Impaired Direction Selectivity in the Nucleus of the Optic Tract of Albino Mice

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Keywords: nucleus of the optic tract, retinotopy, albinism, vision, nystagmus, neuronal tuning

Purpose. Human albinos have a low visual acuity. This is partially due to the presence of spontaneous erroneous eye movements called pendular nystagmus. This nystagmus is present in other albino vertebrates and has been hypothesized to be caused by aberrant wiring of retinal ganglion axons to the nucleus of the optic tract (NOT), a part of the accessory optic system involved in the optokinetic response to visual motion. The NOT in pigmented rodents is preferentially responsive to ipsiversive motion (i.e., motion in the contralateral visual field in the temporonasal direction). We compared the response to visual motion in the NOT of albino and pigmented mice to understand if motion coding and preference are impaired in the NOT of albino mice.

Methods. We recorded neuronal spiking activity with Neuropixels probes in the visual cortex and NOT in C57BL/6J mice (pigmented) and DBA/1J mice with ocuolucenateous albinism (albino).

Results. We found that in pigmented mice, NOT is retinotopically organized, and neurons are direction tuned, whereas in albino mice, neuronal tuning is severely impaired. Neurons in the NOT of albino mice do not have a preference for ipsiversive movement. In contrast, neuronal tuning in visual cortex was preserved in albino mice and did not differ significantly from the tuning in pigmented mice.

Conclusions. We propose that excessive interhemispheric crossing of retinal projections in albinos may cause the disrupted left/right direction encoding we found in NOT. This, in turn, impairs the normal horizontal optokinetic reflex and leads to pendular albino nystagmus.

Albinism is a congenital disorder characterized by a lack of pigment. Its incidence in Europe is 1 in 20,000 and as high as 1 in 1000 in some sub-Saharan populations. Human albinos have lower than normal visual acuity. On average, they can see at 20 m what a pigmented individual sees at 80 m. Foveal hypoplasia, refractive errors, glare, and the presence of spontaneous involuntary eye movements, called pendular nystagmus, contribute to the low acuity in albino vertebrates. The miswiring includes retinal input to the lateral geniculate nucleus (LGN) and the superior colliculus, but the error in input to the nucleus of the optic tract (NOT) within the AOS has been considered the most likely cause of nystagmus in albinism.

The NOT is essential for the normal horizontal OKR that is closely associated with nystagmus. It receives direct input from direction-selective RGCs, responds to temporonasal visual movement in the contralateral visual field, and projects to precerebellar and preocular motor nuclei involved in eye movement control. In albino rabbits and ferrets, the direction tuning of NOT to visual motion is disturbed. These facts strongly suggest that NOT plays a role in albino nystagmus. Aberrant projections in the eye lead to a reduction of retinal ganglion cells (RGCs) in the temporal retina projecting ipsilaterally and a concomitant increase in these RGCs projecting contralaterally, which is present in almost all people with albinism and to some degree in all albino vertebrates. The miswiring includes retinal input to the lateral geniculate nucleus (LGN) and the superior colliculus, but the error in input to the nucleus of the optic tract (NOT) within the AOS has been considered the most likely cause of nystagmus in albinism.
from the ventrotemporal retina could underlie the disturbance in the NOT and the optokinetic problems seen in albinos.

However, the evidence for ipsilateral projections from the retina to NOT is debated. While ipsilateral projections are found in pigmented ferrets, older studies reported ipsilateral projections seem to be weak or absent in rabbits and mice. More recent studies in mice using genetic fluorescent tracers, however, show the presence of ipsilateral retinal axons in the NOT and give new life to the hypothesis that the miswiring of retinal inputs to the NOT underlies optokinetic problems in albinos.

We therefore investigated the neural responses to visual motion of the NOT in pigmented C57BL/6J mice and in DBA/1J mice with oculocutaneous albinism. DBA/1 mice are hypopigmented and have a gray coat color. They carry a mutation in the Tyrp1 gene on the brown coat color locus. Tyrp1 codes for tyrosinase-related protein 1 and is involved, with tyrosinase, in melanin synthesis. In humans, mutations in Tyrp1 are associated with oculocutaneous albinism type 3 (OCA3) and chiasmic misrouting.

Like other albinotic mice, hypopigmented mice display developmental displacement of RGCs in the ventrotemporal retina and aberrant crossing. The advantage of using the hypopigmented DBA/1 mouse over albino strains that lack all pigment is that mice with a Tyrp1 mutation do not have the poor acuity and low visual response that complete albino mice with a mutation in the tyrosinase gene have. Young adult mice with the same Tyrp1 mutation have an acuity matching that of the pigmented BL/6 strain and a visual response in visual cortex that is lower but of the same order as that in pigmented mice.

We recorded from visual cortex and NOT in pigmented and oculocutaneous albinoid (hypopigmented) mice using Neuropixels and found that direction selectivity was impaired in NOT of albino mice while orientation selectivity was preserved. This observation supports the hypothesis that excessive interhemispheric crossing of retinal projections in albinos causes disrupted left/right direction encoding in NOT, which in turn impairs the normal horizontal OKR and leads to albino nystagmus. Finally, we also describe a putative retinotopic map of area NOT based on receptive field mapping in pigmented mice.

METHODS

Acquisition and Preprocessing of Laminar Neuropixels Probe Data

We analyzed repeated-insertion silicon probe recordings with Neuropixels 1.0 in seven pigmented C57BL/6J mice (Janvier) and five DBA/1J mice (Janvier) with oculocutaneous albinism, 2 to 7 months of age. Mice were housed in a normal 12-hour/12-hour dark/light cycle with ad libitum access to food and water. All experiments were approved by the institutional and national animal ethics committees, in compliance with all relevant ethical regulations. For brevity and because of our focus on the early visual system, we will refer to DBA/1J mice with oculocutaneous albinism as “albinos.”

Mice were habituated for 1 to 4 weeks before being implanted with a cranial bar used for head fixation. Mice were anesthetized with isoflurane (3% induction, 1%–1.5% maintenance in 50% O2) and injected subcutaneously with an analgesic and anti-inflammatory compound (Metacam, 2 mg/kg). The eyes were protected from drying by Cavasan eye ointment. They were moved to a stereotact with a thermal mat to keep their core temperature at 37°C, and the fur on their heads was removed. Once anesthesia was sufficiently deep, as indicated by the absence of a toe-pinch reflex, we applied lidocaine locally on the skin of the head and sterilized it with 70% ethanol or betadine. The skin was removed, the skull cleaned, and a small metal head post was fixed to the skull anterior of bregma with the use of blue-light curing dental cement. If necessary, we sutured the skin and let the mice recover for 2 to 7 days.

After the mice recovered as indicated by a return to their preoperative weight, mice were habituated to sitting head-fixed in the electrophysiology rig for 3 to 10 days. Once habituated, they underwent a craniotomy surgery, following the same preparatory steps as described above. Before performing the craniotomy at 2.5 mm lateral of the midline and 1.05 mm posterior to lambda (1.5–3 mm in diameter), we first constructed a small ring of dental cement so the brain could be bathed in saline during recordings to avoid tissue desiccation. Once the craniotomy was complete, this ring was filled with sterile silicone, and the animals were left to recover for at least 16 hours. Over the next 1 to 7 days, we performed repeated-insertion recordings using Neuropixels 1.0 silicon probes (Imec, Leuven, Belgium). The probe was inserted near the center of the craniotomy with an angle that ranged from 0 to 10 degrees medially relative to vertical and 5 to 10 degrees anteriorly. Before each recording, we dipped the probe into a fluorescent dye (DiD, DiO, or Dil; cat. V22889; Thermo Fisher, Waltham, MA, United States), using a different dye each day (and repeating the triplet if necessary). On the last day of recording, we perfused the animal with 4% paraformaldehyde in PBS, and the brains were extracted to perform post hoc tracing of the electrode recording locations to distinguish recordings made on different days. The brains were sliced corologically using a vibratome, and the slices (70 μm thick) were mounted using Vectashield DAPI solution (Vector Laboratories, Newark, CA, United States) to label cell nuclei. Imaging acquisition was performed using a Zeiss Axioscan.Z1 slide scanner (10× objective; Zeiss Plan-Apochromat, 0.16 NA; Zeiss, Oberkochen, Germany) to visualize the different fluorophores (DAPI, 405 nm; DiO, 488 nm; Dil, 555 nm; DiD, 647 nm). Neuropixels recordings were performed using a National Instruments, Austin, TX, United States 1/O PXIe-6341 module and SpikeGLX (https://github.com/billkarsh/SpikeGLX). Visual stimulation was performed as described previously using the Acqupix acquisition toolbox (https://github.com/JorritMontijn/Acqupix). We corrected for screen latencies with high accuracy (<1 ms) using photodiode signals that recorded visual stimulus onsets. Spikes were sorted post hoc using Kilosort2.5 (https://github.com/MouseLand/Kilosort), and we analyzed only clusters of sufficient quality, as defined by a low spike contamination and low nonstationarity of spiking rate across the recording (see next section). The cluster contamination was calculated by counting the number of spikes occurring within 1 ms of another spike, normalized to the number of violations expected if the neuron showed a time-independent Poisson spiking rate. High-quality clusters (i.e., putative neurons) were assigned a brain region using the Allen Brain Atlas in the UniversalProbeFinder toolbox.

We performed pupil detection of the mouse’s eye by positioning a dichroic mirror in front of the (left) eye.
contralateral to the (right) recorded brain hemisphere and illuminating the eye with an infrared LED. Visible light emitted by the screen could pass through the dichroic mirror, while an infrared-sensitive camera mounted at the top of the Faraday cage could record the mouse's pupil by the infrared light reflected by the dichroic mirror. All code used in laminar probe data acquisition and preprocessing is available online at github.com/JorritMontijn/Acuquipix, github.com/JorritMontijn/UniversalProbeFinder, and github.com/JorritMontijn/EyeTracker.

Data Curation and Number of Cells per Area and Mouse Type

In total, we analyzed 21 recordings from seven pigmented mice and 14 recordings from five albino mice. We used eight C57BL/6J(R) (Janvier) and eight DBA/1J(R) (Janvier) mice, of which respectively seven and five animals yielded at least one high-quality data set that contained well-isolated single units in visual cortex/posteromedial visual cortex (V1/PM) and/or in NOT. We discarded all units with a high nonstationarity or a high number of spikes in the refractory period, calculated as described above and online.36 We set the threshold at a nonstationarity < 0.25 and a violation fraction < 0.25. This yielded 3261 well-isolated, stable, single units in cortex from 21 of 21 recordings in pigmented mice, 835 units in cortex from 12 of 14 recordings in albino mice, 137 units in NOT from 10 of 21 recordings in pigmented mice, and 57 units in NOT from 6 of 14 recordings in albino mice. The difference in yield between albino and pigmented mice can be partly explained by the phenotype of albino mice: despite habituating albino mice for longer than pigmented mice in the head-fixed setup prior to recording, they showed a much greater tendency to move than pigmented mice. This occasionally resulted in low-quality recordings due to brain movement that had to be excluded. Moreover, we observed seizure-like activity in two albino mice, one of which showed full tonic-clonic seizures. This is a known phenotype of the DBA/1 strain.39 We excluded both of these mice from further analysis. Finally, histologic reconstructions revealed we did not hit NOT in one pigmented mouse and one albino mouse, nor did we record any visually responsive cells in visual cortex, probably due to recording in a retinotopic location. For all remaining recordings from the remaining 14 mice in the head-fixed setup prior to recording, they showed full tonic-clonic seizures. This is a known phenotype of the DBA/1 strain.39 We excluded both of these mice from further analysis. For all remaining recordings from the remaining 14 mice in the head-fixed setup prior to recording, they showed full tonic-clonic seizures. This is a known phenotype of the DBA/1 strain.39 We excluded both of these mice from further analysis. For all remaining recordings from the remaining 14 mice in the head-fixed setup prior to recording, they showed full tonic-clonic seizures. This is a known phenotype of the DBA/1 strain.39 We excluded both of these mice from further analysis.

Drifting Grating Stimuli

Visual stimuli during Neuropixels recordings were shown at 60 Hz on a 51-cm × 29-cm Dell screen at a 27-cm distance from the animal's eyes using Acuquipix,56 powered by Psychtoolbox 3.10 in MATLAB (MathWorks, Natick, MA, USA). The full screen covered 89.4 (azimuth) by 58.7 (elevation) visual degrees and extended from the ipsilateral central binocular visual field (+25.2 degrees) to the contralateral monococular visual field (~64.2 degrees). Drifting gratings were displayed within a circular 100 visual-degree diameter window with 2 visual-degree cosine edge that faded smoothly into a neutral-gray background to reduce edge effects. Drifting gratings were shown in 24 directions: [0, 15, . . . , 345] degrees at a spatial frequency of 0.06 cycles per degree and a temporal frequency of 1 cycle per second. We presented two blocks of drifting gratings, one at the beginning and one at the end of the recording, with 20 repetitions per direction in each block (2 × 20 × 24).

Sparse Checker Stimuli and Receptive Field Map Calculation

We also performed receptive field mapping using sparse luminance patches in all pigmented mice and a subset of albino mice. On each trial, we presented three white and three black patches on a gray background, chosen randomly from a 10 (horizontal) × 7 (vertical) matrix of evenly divided patches of the computer screen. Each patch was on average 8.9 (azimuth) × 8.4 (elevation) visual degrees and stimulation lasted for 600 ms, followed by a 400-ms gray intertrial blank screen. Patches were presented until each location was displayed at least 14 times (7 white, 7 black). Receptive field (RF) maps were calculated for each neuron in two ways. First, we calculated an RF map by taking the average spiking rate for each patch over all trials where that patch was presented. Comparing these results with a different approach, however, we found that more reliable RF maps could be obtained by using the zeta-test, a statistical test that quantifies the time-locked responsiveness of a neuron.41

The population RF map for a single recording in NOT was then calculated by averaging the RF maps of all neurons that showed a statistically significant visual response to one of the patches (either black or white) at an α of 0.01. The recording’s receptive field center was then determined to be the patch with the most statistically significant response, after averaging over both on and off fields (white and black patches, respectively).

We calculated the retinotopic organization of area NOT by running an optimization algorithm (fminbnd in MATLAB R2019b) on the angle θ that would maximize the correlation between the RF center locations of all recordings and their anatomic location. The correlation we maximized was the average over azimuth and elevation, where we took the first anatomic dimension for azimuth and the second for elevation. This way, the anatomic directions for azimuth and elevation are orthogonal to each other:

$$\arg\max_\theta \frac{1}{2} \left| f_r (c_{az}, \hat{v}_a R \mathbf{X}) \right| + \frac{1}{2} \left| f_r (c_{el}, \hat{v}_e R \mathbf{X}) \right| .$$

Here, $f_r (x,y)$ refers to the Pearson correlation between $x$ and $y$, $\left| \cdot \right|$ indicates the absolute, $c_{az}$ and $c_{el}$ are the azimuth and elevation of the recording’s receptive field center, and $\hat{v}_a$ and $\hat{v}_e$ are the transposed first-dimensional and second-dimensional unit vectors $[1 \ 0]$ and $[0 \ 1]$, respectively. $R_\theta$ is the rotation matrix for angle $\theta$, and $\mathbf{X}$ is the $[2 \times n]$ matrix of n anatomic recording locations in mediolateral (ML) and anteroposterior (AP) coordinates. We found that the best angular fit $\theta$ corresponded to a correlation of $r = 0.72$. To quantify the statistical significance of this correlation, we repeated the above procedure 100,000 times, but for each iteration, we independently shuffled the ML and AP coordinates of matrix $\mathbf{X}$. The resulting sample of 100,000 correlations describes the null distribution if there is no retinotopic organization in area NOT (Fig. 11). Our analysis shows that observing a correlation of $r = 0.72$ is exceedingly unlikely under the assumption that there is no retinotopic map in NOT ($P = 0.0062$).
Calculation of Orientation and Direction Selectivity Metrics

All procedures described here used functions that can be downloaded from https://github.com/JorritMontijn/TuningCurves, specifically the functions getTuningCurve for tuning curve fitting and getOPI for the orientation selectivity calculation.

We calculated the overall tuning strength for each neuron by fitting a double-peaked von Mises function to the cell’s tuning curve averaged over trial repetitions. The double-peaked von Mises has $k = 5$ predictors: the preferred direction $\theta$; concentration parameter $\kappa$, which defines the bandwidth; the direction index $d$; the baseline firing rate $b$; and the tuning curve gain factor $g$:

$$ y(x) = g \cdot ((M(x|\theta, \kappa) + (1 - d) \cdot M(x|\theta + \pi, \kappa)) + b. $$

Here, $M(x|\theta, \kappa)$ is the standard von Mises function at angle $x$ with the circular mean at $\theta$ and concentration parameter $\kappa$. The tuning strength $\sigma$ was defined as the $P$ value of the fit’s explained variance ($R^2$) transformed to standard deviations such that a $P$ value of 0.05 corresponds to a $\sigma$ of 1.96, using the normal inverse cumulative distribution function $F^{-1}$:

$$ \sigma = F^{-1} \left( \frac{p}{2} \right). $$

Here, the $P$ value is computed from the fit’s $R^2$ using the Student’s $t$ cumulative distribution function $T$, the number of
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FIGURE 2. Tuning curves of example cells in the NOT of a pigmented and albino mouse. (A) This cell recorded in a pigmented mouse shows a preference for drifting gratings moving in the temporonasal direction in the contralateral visual field (0 degrees). (B) The cell’s tuning curve (blue) was well described by fitting a von Mises distribution (circular Gaussian, orange). (C) The spiking response to the preferred direction shows an onset peak and a sustained response during the stimulus presentation (gray bar, 0.0–1.0 seconds). (D) A cell recorded in an albino mouse. Note the response to the temporonasal as well as nasotemporal direction. (E) As in B, but for the cell shown in B1. (F) Despite clear changes in tuning curve properties between NOT cells in pigmented and albino mice, there was no difference in average firing rate in response to a neuron’s preferred stimulus (mean rate; pigmented = 15.4 Hz; albino = 11.5 Hz; t-test, P = 0.366).

Decoding Procedure

All decoding analyses used a multiclass logistic regression decoder with leave-one-repetition-out cross-validation. The function `doCrossValidatedDecodingLR` that implements this algorithm can be downloaded from the DecodingAndInformation repository on GitHub: https://github.com/JorritMontijn/DecodingAndInformation.

RESULTS

The NOT Is Retinotopically Organized

We performed repeated-insertion electrophysiologic recordings with Neuropixels in the right hemisphere of seven pigmented C57BL/6J (pigmented) mice and five DBA/1J (albino) mice with oculocutaneous albinism (albino). While our main aim was to record from the NOT and compare its functioning between pigmented and albino mice, we inserted the probe...
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at an angle so we could simultaneously record NOT and primary visual cortex (V1) or PM (Figs. 1A, 1B). This allowed us to compare neurophysiologic changes between visual cortex (V1/PM) and NOT, as well as between pigmented and albino animals. Moreover, by verifying that visual responses in the cortex of the albino mice were mostly unaffected, we ensured that our observations in NOT of albino mice could not be explained by generally poor eyesight.

First, we performed receptive field mapping in pigmented mice to investigate the potential retinotopic organization of the NOT. In 10 recordings from six different pigmented mice, we obtained statistically significant receptive fields from at least one NOT cell. As our penetration angle was almost perpendicular to the mostly two-dimensional organization of NOT in the AP/ML plane (Fig. 1C), we hypothesized that the retinotopic map of NOT, if it existed, would likely lie in the AP/ML plane. To increase our statistical sensitivity, we therefore averaged the receptive fields over all cells from the same recording and defined the location's receptive field center to be that recording's most statistically significant response to sparse black/white checkers (Figs. 1D–F). The best fit for the organization of receptive fields we recorded described a pattern aligned with the principal axes of area NOT, with the midpoint of the visual field located at the anteromedial end. The receptive field azimuth became more temporal in the posteroomedial direction (Fig. 1G), while the elevation became higher in the posterolateral direction (Fig. 1H). However, the estimate of azimuth is somewhat uncertain, as the anatomic recording locations in NOT were mostly centered on the posterolateral part of NOT. We can say with high confidence, however, that this area of NOT responds to the binocular visual field with an elevation above the horizontal midline.

We quantified the statistical significance of this map using a shuffle-permutation test (see Methods). In short, we computed the correlation between anatomic recording location and the recording's receptive field center location for the real data (r = 0.72) and for 100,000 random shuffle iterations, where we randomized the receptive field center location over recording locations (independently for both elevation and azimuth). Using this sample of randomized correlations as the null-hypothesis distribution (null mean r = 0.39), we calculated the P value as the quantile position of the real correlation (P = 0.0062) (Fig. 1I). We therefore conclude that the NOT is retinotopically organized.

Finally, we note that receptive field mapping with sparse on/off flashes in area NOT led to relatively weak responses compared to our experience with receptive field mapping in visual cortex and superior colliculus. As also noted previously, more reliable receptive field mapping in NOT may be obtained by using small drifting stimuli rather than sparse on/off flashes. We recommend future researchers attempting to create a more detailed retinotopic map of NOT to not use sparse checkers, as we only obtained reliable maps after averaging the responses across all neurons in a single recording.

Direction Selectivity Is Impaired in NOT of Albino Mice, While Orientation Selectivity Is Preserved

Next we proceeded to test our main hypothesis—that the right hemisphere NOT in albino shows impaired selectivity for rightward-moving (predominantly temporonasal) over leftward-moving (predominantly nasotemporal) visual stimuli. We analyzed the overall direction selectivity of 3261 well-isolated, stable, single units in visual cortex of pigmented mice, 835 units in visual cortex of albino mice, 137 units in NOT of pigmented mice, and 57 units in NOT of albino mice. We presented 20 to 40 shuffle-randomized repetitions of drifting gratings that moved in 24 directions around the unit circle (so in steps of 15 degrees). We fitted a double-peaked von Mises (circular Gaussian) function to each cell's responses and calculated the tuning strength as the predictive power of this fit (P value of the fit's R² transformed to units of standard deviation; see Methods and Fig. 2). For each significantly tuned cell, we also calculated its OSI and the left/right direction selectivity index (DSI). For all analyses in Figures 2 and 3, we only included significantly tuned cells. Including also untuned cells reduced the statistical power of our analyses but otherwise did not qualitatively alter the results.

First, we investigated visual cortical responses. We compared pigmented animals with albino animals and found that the overall distribution of preferred directions was comparable (Fig. 3A). We observed a textbook-like distribution of tuning in cortex of pigmented mice, where cardinal directions are somewhat overrepresented, and a similar but somewhat noisier pattern in cortex of albino mice. In fact, neither orientation selectivity (two-sample t-test, P = 0.067) nor left/right direction selectivity (P = 0.300) was significantly different between cortex of pigmented and albino mice (Figs. 3B, 3C).

Second, we investigated direction tuning in area NOT. In pigmented animals, we observed an overabundance of (ipsiversive) rightward-moving preferring cells, while in albino animals, we recorded an overrepresentation of rightward- as well as leftward-movement preferring cells (Fig. 3D). Interestingly, cells in NOT of albino mice showed a similar orientation selectivity (Fig. 3E, P = 0.211), suggesting that orientation tuning is preserved in NOT of albino mice. In contrast, the distribution of direction selectivity in the right NOT of pigmented mice was strongly biased to rightward-moving stimuli, while no such bias was observed in the right NOT of albino mice (Fig. 3F, albino versus pigmented DSI, two-sample t-test, P = 9.4 × 10⁻³). In fact, the strongest left/right direction selectivity we found in NOT of albino mice was a cell with DSI = 0.268, whereas in NOT of pigmented mice, we found multiple cells with a DSI of 1.00 (i.e., complete silencing when the nasotemporal direction was presented).

To conclude, we found that overall tuning strength was similar for pigmented and albino animals in visual cortex (P = 0.217) but was impaired in NOT of albino animals compared to pigmented animals (P = 9.1 × 10⁻³) (Fig. 3G). Moreover, we found a significant interaction effect between brain area (cortex/NOT) and animal pigmentation (pigmented/albino): two-way ANOVA, area × pigmentation: P = 7.7 × 10⁻⁴. This effect was not explained by reduced orientation selectivity, which was similar across area and pigmentation: cortex pigmented versus albino, P = 0.067; NOT pigmented versus albino, P = 0.211; interaction area × pigmentation, P = 0.970 (Fig. 3H). Instead, we found that left/right direction selectivity was similar in cortex between pigmented and albino mice (P = 0.300) but observed a strong reduction in left/right direction selectivity in NOT of albino mice compared to NOT of pigmented mice (P = 9.4 × 10⁻³) (Fig. 3I). The interaction effect of area × pigmentation for direction selectivity was also significant (P = 0.022).
**Decoding Analyses Confirm a Lack of Horizontal Direction Differentiation in NOT of Albino Mice**

The analyses described above suggest that neural codes for stimulus direction in visual cortex are similar for pigmented and albino mice but that specifically left/right selectivity is impaired in area NOT of albinos. However, the above analyses were based on different numbers of stimulus repetitions and cells per area and mouse pigmentation type. To confirm these results with a different approach, we therefore performed cross-validated single-trial stimulus decoding on noise-whitened pseudo-populations where we equalized the number of trials and number of neurons in each condition (see Methods). To illustrate the importance of this approach, we first ran a traditional decoding analysis, where we used a logistic regressor independently on each recording and averaged the output over all recordings (Fig. 4A). The resulting confusion matrices suggest that decoding accuracy in the visual cortex (Ctx) of pigmented mice is much higher than in the visual cortex of albino mice (compare the Ctx pigmented with Ctx albino panel). However, this difference can be explained by the...
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Figure 4. Single-trial decoding analyses reveal impaired neural codes in NOT of albino mice for leftward-moving versus rightward-moving stimuli. (A) Confusion matrices of a logistic regression decoder, averaged over all drifting grating recording blocks. The matrices show typical errors in visual cortex (Ctx) of pigmented and albino mice, where the orientation is correctly decoded, but the decoded direction is sometimes incorrect, leading to a heightened band of errors at ±180 degrees of the real stimulus direction (diagonal). This typical pattern is attenuated in NOT pigmented. (B) Average decoding approaches as in panel A are biased by the number of recordings, trials, and neurons. An unbiased decoding approach therefore requires normalizing for these factors, which we achieved using pseudo-populations of neurons (see Methods). These four confusion matrices show the result of four example pseudo-populations. (C) Decoding the stimulus direction recapitulates the results of Figure 3C: NOT of albino mice has reduced decoding accuracy. (D–F) Running 2500 bootstrap iterations of randomly selected pseudo-populations reveals the same pattern: left/right decoding accuracy in NOT of pigmented mice is higher than in NOT of albino mice (pigmented: 89%, albino: 46%, z-test, \(P = 4.5 \times 10^{-5}\)), while cortex shows no difference (pigmented: 65%, albino: 54%, \(P = 0.426\)).

lower number of cells per recording and lower number of recordings in Ctx albino. When we ran the same decoding approach, but now on equalized pseudo-populations, the difference between Ctx pigmented and Ctx albino became much less stark (Fig. 4B).

We ran 2500 iterations of a pseudo-population decoding analysis, where for each iteration, we took a random subsample of a fixed number of neurons (\(N_r = 20\)) and stimulus repetitions (\(N_r = 14\)) from the concatenated and whitened data pooled across all recordings in the same area (cortex or NOT) of the same mouse type (pigmented or albino). Using this approach, we confirmed the results we observed previously (Fig. 4C): direction decoding accuracy did not differ between pigmented and albino in cortex (z-test, \(P = 0.073\)) but was much higher in NOT of pigmented mice than NOT of albino mice (\(P = 6.8 \times 10^{-21}\)). Moreover, decoding accuracy was higher in NOT of pigmented mice than in cortex of pigmented mice (\(P = 1.4 \times 10^{-5}\)) and lower in NOT of albino mice than in cortex of albino mice (\(P = 5.0 \times 10^{-4}\)).

We also used this pseudo-population approach to investigate whether a single-trial decoding analysis would confirm our previous observations on left/right direction selectivity. We therefore reran the decoding analysis as above but now selected only rightward- and leftward-moving stimuli (0- and 180-degree stimuli, respectively) and decoded only these. Note that the chance level is therefore 50%. As before, we found no difference between pigmented and albino in cortex (\(P = 0.426\)) but a much higher performance in NOT of pigmented mice than NOT of albino mice (\(P = 4.5 \times 10^{-5}\)).
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**Figure 5.** Impaired direction selectivity in the NOT of albinos may contribute to albino nystagmus. (A) NOT activation in pigmented animals. **Left:** the right NOT is activated by input from direction-selective ganglion cells preferring left to right temporonasal motion in the contralateral visual field. The activity in the right NOT drives rightward (leftward) motion via its projection to the vestibular-ocular system, resulting in a normal optokinetic response. **Right:** right to left motion activates the left NOT, which in turn drives a leftward motion via its projections to the vestibular-ocular system, resulting in a normal optokinetic response. (B) NOT activation in albino animals. Both left to right and right to left temporonasal motion activate both the right and left NOT (**left and right** images), resulting in a conflicting left/right signal to the vestibulo-ocular system, which results in an impaired optokinetic response. This could lead to albino nystagmus, where random movements are enhanced by a positive feedback loop.

(Figs. 4D–F). In fact, left/right direction decoding in NOT of albino mice was not significantly above chance (z-test vs. 0.5, $P = 0.360$). Two independent analyses therefore converge to the same conclusion: neuronal direction tuning curves in cortex of albino mice are preserved, while neurons in NOT of albino mice respond to both leftward-drifting and rightward-drifting stimuli.

**A Conceptual Model of the Role of NOT in Albino Nystagmus**

NOT receives direct inputs from horizontal direction-selective retinal ganglion cells (DSGCs). The simultaneous impairment of direction selectivity and preservation of orientation selectivity in NOT of albino mice may be explained by the altered input from these DSGCs. In albino animals, the embryonic development of RGCs in the ventrotemporal is delayed, which affects their hemispheric crossing, causing increased contralateral and reduced ipsilateral projections. Assuming this holds for all direction-selective input to the AOS, then a simple model accounts for our and previous observations in other species (Fig. 5). In the normal, pigmented configuration, NOT receives converging input from DSGCs in both retinas with aligned horizontal direction selectivity. This would create the observed bias for temporonasal (ipsiversive) directions in NOT neurons. In albino animals, however, the aberrant contralateral inputs carry a preference for the opposite direction. Neurons in NOT of albino mice therefore still receive inputs that bias their responses to horizontal versus vertical motion, but their left/right selectivity is lost for stimuli covering the binocular visual field, as inputs from left- and rightward motion preferring RGCs lead to equal responses in the NOT. NOT responses are integrated in the vestibulo-ocular system directing horizontal oculomotor responses. This means that when both leftward-moving and rightward-moving stimuli elicit responses in the NOT in both hemispheres, the oculo-motor system receives conflicting input as to move the eyes leftward or rightward, resulting in an impaired optokinetic response. Any spontaneous small eye movement causes visual motion in the opposite direction. The resulting activity in the NOT in either hemisphere of the albino animal can therefore lead to motion that does not stabilize the retinal image but instead induces further visual motion. This positive feedback could be the origin of the spontaneous pendular nystagmus in albinos.

**DISCUSSION**

We investigated neuronal response properties of the NOT in pigmented and albino mice. We found strong evidence that NOT is organized in a retinotopic manner (Fig. 1). Moreover, we found that in albino mice, neuronal tuning to visual motion was impaired (Figs. 2, 3). Specifically, the preference for temporonasal over nasotemporal motion directions in the contralateral visual field was lost. Interestingly, we found that NOT cells in albinos still preferred horizontal over vertical motion (Fig. 3D). We confirmed these results using a logistic decoder analysis on pseudo-populations to remove any statistical biases in comparing results across mice and brain areas (Fig. 4). These results suggest that NOT may
Impaired Direction Selectivity in Albino NOT

One potential confound that could bias our results is the placement of our Neuropixels within area NOT. The structure and unitary nature of area NOT has long been debated, and it is often referred to as the compound structure NOT-dorsal terminal nucleus (DTN) in cats and primates,48 which combines the NOT with the DTN. If NOT is not functionally homogeneous but shows a varying degree of directionally selective cells depending on the anatomic location within area NOT, and our recordings in pigmented and albino animals are distributed differently, then any difference we attribute to animals being pigmented or albino could also be explained by the difference in recording location. We believe this is unlikely, however, for several reasons. First, no one has reported such heterogeneous direction selectivity area NOT in pigmented animals. Second, while our recordings were biased to the lateral end of area NOT, this was true for both pigmented and albino mice (Fig. 1C). Finally, even if functional heterogeneity within NOT would bias our results, this is unlikely to result in the effect we observed. The strongest left/right direction selectivity of any neuron we found in NOT of albino mice was 0.27, whereas in NOT of pigmented mice, it was 1.00. Moreover, 27% of all NOT of pigmented mice cells showed a direction selectivity stronger than this most strongly direction-selective NOT of albino mouse cell. The likelihood of such differences occurring due to random sampling from the same distribution of direction selectivities is vanishingly small.

Another potential confound is that data quality between pigmented and albino animals may have biased our findings. If our recordings in albino animals were much noisier than in pigmented animals (e.g., because of differences in body movement), the estimate for direction selectivity in albino animals would also be reduced. However, this is unlikely to be the cause of our results. First, low data quality would affect cortical responses as much as NOT responses, but we found little difference in functional response properties between pigmented and albino animals in visual cortex (Fig. 3G). Second, data quality would affect the estimate of orientation selectivity as much as the estimate of direction selectivity, but we found no difference in orientation selectivity in NOT between pigmented and albino mice (Fig. 3H). More important, we used equalized pseudo-populations to reduce data quality biases as much as possible and found the exact same results as before (Fig. 4). We are therefore confident that the differences between NOT of pigmented and albino mice we report here cannot be explained by differences in data quality. The intact responses in the visual cortex and the preference for horizontal over vertical motion in the NOT of albino mice also suggest that the loss of preference for temporonasal motion is a specific defect in the NOT of albino mice and not reflecting a generic loss of acuity due to the lack of pigment or subsequent photoreceptor loss.

Our results are in agreement with the hypothesis that the nystagmus and disturbed OKR in albino animals is due to miswiring of axons of the ventrotemporal retina to the contralateral NOT. The loss of ipsiversive direction preference in albino animals would also be explained by this miswiring. We observed two peaks in the direction preference of neurons in the NOT of albino mice: one in the ipsiversive direction, as observed in pigmented animals, and another peak in the opposite direction. It is puzzling how the small projection from the ventrotemporal retina preferring this opposite direction would cause the ipsiversive preference in NOT to be completely lost. We can speculate that the projection is only small in number of axons, but plasticity and developmental changes amplify its postsynaptic effect. This could be at the level of the RGC-to-NOT synapse or by changes in connectivity or intrinsic properties of the NOT. A more depolarizing effect of GABA that was found in the NOT of albino rats could be a mechanism that amplifies the small erroneous input.

As there are multiple types of DSGCs with different preferred directions,45,50,51 another possibility is that the loss of direction selectivity in albinos is not due to a wiring error in the DSGCs responsive to temporonasal motion that normally project to area NOT. Rather, in albinos, there may be increased projections from DSGCs tuned to nasotemporal motion. This hypothesis is supported by the observation that the orientation selectivity in NOT of albino mice was unaltered (Fig. 3E). Future research may test this hypothesis by mapping the direction selectivity of inputs into NOT in pigmented and albino animals. While retina-to-NOT miswiring is one possible cause, alternative hypotheses have also been formulated for this loss of direction selectivity, in which the connections from the retina to NOT are not the primary cause.7,21

One possibility is that aberrant input from the visual cortex to the NOT is responsible. Connections from the visual cortex to the NOT are present in the mouse and substantial in the monkey.14 In albino mice, the wiring defect that affects projections to the AOS also affects the retinal projections to the LGN and therefore indirectly also the visual cortex. However, we did not find a significant change in direction selectivity or preference in the visual cortex of albino mice compared to pigmented mice. Furthermore, under normal circumstances, OKR is undisturbed if visual cortex is silenced in mice.17 Together, this makes it unlikely that corticofugal connections from visual cortex to NOT play a major role in the disturbed direction selectivity in the NOT of albino mice.

Another hypothesis that has been suggested is that the reduction of direction selectivity in the NOT is due to disturbed direction selectivity in the albino retina, rather than caused by erroneous retinal projections. Absence of direction selectivity and spontaneous oscillations in ON direction-selective RGCs can cause nystagmus and impaired OKR in a mice model not associated with albinism.52,53 ON-center direction-selective ganglions cells are, however, present in the albino rabbit and in the albino rat, ON-center RGCs projecting to the AOS are similar in direction selectivity to those in the pigmented rat, suggesting that direction selectivity in the albino mouse retina would be normal. A change in direction selectivity of the RGCs from the nasal retina projecting to the NOT, however, cannot be completely ruled out.

Combining all the above, this leaves our hypothesis of excessive crossing of retinal projections in albinos to be the most likely cause of the disrupted left/right direction encoding in NOT (Fig. 5). Even though in mice, the anatomic evidence of aberrant projections from the ventrotemporal retina to the contralateral NOT is scarce, it is found in almost all albino vertebrates, so it is likely to also occur in mice. Our proposal for the etiology of albino nystagmus is therefore the following. The aberrant crossing of retinal DGCs simultaneously activates left and right NOTs, providing conflicting left/right horizontal direction signals. The NOTs, in turn, send this conflicting output to nuclei of the vestibulo-ocular system in the brainstem, such as...
the medial vestibular nucleus and the nucleus prepositus hypoglossi. This, in turn, leads to an impaired horizontal OKR and spontaneous pendular nystagmus through a positive feedback loop. We therefore believe that the NOT could be a potential target for treatment, when other interventions to reduce the nystagmus are unsuccessful.

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