–1,000 µm) and the largest MTN injection (a–p extent 2,500 µm). The smallest NOT-DTN injection (Fig. 1a) did not result in anterograde labelling in the IO but the larger injection (Fig. 1b) did (see Table 1; Fig. 3d). For comparison, Fig. 2 presents representative injections in albino ferrets. A mid-range NOT-DTN injection (a–p extent 500 µm) and the largest MTN injection (a–p extent 3,600 µm) are shown in Fig. 2a, the smallest NOT-DTN injection (a–p extent 400 µm) and a mid-range MTN injection (a–p extent 2,500 µm) in Fig. 2b, and the largest NOT-

Table 2 Data summary albino ferrets; percentage of double labelled cells refers to the smaller population of labelled cells

Animal	NOT	IO-label	Retinal label co	# Cells co retina	# Cells ipsi retina	Co:ip ratio	MTN	Retinal label co	# Cells co retina	# Cells ipsi retina	Co:ip ratio	# Double labellled co	# Double labelled ip
432233a	Lateral close to pul?	Sparse medial not dorsal	Streak + dorsal	3,471 + 160α	92 + 3α	37.7:1	–	–	–	–	–	–	–
238658a	Tiny, good	–	Streak + dorsal	1,075 + 28α	16	67.2:1	–	–	–	–	–	–	–
429149a	Small, superfic	–	Streak + dorsal + ventral	1,431 + 40α	17	84.5:1	–	–	–	–	–	–	–
250965a	Tiny, superficial	–	Streak + dorsal + ventral	44 + 3α	6	7.3:1	Large, lateral	Streak + dorsal + ventral	642	79	8.1:1	6 13.6%	0
411113a	Small, not quite superficial	Sparse mediodors	Mainly dorsal	636 + 9α	26	24.5:1	Large, good	Mainly dorsal	150	7	21.4:1	3 1.96%	0
212233a	Good, lateral close to pul	Sparse, fibers med + lat	Streak + dorsal + ventral	6,209 + 578α	732 + 2α	8.5:1	Large, medial	Mainly dorsal	40	52	0.8:1	9 18.4%	2 3.7%
422270a	Good, lateral close to pul	Sparse, fibers med	Streak + mainly dorsal	1,870 + 110α	27	69.3:1	Large, medial	Mainly dorsal	67	60	1.1:1	1 1.47%	0
355924a	Small, superficial	Mediodors + middle	Streak + dorsal + ventral	103 + 5α	45	2.3:1	Large, good	Streak + dorsal + ventral	842	29	29:1	0	0
421399a	Large, deep	Mediodors + laterodors	Streak + dorsal + ventral	1,641 + 48α	21 + 1α	78.1:1	Large halo around aqueduct	Mainly dorsal	77	28	2.8:1	12 13.5%	0
424859a	Small, medial	Mediodors + laterovent	Streak + dorsal + (vent)	881 + 62α	36 + 1α	24.5:1	Large, medial	Streak + dorsal	30	15	2:1	0	0

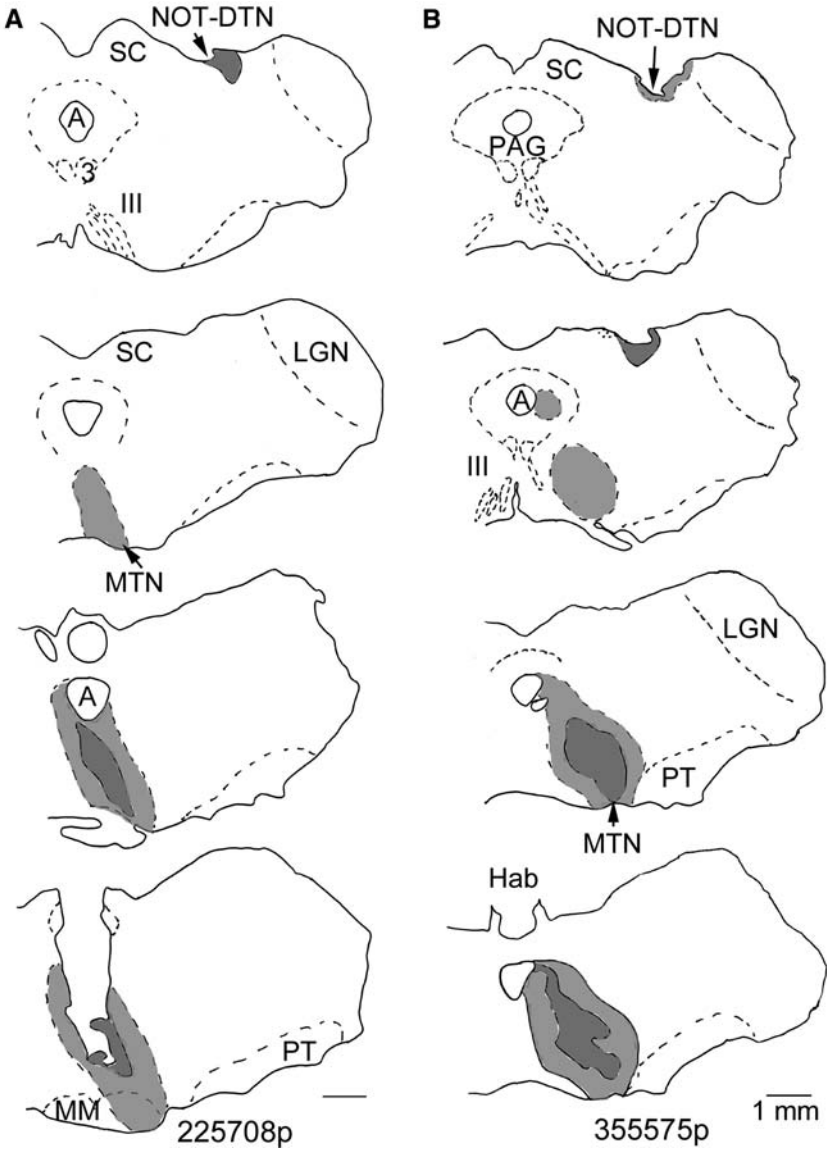

Fig. 1 Reconstruction of the injection sites in two pigmented ferrets. **a** Smallest NOT-DTN injection with an a–p extent of 400 µm and a mid-range MTN injection with an a–p extent of 1,800 µm. **b** Mid-range NOT-DTN injection with an a–p extent of 800–1,000 µm and the largest MTN injection with an a–p extent of 2,500 µm. The frontal sections through the midbrain are arranged from posterior (*top*) to anterior (*bottom*), the intersection distance in both cases is 600 µm. The centers of the injection sites are indicated by *dark grey*, the periphery of the injection sites by *light grey* shading. *A* aqueduct, *3* nucleus oculomotorius, *III* nervus oculomotorius, *Hab* habenulae, *LGN* lateral geniculate nucleus, *MM* mammillary body, *PAG* periaqueductal grey, *PT* pyramidal tract. *Numbers* below rows of sections indicate the animal number. *Scale bars* represent 1 mm

DTN injection (a–p extent 1,000 µm) and the smallest MTN injection (a–p extent 1,200 µm) in Fig. 2c. Again, the smallest NOT-DTN injection (Fig. 2b) yielded no anterograde labelling of the IO, whereas the other injections did (see Table 2; Fig. 4d). From these figures, as well as Tables 1 and 2 it becomes clear that the size, as well as the exact location of the center of the injections was variable. Nevertheless, all MTN injections included the region of the exit of the third nerve. All NOT-DTN injections penetrated and included the BSC. To what extent fibers of passage in the BSC were disrupted is difficult to assess but can certainly not be excluded.

Retinal input to the NOT-DTN

We did not attempt to allocate the labelled ganglion cells to certain ganglion cell classes other than differentiating between alpha-cells and smaller cells because dendritic labelling was usually incomplete. Tables 1 and 2 indicate the number of small labelled ganglion cells and, in addition, the number of labelled alpha-cells. On average, the labelled alpha-cells in the contralateral retina amounted to $4.2 \pm 3.9\%$ of the labelled population in pigmented ferrets and to $4.6 \pm 2.2\%$ in albinos.

Figures 3 and 4 give typical examples of retinal labelling after NOT-DTN and MTN injections in pigmented and albino ferrets, respectively. For the sake of clarity, alpha-cells are not demonstrated. However, their distribution followed the one of the small labelled ganglion cells in the contralateral retina in all cases. The injection sites are demonstrated in the frontal sections through the midbrain (Figs. 1b, 2c, 3c, 4c). The anterograde labelling in the IO is shown in Figs. 3d and 4d.

After NOT-DTN injections, labelled ganglion cells (red symbols) were found in the visual streak and the dorsal and,

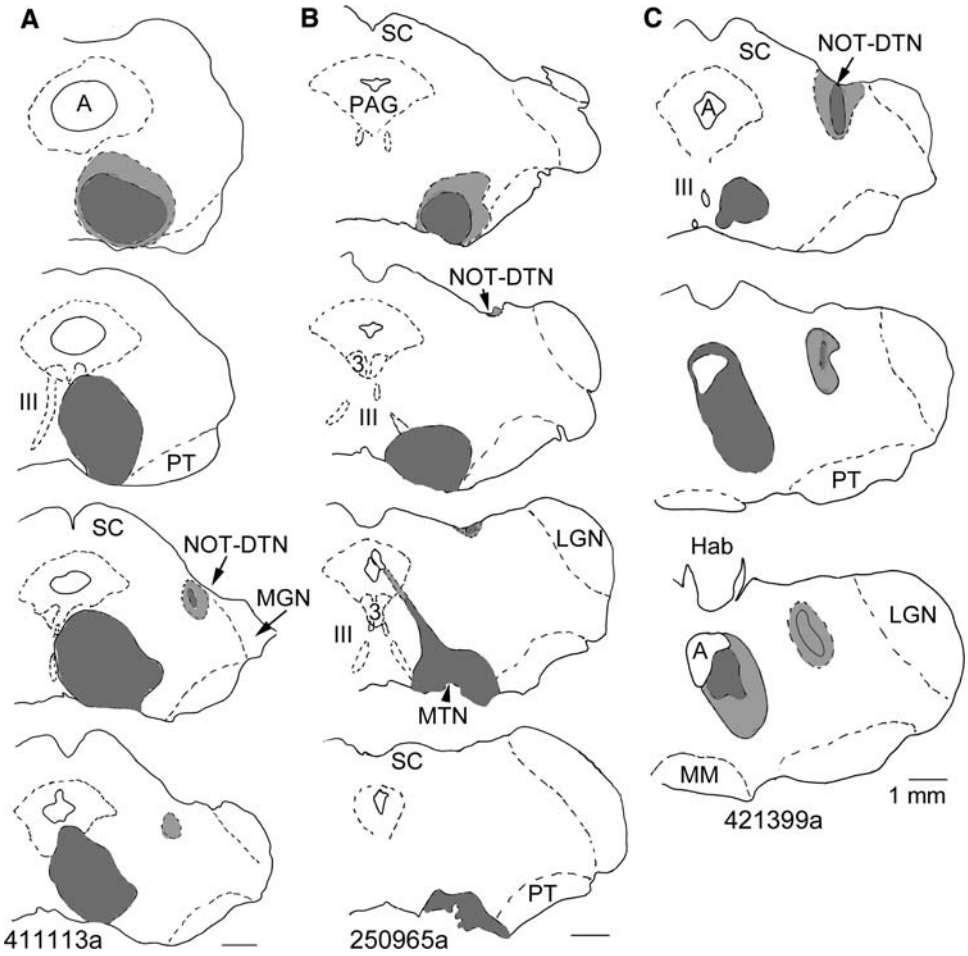

Fig. 2 Reconstruction of the injection sites in three albino ferrets. **a** Mid-range NOT-DTN injection with an a–p extent of 500 µm and largest MTN injection with an a–p extent of 3,600 µm. Intersection distance is 900 µm. **b** Smallest NOT-DTN injection with an a–p extent of 400 µm and mid-range MTN injection with an a–p extent of 2,500 µm; intersection distance is 600 µm. **c** Largest NOT-DTN injection with an a–p extent of 1,000 µm and smallest MTN injection with an a–p extent of 1,200 µm; intersection distance is 600 µm. *MGN* medial geniculate nucleus. For all other conventions see Fig. 1

often to a lesser extent, in the ventral retina of the contralateral eye (Figs. 3a, 4a). This was true for both pigmented and albino animals. In the ipsilateral retina (Figs. 3b, 4b), labelled ganglion cells were mainly located in the temporal retina. However, isolated labelled cells were also present in other regions in albinos (nine of ten cases), but also in pigmented animals (five of seven cases). On average, the region labelled in the ipsilateral temporal retina was not significantly smaller in albino than in pigmented ferrets.

We also analyzed the ratio of ganglion cells labelled after NOT-DTN injection in the contra- and ipsilateral retina (Tables 1 and 2). Unexpectedly, on average this ratio did not differ significantly between pigmented and albino ferrets (pigmented $x = 33.6{:}1$, ± 33.5; albino $x = 40.4{:}1$, ± 31.6; *T*-test, $P > 0.05$).

Retinal input to the MTN

After MTN injections, a homogenous population of small ganglion cells (blue symbols) was labelled mainly in the visual streak and the dorsal retina of the contralateral eye. In most cases, also the ventral retina was labelled (Figs. 3a, 4a; Tables 1, 2). Ipsilaterally, labelled ganglion cells were located mainly in the temporal and in the dorsal retina both in pigmented and albino ferrets. Again, isolated labelled ganglion cells in other parts of the retina were present in both phenotypes (Figs. 3b, 4b).

Note that the evidently different labelling from the NOT-DTN and the MTN shown in Figs. 3 and 4 does not indicate different input strength to one or the other nucleus between pigmented and albino ferrets. Rather, the different labelling is due to the individual size and location of the injection sites in the two cases demonstrated in Figs. 3 and 4.

A nearest neighbour analysis was undertaken in order to judge the regularity of the distribution of labelled ganglion cells. For the NOT-DTN projecting population this analysis is confounded by the fact that due to the location of the retinal slip cells in and below the BSC inevitably more than one population of ganglion cells are labelled. Thus, we concentrated the nearest neighbour analysis on the MTN projecting cells. Figure 5 shows the distributions of nearest neighbour distances for a pigmented (Fig. 5a) and an albino ferret (Fig. 5b), where the injections yielded a sufficiently large number of labelled ganglion cells in the contralateral retina. We omitted the larger distances i.e. values beyond 400 µm from the analysis because they presumably represent

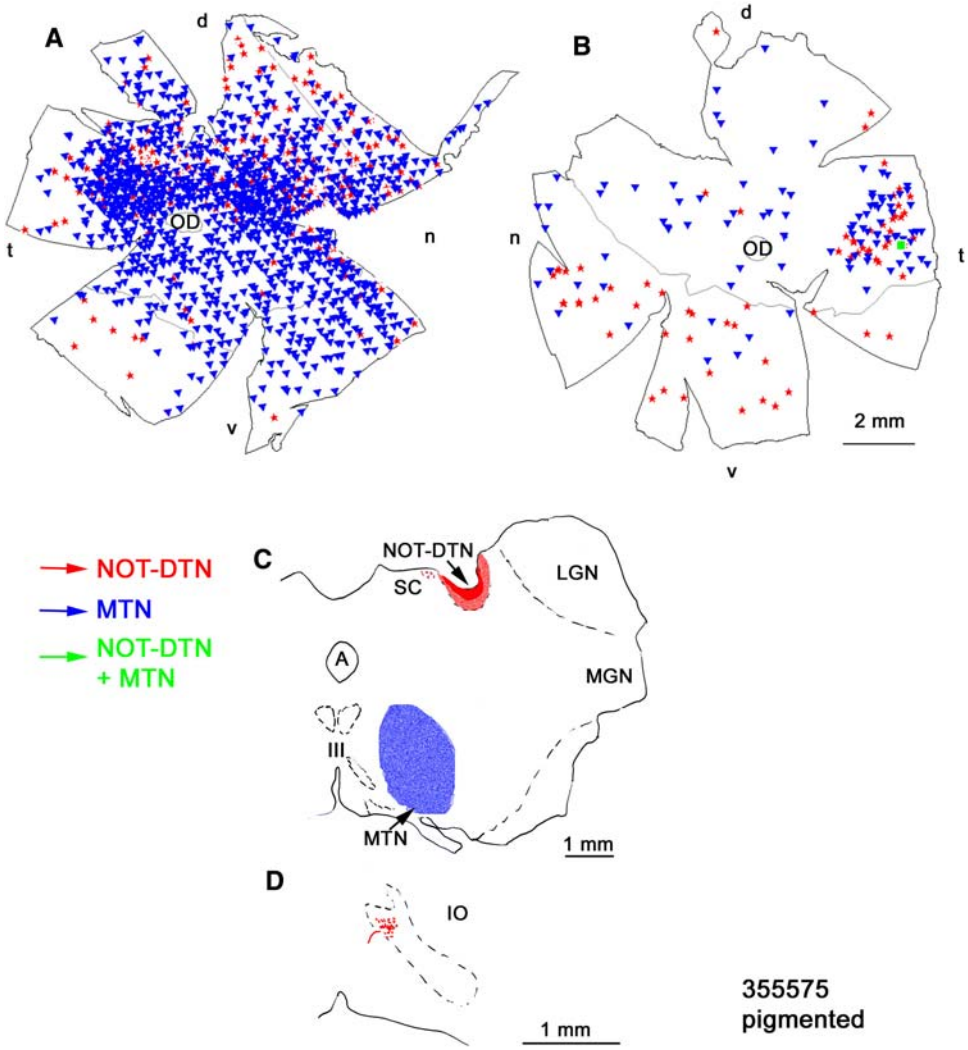

Fig. 3 Ganglion cells in the contra- (**a**) and the ipsilateral (**b**) retina of a pigmented ferret (355575) retrogradely labelled after RD-injection into the NOT-DTN (*red symbols*) and GB-injection into the MTN (*blue symbols*). There is only one double labelled cell (*green symbol*) in the ipsilateral retina of this animal. **c** Frontal section demonstrating the location of the injection sites (*coloured areas*). **d** Frontal section through the brainstem demonstrating the anterograde labelling after NOT-DTN injection in this case. *d* Dorsal, *n* nasal, *t* temporal, *v* ventral, *III* oculomotor nucleus, *A* aqueduct, *IO* inferior olive, *LGN* lateral geniculate nucleus, *MGN* medial geniculate nucleus, *MTN* medial terminal nucleus, *NOT-DTN* nucleus of the optic tract and dorsal terminal nucleus, *SC* superior colliculus

gaps caused by incomplete labelling of the entire population. The continuous lines indicate the Gaussian curve fitted to the data. For both populations a normal distribution was not rejected by a Shapiro-Wilk test ($P > 0.05$). Again, there was no obvious difference between pigmented and albino ferrets.

Taken together, our data indicate that the distribution of ganglion cells projecting to the NOT-DTN and to the MTN, respectively, is very similar in ferret. In addition, there is no difference in the distribution of these cells between pigmented and albino ferrets.

Specificity of retinal projections to the NOT-DTN and the MTN

To judge the specificity of the retinal projections to the NOT-DTN and to the MTN we analyzed the occurrence of ganglion cells simultaneously labelled by tracer injections into these nuclei. In pigmented ferrets, the retinal input to the two nuclei was completely distinct, only one double labelled cell was found in the ipsilateral retina of one pigmented animal (355575, Fig. 3b; Table 1). By contrast, in five out of seven albino ferrets double labelled ganglion cells were present (Fig. 4; Table 2). The proportion of double labelled ganglion cells relative to the smaller population of labelled cells varied between 1.5 and 18.4%. Thus, the specificity of the retinal projections to the accessory optic system indeed seems to be reduced in albino ferrets. The quantity of double labelled cells seems to depend on the exact location and size of the injection sites.

Discussion

Using a dual tract tracing approach, we investigated the distribution of retinal ganglion cells projecting to the accessory optic system, as well as the specificity of this projection in pigmented and albino ferrets. We could show that in ferret ganglion cells projecting to the NOT-DTN and to the MTN occupy the same retinal regions, namely the visual streak and the upper retina, and often to a lesser

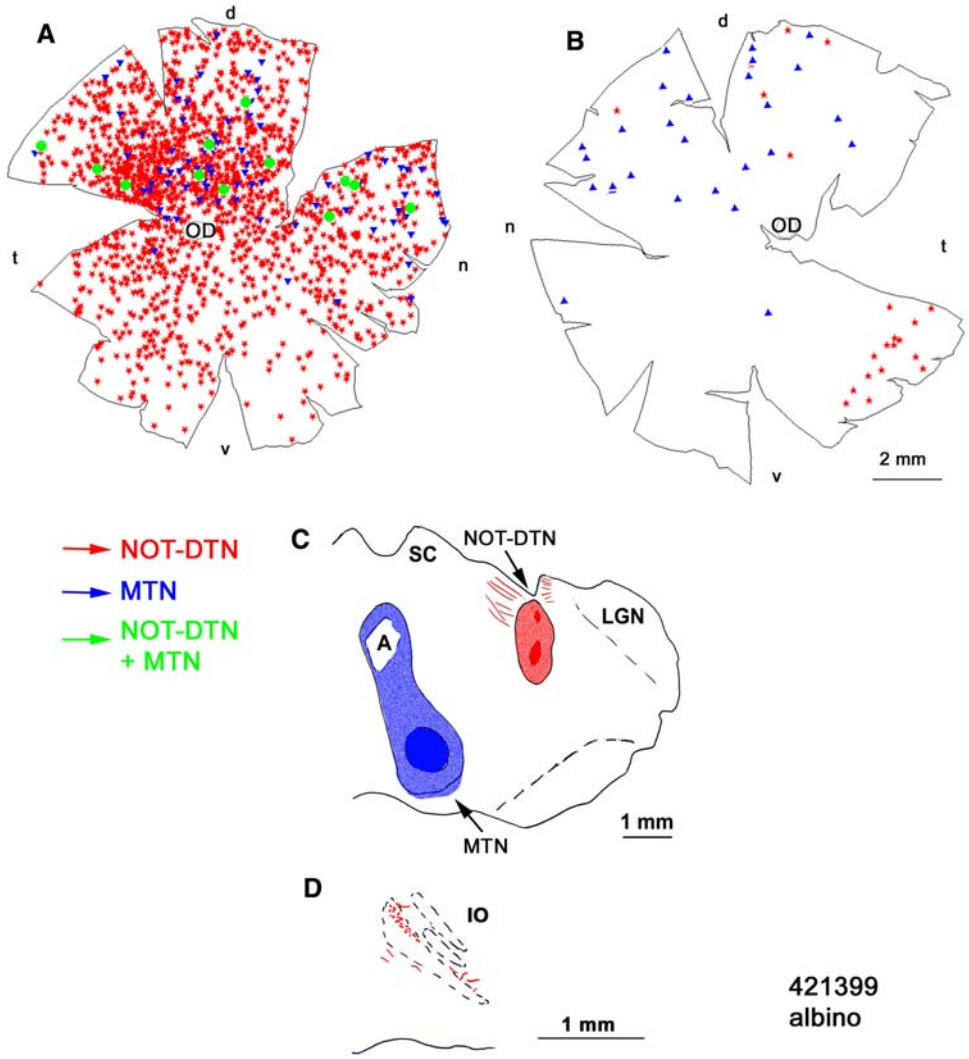

Fig. 4 Retrogradely labelled ganglion cells in the contra- (**a**) and ipsilateral (**b**) retina of an albino ferret (*421399*). Conventions as in Fig. 3

extent, the ventral retina. No difference was found between pigmented and albino ferrets. Whereas in pigmented ferrets the retinal projections to the NOT-DTN and to the MTN were completely separate, in albinos up to 18% of retinal ganglion cells projected simultaneously to both targets. Thus, the specificity of the retinal projection to the accessory optic system seems to be reduced in albino ferrets.

Retinal input to the accessory optic system in pigmented and albino ferrets

Tracer injections into the NOT-DTN revealed an inhomogenous distribution of labelled ganglion cells in the contralateral retina with the highest density in the visual streak. The majority of cells had small somata corresponding to the electrophysiological findings that ganglion cells projecting to the NOT-DTN in ferrets have slow conduction velocities (Klauer et al. 1990). We did not attempt to further classify these input neurons because, first, dendritic labelling was insufficient in most cases and, second, ganglion cell classes in ferret cannot be clearly distinguished based on soma size (Henderson 1985). In addition, in most cases alpha-cells were labelled after NOT-DTN injections almost certainly resulting from labelling of fibers terminating in the NOT (Ballas and Hoffmann 1985) or of fibers of passage that travel in the BSC towards the SC. There is no electrophysiological evidence for direct input from alpha-cells to the direction selective cells in the NOT-DTN in any of the species investigated so far. Due to the location of the retinal slip cells of the NOT-DTN between and below the BSC (Zhang and Hoffmann 1993; Telkes et al. 2001) an unintentional labelling of additional ganglion cell populations is unavoidable. However, it does not seem feasible to accurately judge the amount of "unspecifically" labelled neurons. No difference was found in the distribution of ganglion cells projecting to the NOT-DTN between pigmented and albino ferrets.

In the ipsilateral retina, labelled ganglion cells were concentrated in the temporal retina. However, isolated neurons could also be found in other regions. While this result was even more pronounced in albinos than in pigmented animals,

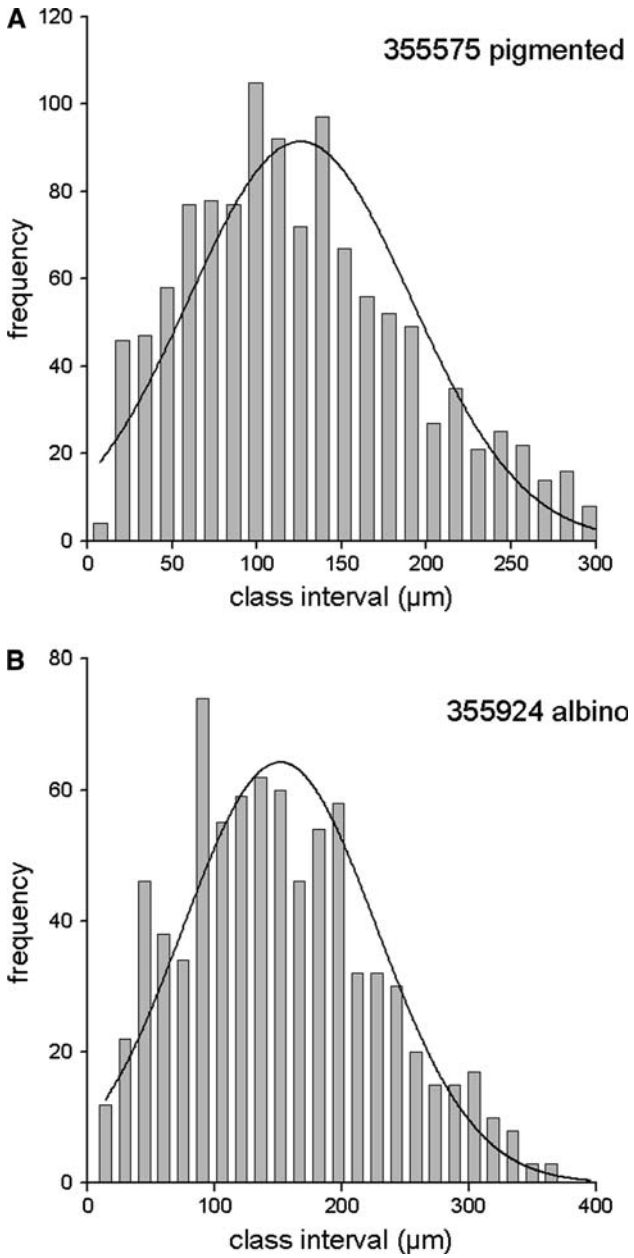

Fig. 5 Frequency distributions of nearest neighbour distances of ganglion cells retrogradely labelled after MTN injections in a pigmented (**a**, *355575*) and an albino ferret (**b**, *355924*). The *continuous lines* represent the Gaussian curves fitted to the data. Ordinate: frequency of distances, abscissa: class intervals of distances (µm)

i.e. more isolated cells were found outside the temporal retina, there was no significant difference in the extent of the densely labelled region in the temporal retina between the two phenotypes. This phenomenon was already described for the retinogeniculate projection (Morgan et al. 1987; Cucchiaro 1991): isolated ganglion cells were found beyond the temporal retina, and the decussation line was not as clearly formed in albinos than in pigmented animals.

Unexpectedly, we found no significant difference in the ratio of contra- and ipsilaterally projecting ganglion cells after NOT-DTN injections between pigmented and albino ferrets. This clearly does not correspond to the findings for the retinogeniculate projection (Morgan et al. 1987; Cucchiaro 1991), where 3–4 times more ganglion cells project ipsilaterally in pigmented than in albino ferrets. Possibly the decussation pattern differs for different subpopulations of ganglion cells projecting to different targets as has been suggested for rats. In congenic albino and pigmented rats retinal input to the ventral preoptic area, the olivary pretectal nucleus, and the ventral lateral geniculate nucleus does not differ between the phenotypes (Fleming et al. 2006). Unfortunately, the accessory optic system was not analyzed in this study. By contrast, with anterograde labelling Zhang and Hoffmann (1993) could not detect any ipsilateral projection to the accessory optic system in albino ferrets probably due to the methodical differences.

The distribution of retinal ganglion cells projecting to the MTN was largely similar to the distribution of NOT-DTN projecting cells both in the contra- and ipsilateral retina. In addition, no difference could be recognized between pigmented and albino animals. Due to the location of the MTN at the base of the brain the risk of contamination of the injection site by other visual structures is minimal. Thus, we performed a nearest neighbour analysis on cases, where large numbers of ganglion cells were labelled. Nevertheless, we eliminated distances larger than 400 µm assuming that these distances were due to incomplete labelling of the MTN. In all cases, regardless of the phenotype, the distribution closely resembled a Gaussian distribution. There was no obvious difference in nearest neighbour distances between pigmented and albino ferrets. This finding decimates the hypothesis that the loss of direction selectivity presumably also present in the MTN of albino mammals may be caused by simultaneous projections of the horizontally coding and the vertically coding subpopulations of direction selective ganglion cells. Supposing that the retina is covered by different ganglion cell populations at a constant cell specific coverage factor (Buhl and Peichl 1986; Dann and Buhl 1987; Cook and Podugolnikova 2001) and fairly regularly spaced cells, the nearest neighbour analysis should reveal smaller distances between neighbouring cells if two independent populations, i.e. horizontally coding and vertically coding ganglion cells were regarded together. This was not the case in our data. Therefore, it seems unlikely that large numbers of different direction selective ganglion cell populations project to the same target, e.g. the MTN or the NOT-DTN, respectively.

Specificity of retinal input to the accessory optic system

Specificity of the retinal projections could be hampered by, first, inappropriate projection patterns of specific subpopulations (see above) or, second, by bifurcation of specific

cells to two different targets. The first possibility is not supported by our nearest neighbour data. However, the second scenario seems at least to play a role in the annihilation of direction selectivity in the accessory optic system. In pigmented ferrets, the retinal projections to the NOT-DTN and the MTN are completely distinct, whereas in most albinos cells were found that bifurcate to project to both targets. This bifurcating population amounted to 1.5–18.4% of the labelled ganglion cells. A recent study has shown that the amount of double labelled neurons after dual tracer injections also depends on the tracers used (Schofield et al. 2007). We used the same tracer combinations in pigmented and albino animals. Thus, even if our results are an underestimate due to the choice of tracers used the difference between the phenotypes is not affected by that.

Especially in the cases with substantial numbers, i.e. more than 10%, of double labelled cells this bifurcation may significantly contribute to the loss of direction selectivity in the accessory optic system. Whether this mechanism is sufficient to cause a complete loss of direction selectivity is not clear. It is practically impossible to obtain data on quantitative differences of retinal projections between individual ferrets because that would require exactly the same injection sites and injection sizes across animals, and the same yield of dye transport. Hence, we consider it very likely that the observed variations in the proportion of bifurcating ganglion cells are largely due to differences in the injections, and that even the largest proportions of double labelled cells are underestimates.

What causes the loss of direction selectivity in the albino accessory optic system?

It is evident from the literature that cholinergic starburst amacrine cells and in consequence direction selective retinal ganglion cells play a key role in the establishment of direction selectivity in the accessory optic system and in the normal function of the optokinetic system (e.g. Oyster et al. 1972, 1980; Hoffmann and Stone 1985; Reymond and Morgan 1990; Yang and Morgan 1990; Rosenberg and Ariel 1991; Yoshida et al. 2001; Amthor et al. 2002). In an immunohistochemical approach we found a slight but significant reduction of GABAergic starburst amacrine cells in albino rat retina but not in albino ferrets (Blaszczyk et al. 2004). As the difference in our immunohistological study was rather small we proposed that this effect by itself should not be sufficient to eliminate direction selectivity in the NOT-DTN. In a second approach, investigating the motion sensitive cortical area PSS in ferret, we found a significant reduction of direction selective neurons and a reduced direction selectivity in area PSS of albino ferrets (Philipp et al. 2006). Area PSS has been shown to provide strong cortical input to the accessory optic system (Distler et al. 2006). But again, the effect seemed to be too small to be wholly responsible for the optokinetic defects seen in albino ferrets.

The present study reveals that retinal input to the NOT-DTN and the MTN is completely separate in pigmented ferrets, whereas in albino ferrets a significant number of ganglion cells bifurcate to both targets. This bifurcation could contribute to the loss of direction selectivity by combining input with e.g. horizontal and vertical preferred direction onto one cell thus causing unspecific responses to a large range of stimulus directions. Assuming that the amount of bifurcating ganglion cells found in the present study is probably an underestimate, a reduced specificity of the projection pattern can present a crucial factor for the loss of direction selectivity in the accessory optic system. Whether this mechanism is indeed primarily responsible for the loss of direction selectivity in the NOT-DTN or to what extent the other mechanisms mentioned above contribute remains to be elucidated.

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References

Amthor FR, Keyser KT, Dmitrieva NA (2002) Effects of the destruction of starburst-cholinergic amacrine cells by the toxin AF64A on rabbit retinal directional selectivity. Vis Neurosci 19:495–509

Ballas I, Hoffmann K-P (1985) A correlation between receptive field properties and morphological structures in the pretectum of the cat. J Comp Neurol 238:417–428

Birch D, Jacobs GH (1976) Spatial contrast sensitivity in albino and pigmented rats. Vision Res 19:933–937

Blake R, Antoinetti DN (1976) Abnormal visual resolution in the siamese cat. Science 194:109–110

Blaszczyk WM, Telkes I, Distler C (2004) GABA-immunoreactive starburst amacrine cells in pigmented and albino rats. Eur J Neurosci 20:3195–3198

Blaszczyk WM, Arning L, Hoffmann K-P, Epplen JT (2005) A tyrosinase missense mutation causes albinism in the Wistar rat. Pigment Cell Res 18:144–145

Blaszczyk WM, Distler C, Dekomien G, Arning L, Hoffmann K-P, Epplen JT (2007) Identification of a tyrosinase (TYR) exon 4 deletion in albino ferrets (*Mustela putorius furo*). Animal Genet 38:421–423

Buhl EH, Peichl L (1986) Morphology of rabbit retinal ganglion cells projecting to the medial terminal nucleus of the accessory optic system. J Comp Neurol 253:163–174

Collewijn H, Winterson BJ, Dubois MF (1978) Optokinetic eye movements in albino rabbits: inversion in anterior visual field. Science 199:1351–1353

Collewijn H, Apkarian P, Spekreijse H (1985) The oculomotor behaviour of human albinos. Brain 108:1–18

Cook JE, Podugolnikova TA (2001) Evidence for spatial regularity among retinal ganglion cells that project to the accessory optic system in a frog, a reptile, a bird, and a mammal. Vis Neurosci 18:289–297

Cucchiaro JB (1991) Early development of the retinal line of decussation in normal and albino ferrets. J Comp Neurol 312:193–206
Dann JF, Buhl EH (1987) Retinal ganglion cells projecting to the accessory optic system in the rat. J Comp Neurol 262:141–158
Distler C, Korbmacher H, Hoffmann K-P (2006) Neuronal connections of motion sensitive area PSS of the ferret (*Mustela putorius furo*). FENS Forum Abstr 3:A216.4
Elekessy EI, Campion JE, Henry GH (1973) Differences between the visual fields of siamese and common cats. Vision Res 13:2533–2543
Fleming MD, Benca RM, Behan M (2006) Retinal projections to the subcortical visual system in congenic albino and pigmented rats. Neuroscience 143:895–904
Garipis N, Hoffmann K-P (2003) Visual field defects in albino ferrets (*Mustela putorius furo*). Vision Res 43:793–800
Girelli M, Campara D, Tassinari G, Marzi CA (1995) Abnormal spatial but normal temporal resolution in the Siamese cat: a behavioural correlate of a genetic disorder of the parallel visual pathways. Can J Physiol Pharmacol 73:1348–1351
Grasse KL, Ariel M, Smith ID (1990) Direction-selective responses of units in the dorsal terminal nucleus of cat following intravitreal injections of bicuculline. Visual Neurosci 4:605–617
Hahnenberger RW (1977) Differences in optokinetic nystagmus between albino and pigmented rabbits. Exp Eye Res 25:9–17
Henderson Z (1985) Distribution of ganglion cells in the retina of adult pigmented ferret. Brain Res 358:221–228
Hoffmann K-P, Stone J (1985) Retinal input to the nucleus of the optic tract of the cat assessed by antidromic activation of ganglion cells. Exp Brain Res 59:395–403
Hoffmann K-P, Garipis N, Distler C (2004) Optokinetic deficits in albino ferrets (*Mustela putorius furo*): a behavioural and electrophysiological study. J Neurosci 24:4061–4069
Hupfeld D, Hoffmann K-P (2006) Motion perception in rats (*Rattus norvegicus* sp.): deficits in albino Wistar rats compared to pigmented Long-Evans rats. Behav Brain Res 170:29–33
Hupfeld D, Distler C, Hoffmann K-P (2006) Motion perception deficits in albino ferrets (*Mustela putorius furo)*. Vision Res 46:2941–2948
Jeffery G (1997) The albino retina: an abnormality that provides insight into normal retinal development. Trends Neurosci 20:165–169
Klauer S, Sengpiel F, Hoffmann K-P (1990) Visual response properties and afferents of nucleus of the optic tract in the ferret. Exp Brain Res 83:178–189
Knapp AG, Ariel M, Robinson FR (1988) Analysis of vertebrate eye movements following intravitreal drug injections. I. Blockade of retinal ON-cells by 2-amino-4-phosphonobutyrate eliminates optokinetic nystagmus. J Neurophysiol 60:1010–1021
Lannou J, Cazin L, Precht W, Toupet M (1982) Optokinetic, vestibular, and optokinetic-vestibular responses in albino and pigmented rats. Pfluegers Arch 393:42–44
Lopez VM, Decatur CL, Stamer WD, Lynch RM, McKay BS (2008) L-DOPA is an endogenous ligand for OA1. PLoS Biol 6:e236. doi:10.1371/journal.pbio.0060236
Morgan JE, Henderson Z, Thompson ID (1987) Retinal decussation patterns in pigmented and albino ferrets. Neuroscience 20:519–535
Oyster CW, Takahashi E, Collewijn H (1972) Direction-selective retinal ganglion cells and control of optokinetic nystagmus in the rabbit. Vision Res 12:183–193
Oyster CW, Simpson JI, Takahashi ES, Soodak RE (1980) Retinal ganglion cells projecting to the rabbit accessory optic system. J Comp Neurol 190:40–61
Philipp R, Distler C, Hoffmann K-P (2006) A motion-sensitive area in ferret extrastriate visual cortex: an analysis in pigmented and albino animals. Cereb Cortex 16:779–790
Precht W, Cazin L (1979) Functional deficits in the optokinetic system of albino rats. Exp Brain Res 37:183–186
Reymond E, Morgan IG (1990) Destruction of cholinergic amacrine cells in the retina abolishes opto-motor responses in chickens. Proc Australian Neurosci Assoc 1:122
Rosenberg AF, Ariel M (1991) Electrophysiological evidence for a direct projection of direction-selective retinal ganglion cells to the turtle's accessory optic system. J Neurophysiol 65:1022–1033
Schofield BR, Schofield RM, Sorensen KA, Motts SD (2007) On the use of retrograde tracers for identification of axon collaterals with multiple fluorescent retrograde tracers. Neuroscience 146:773–783
Simoni A, Sprague JM (1976) Perimetric analysis of binocular and monocular visual fields in siamese cats. Brain Res 111:189–196
St. John RS, Fisk JD, Timney B, Goodale MA (1984) Eye movements of human albinos. Am J Optometry Physiol Optics 61:377–385
Telkes I, Garipis N, Hoffmann K-P (2001) Morphological changes in the neuronal substrate for the optokinetic reflex in albino ferrets. Exp Brain Res 140:345–356
Winterson BJ, Collewijn H (1981) Inversion of direction selectivity to anterior fields in neurons of nucleus of the optic tract in rabbits with ocular albinism. Brain Res 220:31–49
Yang G, Morgan IG (1990) Destruction of cholinergic amacrine cells in the retina silences directionally selective units in the nucleus of the basal optic root of chicken. Proc Australian Neurosci Assoc 1:142
Yoshida K, Watanabe D, Ishikane H, Tachibana M, Pastan I, Nakanishi S (2001) A key role of starburst amacrine cells in originating retinal direction selectivity and optokinetic eye movement. Neuron 30:771–780
Zhang HY, Hoffmann K-P (1993) Retinal projections to the pretectum, accessory optic system and superior colliculus in pigmented and albino ferrets. Eur J NeuroSci 5:486–500