Repeated nuclear translocations underlie photoreceptor positioning and lamination of the outer nuclear layer in the mammalian retina

Graphical abstract

Highlights
- Photoreceptor nuclei exhibit apico-basal motility in the developing mouse retina
- Rapid apically directed nuclear translocation is powered by dynein 1
- Dynein 1 disruption impedes rapid apical nuclear translocation, causing displacement
- Synapse formation is disrupted in basally displaced photoreceptors

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In brief
Photoreceptors occupy a defined layer in the retina. Aghaizu et al. show that mammalian rod and cone photoreceptors use repeated, dynein-1-driven apically directed nuclear translocations for fine-positioning and retention within this layer. Dynein 1 disruption in photoreceptors results in ectopic displacement beyond the photoreceptor layer and impaired synapse formation.
Repeated nuclear translocations underlie photoreceptor positioning and lamination of the outer nuclear layer in the mammalian retina

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SUMMARY

In development, almost all stratified neurons must migrate from their birthplace to the appropriate neural layer. Photoreceptors reside in the most apical layer of the retina, near their place of birth. Whether photoreceptors require migratory events for fine-positioning and/or retention within this layer is not well understood. Here, we show that photoreceptor nuclei of the developing mouse retina cyclically exhibit rapid, dynein-1-dependent translocation toward the apical surface, before moving more slowly in the basal direction, likely due to passive displacement by neighboring retinal nuclei. Attenuating dynein 1 function in rod photoreceptors results in their ectopic basal displacement into the outer plexiform layer and inner nuclear layer. Synapse formation is also compromised in these displaced cells. We propose that repeated, apically directed nuclear translocation events are necessary to ensure retention of post-mitotic photoreceptors within the emerging outer nuclear layer during retinogenesis, which is critical for correct neuronal lamination.

INTRODUCTION

The central nervous system is characterized by its stratified organization, and the arrangement of newly born neurons into distinct layers is critical for synaptic connectivity and function. In the vertebrate retina, photoreceptors (PRs) reside exclusively in the outer nuclear layer (ONL) (Figure 1 A). They are bordered apically by the retinal pigment epithelium (RPE), which supports PR function and survival. Basal to the ONL lies the interneurons of the inner nuclear layer (INL), which form synaptic connections to PRs within the outer plexiform layer (OPL). The accurate positioning of PRs between the RPE and INL facilitates the establishment of correctly located PR synapses, which is essential for vision (Dick et al., 2003; Maddox et al., 2015; Sarin et al., 2018).

In development, retinal progenitor cells (RPCs) undergo mitosis at the apical limit of the retina (Figure 1 A). Consequently, most post-mitotic daughter cells must move basally into their designated strata and do so by using a variety of methods; retinal ganglion cells (RGCs) migrate across the entire retinal radial width by using fast, unidirectional somal translocation (Icha et al., 2016; Poggi et al., 2005; Zolesi et al., 2006), whereas horizontal cells initially overshoot the INL during bipolar migration, before returning apically by multipolar migration (Chow et al., 2015; Edqvist and Hallböök, 2004). The nascent ONL overlaps with the neuroblastic layer (NBL) that exists before the ONL and INL separate with the forming OPL (Sarin et al., 2018). Cone PRs are born early in development and are initially displaced throughout the NBL (Suzuki et al., 2013), before becoming restricted to their adult location at the apical margin of the ONL (Rich, et al., 1997).

Cone PR nuclei achieve this apical position by a nuclear translocation mechanism that involves Linker of Nucleoskeleton and Cytoskeleton (LINC). LINC complexes are involved in centrosome-mediated nuclear translocations, for which mechanical forces are exerted by kinesin-1 and dynein, which interact with KASH (Klarsicht, ANC-1, Syne homology) proteins (Fridolfsson et al., 2010; Fridolfsson and Starr, 2010; Zhang et al., 2009). Disruption of LINC complexes resulted in partially mis-localized PR nuclei in the adult mouse (Razafsky et al., 2012; Yu et al., 2011), whereas in zebrafish, exogenous expression of the KASH domain of Syne2a induced PR nuclei mispositioning (Tsujikawa et al., 2007). Similar phenotypes are reported in Drosophila klarsicht (Nesprin) (Patterson et al., 2004), klaroid (Sun) (Kracklauer et al., 2007), and glued (dyncatin) (Whited et al., 2004) and in zebrafish mok (dyncatin) mutants (Tsujikawa et al., 2007).

Unlike cones, rod somata are distributed throughout the ONL, but there is a gap in our understanding regarding potential migratory phenomena required for rod somal positioning and/or retention within the ONL. Histological studies in mice (Akimoto et al., 2006; Sarin et al., 2018; Young, 1984) and in...
Figure 1. Rod photoreceptor nuclei are motile during retinogenesis
(A) Location of rods (green) in the developing and adult Nrl::GFP+/- retina.
(B) Individual rod in P2 Nrl::GFP+/- retina. Soma is highlighted (white).
(C) Schematic of retinal preparation for time-lapse live imaging to track rod nuclear motions (red dots) in Nrl::GFP+/- retinae.
(D) Nucleus (red dot) of a segmented rod (green) migrating apico-basally in P3 Nrl::GFP+/- retina (grayscale).
(E) Overlaid example trajectories depicting apico-basal rod nuclear motility at P3. Basal ONL limit indicated (Ferguson et al., 2013).
(F) Representative kinetics of apico-basal rod nuclear motility at P3. Nuclear position, velocity, and acceleration plotted against time. Real data points (position: black dots/red line; velocity and acceleration: gray lines) and moving average (position: blue; velocity and acceleration: black) are shown. Periods of apical- and basal-directed movement are shaded green and red, respectively. Local velocity minima indicate peaks of rapid apically directed translocation phase (middle dotted line). Local acceleration minima/maxima indicate initiation and cessation of rapid apically directed translocation (outer dotted lines).
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human-stem-cell-derived retinal organoids (Kaewkhaw et al., 2015) indicate that early in development many newborn rod nuclei are basal to the limits of the eventual ONL. Similarly, in zebrafish, rod and cone PR somata can be found basal to the nascent ONL early in development (Suzuki et al., 2013). Because neither are found outside the ONL in the adult retina, it has been hypothesized that these displaced nuclei must move apically or back into the ONL or that the cells die (Young, 1984). We therefore sought to determine whether displaced PRs undergo a form of nuclear translocation and how this contributes to stratification of the mammalian ONL.

Here, we report that rod and cone PR nuclei undergo dynaemin-dependent apically directed nuclear translocation. More importantly, this is not a single positioning event, as perhaps envisaged by earlier descriptions of cone stratification. Instead, PR nuclei undergo repeated apical translocations as part of an oscillatory apico-basal cycle throughout early retinogenesis. Dynein 1 disruption impedes apical translocation and leads to lamina defects, with PRs being displaced into the OPL and INL and displaying compromised synapse formation. We propose that repeated, apical nuclear translocation events represent a pattern of movement not previously described for a post-mitotic neuron. In the retina, this serves to retain PRs within the forming ONL during retinogenesis and ensures correct neural stratification.

RESULTS

Rod PR nuclei are motile during retinogenesis

To study rod PR motility during mouse development, we examined Nrl.Gfp+/− mice from embryonic day 16 (E16) to postnatal day 10 (P10). In the retina, these mice express GFP specifically in post-mitotic rods under control of the Nrl promoter (Akimoto et al., 2006; Kim et al., 2016; Figure 1A). At this stage, rod PRs exhibit spindle-shaped somata (Figure 1B) and the nucleus is the principal occupant of the rod soma by volume (Figure 1B). Thus, somata position may be taken as the position of the nucleus. Imaging of retinal explants revealed that rod somata were permanently anchored to the limit of the retina by an apical process. Conversely, basal processes were frequently too thin and/or weakly labeled to be detectable based on cytoplasmic GFP; instead, we used membrane labeling (Nrl.myr/palm-mCherry) for visualization (Figure S1A), which showed them to be ubiquitous from P7, a time that coincides with the ramification of the OPL (Huckfeldt et al., 2009; Morgan et al., 2006).

Real-time imaging of explanted retinae revealed that between P1–P7 most rod nuclei are highly motile, moving in the apico-basal (radial) axis, with little or no lateral movement (Figure 1D; Figure S1E; Video S1). At P1, we detected 493 basally and 307 apically directed movements (classified by exhibiting persistent positive and negative velocities, respectively, for at least 1 h) within a total population of 394 rod nuclear trajectories (N = 3 retinal explants). Each of them could be interspersed with periods of little/no net movement (Figures 1D and 1E; Figure S1C and Videos S2, S3, and S4 show representative examples of these movements at P3). Strikingly, a small proportion of rod nuclei could be observed undergoing more than one complete oscillation within the 12-h imaging period (~3%); n = 11/394 nuclei, N = 3 at P1; Figures 1D–1F; Video S5). This corresponds to 0.1 ± 0.0 oscillatory events per 1,000 recording mins (event count normalized by cumulative trajectory recording time per live imaged retina). In these presumptive repeated oscillations, the nuclei translocated rapidly in the apical direction, before moving more slowly in the basal direction, followed by another rapid apical translocation.

Within the population of net apically directed nuclear movements, we identified a distinct subgroup of high-speed (>−0.3 μm/min; range, −0.3 to −1.2) unidirectional translocations (Figure 1G; rapid apical phase, green), which when normalized for onset of movement, exhibited a highly uniform profile (Figure 1H). These events were non-synchronized and typically lasted ~1 h (Figures 1F and 1H). At P3, we observed 0.3 ± 0.1 rapid apical events per 1,000 recording mins (Table S1). Conversely, we found no evidence of an equivalent subgroup of comparably fast translocations in the basal direction (Figure 1G).

Here, the term rapid apical defines the group of fast, highly uniform, apically directed nuclear translocations. To differentiate between periods of little/no net movement and slow but persistent basal movements, we applied a threshold criterion for net basal movement of 15 μm (2 rod somal lengths) per 2 h. Where interventions were made, we compared the effects on total movement (rapid apical, basal, and non-directional), as well as the effects on rapid apical movements and basal movements, specifically.

We next addressed whether initiation of rapid apical translocation relates to a cell’s depth within the retina (Figures 1I and S1E). Based on fixed sections, the P3 retina is ~160 μm thick. At this age, most rapid apical translocation events were initiated from 45–50 μm away from the apical margin, but a wide range...
Figure 2. Rod nuclear motility does not require dynamic microtubule polymerization
(A) Rod MT plus ends labeled with Nrl.EB3-tdTomato (red) in segmented Nrl.GFP+ve rods (green) at P4 and P8.
(B) Time-lapse series of dynamic MT plus ends in rods labeled with Nrl.EB3-tdTomato (red) at 4 days post-retinal electroporation at P0. White arrowheads show moving EB3-tdTomato foci; yellow arrowheads demarcate starting position in the peri-nuclear fork (green region of interest [ROI] and growing axon (magenta ROI).
(C) P3 Nrl.GFP+/+ retina (green) following exposure for 10 h to 45 nM demecolcine leading to accumulation of PH3+ve mitotic figures (red).
(D) Time-lapse series of a segmented rod (green) nucleus (red dot) moving apico-basally in a P3 Nrl.GFP+/+ retina (grayscale) exposed to demecolcine, added at 120 min.

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(10–108 μm) was observed, with the deepest originating from a depth level with the future IPL (Ferguson et al., 2013). A similar distribution was observed at P1 and P7. Rapid apical translocations were virtually absent by P10; those that did occur were initiated from positions deep within the retina, basal to the nascent OPL. Thus, initiation of rapid apical translocations is not apparently tied to a specific apico-basal position within the tissue.

An analysis of all full-length rod nuclear trajectories at P3 revealed a quasi-Gaussian distribution of measured instantaneous velocities with a mean of 0.0 μm/min (Figure 1J). However, isolated rapid apical or basal nuclear movements exhibited instantaneous velocity profiles that were biased and skewed toward negative and positive velocity values, respectively.

Rod nuclear motility declines during development and becomes stationary by P10 (Figures 1K and 1L; Figure S1D; N = 3 experimental repeats (retinas) for each time point; P1: n = 394 individual nuclear trajectories, P3: n = 324, P7: n = 262, P10: n = 263; acquisition time = 10–12 h per retina; acquisition interval, Δt = 10 min). As noted above, apical and basal movements can be interspersed with considerable periods of little/no net movement. This manifests in a linear relationship between mean squared displacement (MSD) of rod nuclei with increasing time lag (Figures 1L and S1F for comparison with E16; see STAR Methods), a feature typical of particles undergoing non-directional motion (Rutherford et al., 2011). The decline in overall nuclear motility was quantified by comparing the coefficient of movement, which is directly proportional to the slope of the MSD curves (see STAR Methods), and results are as follows: 0.234 ± 0.017 μm² min⁻¹ at P1, 0.257 ± 0.030 μm² min⁻¹ at P3, 0.178 ± 0.035 μm² min⁻¹ at P7, and 0.034 ± 0.006 μm² min⁻¹ at P10. Significant differences were found between ages (two-way ANOVA, p = 0.029), but not within groups (p = 0.479) (Figures 1M and S1G). The reduction in the coefficient of movement during development was highly significant according to post hoc permutation testing.

Taken together, these data suggest that post-mitotic rod PR nuclei undergo repeated rapid, apically directed translocation events, which are followed by periods of persistent basal drift that may be interspersed with periods of little/no net movement.

**MT dynamic behavior is not required for rod nuclear oscillations**

To investigate the molecular mechanisms underlying PR nuclear movements, we first examined the organization of the cytoskeleton (Kosodo, 2012). The microtubule (MT) cytoskeleton is comprised of MT filaments and MT organizing centers (MTOCs; centrosomes). In post-mitotic mammalian epithelial cells, the centrosome converts into the basal body (Hoyer-Fender, 2010). In developing rod PRLs, the MTOC remains stationary at the top of the apical process at all times (Figures S2A–S2D). MT filaments contain a stable minus end located at the MTOC and a dynamic plus end that can extend or retract. To visualize MT plus-end dynamics, we expressed the plus-end binding protein EB3 fused to tdTomato (Nrl.EB3-tdTomato) (Merriam et al., 2013) in rods by ex vivo electroporation at P0–P1. TdTomato-labeled MTs were observed in transfected rods both at earlier (4 days in vitro [DIV]) and later stages (8 DIV) of retinogenesis (Figure 2A). Labeled MT bundles were seen in the apical process, wrapping around the nucleus (the perinuclear fork), and in the growing basal process (Figure 2A). MT plus ends (tdTomato⁺⁺ foci) moved basally, indicating active MT polymerization (Figure 2B; Videos S6 and S7; Stepanova et al., 2003).

We next tested whether dynamic MT plus ends generate sufficient mechanical forces to translocate rod nuclei, as described for other cell types (Inoué and Salmon, 1995; Tran et al., 2001) by treating P3 retinal explants with demecolcine. MT-targeted drugs like demecolcine destabilize MTs at high doses (μM range). Indeed, 25 μM demecolcine led to tissue disintegration (data not shown). However, at low concentrations (nM range), demecolcine suppresses MT dynamics by attenuating MT plus-end (de-)polymerization without affecting cellular MT mass (Jordan and Wilson, 2004; Panda et al., 1995; Picone et al., 2010). Effective drug action at 45 nM was confirmed by the accumulation of PH3⁺⁺ mitotic nuclei (which require dynamic MT plus-end polymerization for mitotic spindle formation) at the apical limit of the retina (Figure 2C) and by cessation of movement of EB3-tdTomato foci (Video S8).

Conversely, rod PR nuclear movement continued in the presence of 45 nM demecolcine (N = 3; n = 307; Figures 2D and 2E). The frequency distribution of total measured velocities was unaffected (Figure 2F), and although there was a small reduction in total movement, as shown by MSD and coefficient of movement analysis (Figures 2G and 2H), it was not statistically significant (DMSO: 0.257 ± 0.030 μm² min⁻¹, demecolcine: 0.186 ± 0.066 μm² min⁻¹; unpaired t test, p = 0.165). We next investigated whether demecolcine specifically affected either rapid apical or basal nuclear movements. There were no notable changes in event frequency and velocity profiles of rapid apical and basal movements in demecolcine- versus control-treated retinae (Figures 2I, 2J, 2M, and 2N; Table S1). Furthermore, we obtained MSD profiles for rapid apical and basal movements in both drug-treated and DMSO-control-treated retina that could be fitted with quadratic functions, yielding the associated quadratic coefficient (see STAR Methods; Figures S3B and S3C). There was a trend toward demecolcine reducing basal movement (Figures 2K, 2L and S3C; quadratic coefficients for DMSO: 0.009 ± 0.002, demecolcine: 0.006 ± 0.001; unpaired (E) Representative, overlaid apico-basal rod nuclear trajectories at P3 in the presence of demecolcine after 120 mins. (F–H) Effect of demecolcine versus DMSO on velocity distribution (F), MSD profiles (G), and coefficients of movement (H) for total rod nuclear movements. Data points represent experimental repeats; unpaired t test. (I–L) Effect of demecolcine versus DMSO on frequency of basal events (I), velocity distribution (J), MSD profiles (K), and MSD profile-derived quadratic coefficients (L) for basal movements. (M–P) Effect of demecolcine versus DMSO on frequency of apical events (M), velocity distribution (N), MSD profiles (O), and MSD profile-derived quadratic coefficients (P) for rapid apical nuclear translocations. Scale bars, 5 μm (A and B) and 10 μm (C and D). Unpaired t test; *p < 0.05; **p < 0.01. Data show mean ± SEM (G, K, and O). See also Figures S2B, S3B, and S3C.
confirmed by an increase in PH3 +ve, M-phase arrested RPCs.

II selective antagonist blebbistatin (25 m

on published literature, including murine PRs) (Kovács et al., 2004; Norden et al., 2009; Reidel et al., 2008). Drug action was confirmed by an increase in PH3

The motor protein myosin II has been variously reported to

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Rod nuclear oscillations do not require myosin II

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The event frequency and velocity profiles of rapid apical and basal movements remained largely unchanged following drug administration (Figures 3G, 3H, and 3L; Table S1). There was a reduction in average displacement for basal movement (Figures 3I, 3J, 3S, and 3G; quadratic coefficient for DMSO: 0.009 ± 0.001, blebbistatin: 0.006 ± 0.001; unpaired t test, p = 0.045), whereas no reductions in average displacement for rapid apical nuclear translocations were observed (Figures 3M, 3N, 3B, and 3G; quadratic coefficient for DMSO: 0.292 ± 0.044, blebbistatin: 0.251 ± 0.073; unpaired t test, p = 0.454). Taken together, blocking myosin II constrictions mildly attenuates the kinetics of basally directed, but not rapid apical, nuclear movements, although the overall frequency of basal events was not affected.

Dynein 1 mediates rapid apical translocation of PR nuclei

As the MT polarity of rod PRs is similar to that of cortical progenitor cells (Kosodo et al., 2011; Troutt and Burnside, 1988; Tsai et al., 2010), which use dynein 1 to drive the nucleus apically during G2 of the cell cycle (Tsai et al., 2005, 2010), and LINCs complexes appear to be involved in cone PR positioning (Razafsky et al., 2012; Tsujikawa et al., 2007), we considered dynein 1 to be a strong candidate to mediate repeated apically directed nuclear translocation in post-mitotic PRs.

We performed time-lapse live-imaging recordings on explanted P3 retinae treated with the dynein selective antagonist ciliobrevin D (25 μM; dose selected based on published literature) (Firestone et al., 2012; Herbert et al., 2017; Sainath and Gallo, 2014). Drug action was confirmed by basal mislocalization of the ciliary transport protein IFT88 (Figure 4A), which normally accumulates in the connecting cilium in a dynein-dependent manner (Sedmak and Wolfrum, 2011). In our live-imaging experiments, ciliobrevin D prevented most rapid apical nuclear movements within 20 mins of application (N = 3; n = 322; Figures 4B, 4C, and 3S; Table S1; Video S9). We also detected a robust concomitant attenuation of basal movements. Post-washout, rapid apical movements were restored (Figures 4D, 4F, and 4G; N = 2, n = 205). Note that prolonged exposure (>6 h) to ciliobrevin D led to tissue breakdown and cell death.

An analysis of total nuclear movements revealed a near-complete loss of higher-velocity measurements (Figure 4E). The slope of the MSD curve for ciliobrevin-D-treated retinae was reduced markedly relative to control (Figure 4F), together with a significant 81% reduction in the coefficient of movement (0.257 ± 0.030 versus 0.044 ± 0.007 μm² min⁻¹, respectively; unpaired t test, p = 0.0001; Figure 4G). Ciliobrevin D wash out restored movement to similar levels as DMSO control wash out (0.179 ± 0.015 versus 0.151 μm² min⁻¹, respectively; N = 1, n = 100). This finding indicates that short-term ciliobrevin D treatment is non-cytotoxic and fully reversible. That the coefficients of movement for ciliobrevin D and, especially, DMSO washout were below that of DMSO controls without washout most likely reflects the detrimental effect of prolonged tissue handling in the washout protocol.

Basal movements continued with a frequency similar to that seen in controls (Figure 4H; Table S1) but with lower velocities (Figure 4I) and shorter average displacement (Figures 4J, 4K, 3S, and 3E; quadratic coefficient for DMSO: 0.009 ± 0.002, ciliobrevin D: 0.003 ± 0.001; unpaired t test, p = 0.006). Rapid apical movements were almost completely abolished (n = 3 apical movements from N = 3 retinae; Figure 4L; Table S1). This makes the quantitative assessment of the kinetics of these few remaining events of limited value and accuracy (Figure 4M; note large error bars in Figure 4N). A quadratic coefficient analysis is provided in Figure S3F for completeness, but statistical comparisons were omitted.

Together, these initial pharmacological interventions support the hypothesis that dynein 1 is involved in rapid apical translocations of post-mitotic rod PR nuclei. Nota bene (N.B.) much higher doses of blebbistatin and demecolcine may also affect PR nuclear translocation, but given the significant effect of low doses of ciliobrevin, here, we focus on the role of dynein 1 in, and the biological purpose of, rapid apical translocation.

Cone PR precursors undergo apico-basal nuclear translocations that are kinetically and mechanistically similar to those of rods

As newly born mammalian cone PR nuclei are initially dispersed throughout the NBL (Figure 4A; Rich et al., 1997; Smiley et al., 2016; Waldron et al., 2018), we assessed whether cone nuclei also undergo repeated oscillatory movements, like rods. We performed real-time imaging of explanted retinae from Chrnb4.EGFP reporter mice (Gong et al., 2003), in which EGFP expression is predominantly restricted to cone PRs (Waldron et al., 2018). Cone nuclei exhibited apico-basal motility very similar to that of rods (Figure 4B; Video S13; N = 3, n = 341 cells). The velocity profile of total cone nuclear trajectories revealed a quasi-Gaussian distribution (mean, 0.0 μm/min), albeit with a notably increased low velocity contribution, compared
with rod nuclei of a similar age (Figure S4C). Conversely, the average MSD was similar between cone and rod nuclei (Figure S4D). Next, we examined the effects of ciliobrevin D on cone nuclear motility (25 μM; N = 3; n = 325 cells); rapid apical translocations were virtually abolished upon drug exposure, whereas basal movements were attenuated, which is very similar to that seen for rods (compare Figure S4E with Figure 4). MSD analysis revealed a marked reduction in the total, average displacement (Figure S4F), similar in extent to that observed for rod nuclei, although this reduction was not statistically significant (DMSO: 0.206 ± 0.049 μm² min⁻¹, ciliobrevin D: 0.093 ± 0.062 μm² min⁻¹; unpaired t test, p = 0.068). Thus, repeated, dynein-dependent rapid apical movements are common to all post-mitotic PRs during retinogenesis.

Figure 3. Rod nuclear translocation does not require actomyosin constrictions
(A) P3 Nrl.GFP⁺⁺⁺ retina (green) following 10-h exposure to 25 μM blebbistatin resulting in accumulation of PH3⁺⁺⁺ mitotic figures (red).
(B) Time-lapse series of a segmented rod (green) nucleus (red dot) migrating apico-basally in P3 Nrl.GFP⁺⁺⁺ retina (grayscale) exposed to blebbistatin, added after 120 min.
(C) Representative, overlaid apico-basal rod nuclear trajectories at P3 in the presence of blebbistatin after 120 mins.
(D–F) Effect of Blebbistatin versus DMSO on velocity distribution (D), MSD profiles (E), and coefficients of movement for MSD profiles (F) of total rod nuclear movements. Data points represent experimental repeats, unpaired t test.
(G–J) Effect of blebbistatin versus DMSO on frequency of basal events (G), velocity distribution (H), MSD profiles (I), and MSD-profile-derived quadratic coefficients (J) for basal movements.
(K–N) Effect of blebbistatin versus DMSO on frequency of apical events (K), velocity distribution (L), MSD profiles (M), and MSD-profile-derived quadratic coefficients (N) for rapid apical nuclear translocations. Scale bars, 10 μm (A) and 5 μm (B). Unpaired t test; *p < 0.05; **p < 0.01. Data show mean ± SEM (E, I, and M). See also Figures S3B and S3G.
Dynein 1 loss of function results in displaced PRs and disrupted ONL stratification

The existence of energy-costly active nuclear translocation within PRs is striking. A failure to translocate apically might be expected to have significant consequences for ONL lamination. To assess whether impairment of rapid apical nuclear translocation affects ONL stratification, we perturbed dynein 1 function specifically in rod PRs by using conditional RNAi and examined the effects at short and long time intervals.

First, we electroporated a floxed short hairpin RNA (shRNA) construct against *Dync1h1*, which encodes an essential dynein 1 subunit, into P1 *Nrl.Cre+/* mice. Figure 4 shows representative time-lapse recordings of total rod nuclear movement in the presence of ciliobrevin D, a known inhibitor of intracellular transport. The data indicate that ciliobrevin D significantly affects both the velocity and diffusion of rod nuclear movements, with a more pronounced effect on basal movements.

Figure 4. Dynein 1 mediates rapid apical translocation of photoreceptor nuclei

(A) P3 *Nrl.GFP*/+ retina (green), counterstained with DAPI (blue) following 10-h exposure to 25 μM ciliobrevin D resulting in targeting defects of the ciliary transport protein IFT88 (red).

(B) Time-lapse recording of a segmented rod (green) nucleus (red dot) migrating apico-basally in P3 *Nrl.GFP*/+ retina (grayscale) exposed to ciliobrevin D, added after a control period of 120 min.

(C) Representative, overlaid apico-basal rod nuclear trajectories at P3 in presence of ciliobrevin D after 120 mins.

(D) Representative, overlaid apico-basal trajectories at P3 following 30-min ciliobrevin D treatment and subsequent wash out (4 × 30 min).

(E–G) Effect of ciliobrevin D versus DMSO on velocity distribution (E), MSD profiles (F), and coefficients of movement (G) for total rod nuclear movements, including following respective washouts. Data points represent experimental repeats. Unpaired t test.

(H–K) Effect of ciliobrevin D versus DMSO on frequency of basal events (H), velocity distribution (I), MSD profiles (J), and MSD-profile-derived quadratic coefficients (K) for basal movements.

(L–N) Effect of ciliobrevin D versus DMSO on frequency of apical events (L), velocity distribution (M), and MSD profiles (N). N.B. (N) reflect values from only n = 3 recorded rapid apical movements. Scale bars, 5 μm. Unpaired t tests. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; #, no statistical test performed due to insufficient data points. Data show mean ± SEM (F, J, and N). See also Figures S3B and S3D–S3F.
et al., 2016). The target sequence for shDyn1h1 was obtained from Tsai et al. (2007) (Figure SSA), and Cre expression is restricted to rod PRs in the Nrl.Cre^{+/--} mouse (Figure S5C). Thus, Cre-mediated recombination yields expression of shDyn1h1, alongside a fluorescent reporter, only in transfected rods. To assess the role of dynein 1 in individual rods, we aimed for relatively sparse transfection (representative transfection levels shown in Figure 5A).

Following electroporation, rod nuclear apico-basal position was assessed by confocal microscopy of fixed tissue (Figures 5A, 5P–5R, S5D, and SSE) and real-time live imaging (Figures 5C–5O) after 4–6 DIV. Real-time imaging showed a reduction in the overall MSD profile of shDyn1h1/EGFP- versus shCtrl/ DsRed-expressing cells (shCtrl/DsRed: N = 3 retinae, n = 273 cells; shDyn1h1/EGFP: N = 3, n = 223; Figure 5F), manifesting as a significant reduction in the coefficient of movement, from 0.224 ± 0.050 μm² min⁻¹ to 0.093 ± 0.015 μm² min⁻¹ (unpaired t test, p = 0.013; Figure 5G). The instantaneous velocity profile remained largely unchanged (Figure 5E). Basally directed nuclear movements were reduced compared to those of the control, but the effect was not significant (Figures 5H–5K and S3H; Table S1; no. of basally directed events/1,000 recording mins: 3.4 ± 0.6 [shCtrl/DsRed] versus 2.4 ± 0.3 [shDyn1h1/EGFP], unpaired t test, p = 0.052; quadratic coefficient: 0.006 ± 0.002 [shCtrl/DsRed], 0.005 ± 0.002 [shDyn1h1/EGFP], unpaired t test, p = 0.052). However, shDyn1h1/EGFP expression significantly reduced the number of rapid apically directed nuclear migration events, from 0.4 ± 0.0 to 0.1 ± 0.1 events/1,000 recording mins (unpaired t test, p = 0.009; Figure 5L; Table S1). Kinetic data on instantaneous velocity profile, MSD analysis, and quadratic coefficient are shown for the sake of completeness but are of limited power due to the low remaining event count (Figures 5M–5O).

Consistent with these data, histological analysis showed that nuclei of shCtrl/DsRed^+ve rods were distributed throughout the radial extent of the ONL, occasionally extending into the OPL and INL (N = 3, n = 430 cells) (Figures 5A, 5P, and S5D), as also seen in Nrl.GFP mice and Nrl.Cre^{+/--} x Ai9 mice of the same or similar age (Figures S5C and S5F). In marked contrast, many nuclei of shDyn1h1/EGFP^+ve rods were significantly shifted basally and even ectopically displaced into the OPL and INL (N = 4, n = 817 cells) (Figures 5A, 5P, and SSE). shCtrl/ DsRed- and shDyn1h1/EGFP-expressing rod nuclei were found at an average apico-basal position corresponding to 66% ± 34% and 83% ± 36% of the developing ONL, respectively (Mann-Whitney test, p < 0.0001; Figure 5Q). This finding was accompanied by an increased number of nuclei mis-localized beyond the margins of the nascent ONL, from 0.9 ± 0.5 (equivalent to 14% ± 4% of transfected cells) to 3.1 ± 1.4 nuclei (32% ± 14% of transfected cells) per 1,000 μm², as viewed from the apico-basal retinal axis (unpaired t test, p = 0.044; Figure 5R).

We next sought to investigate the long-term consequences of dynein 1 perturbation in a large population of rod PRs. AAV2/8 shDyn1h1/EGFP or AAV2/8 shCtrl/DsRed was injected sub-retinally into the eyes of P1 Nrl.Cre^{+/--} mice (see Figure 6A for representative transduction levels). To confirm knockdown of Dyn1h1, we performed qRT-PCR of fluorescence-activated cell sorting (FACS)-sorted DsRed^+ve or EGFP^+ve PRs from AAV2/8 shCtrl/DsRed and AAV2/8 shDyn1h1/EGFP treated retinae, respectively, at 10 days post-transduction. Dyn1h1 RNA levels were reduced by 46% in cells transfected with AAV2/8 shDyn1h1/EGFP versus AAV2/8 shCtrl/DsRed (Figure 5B; N > 6 pooled retinae), which is very similar to that achieved by Tsai and colleagues using the same RNAi target sequence (Tsai et al., 2007).

Histological analysis at 3-weeks post-viral administration revealed that the nuclei of rods transfected with AAV2/8 shCtrl/ DsRed were exclusively found within the ONL and were evenly distributed within its depth (N = 3 experimental repeats, 6 eyes per condition in total; Figure 6A, top). Rod BCs, whose nuclei usually locate to the INL, form synaptic connections with rods in the OPL. Accordingly, we detected only minimal fluorescence signal overlap between shCtrl/DsRed^+ve rod PRs and rod BCs (PKCα^+ve), and this corresponded to their synapses (Figures 6B and 6C, top). In contrast, a significant proportion of nuclei of rods transfected with AAV2/8 shDyn1h1/EGFP was ectopically located (“basally displaced”) outside the ONL and within the OPL, frequently invading domains usually occupied by rod BC dendrites and nuclei (Figure 6A, bottom). This invasion resulted in increased fluorescence signal overlap between these cell populations (Figures 6B and 6C, bottom). In areas of high AAV2/8 shDyn1h1/EGFP viral transduction (see representative images in Figure 6A), the number of basally displaced rod PR nuclei increased from 0.1 ± 0.2 to 6.9 ± 2.5
Figure 6. Long-term dynein 1 loss of function in rods results in ectopically located photoreceptors and impaired retinal lamination
(A) Virally transduced (AAV2/8) rods in 3-week-old Nrl.Cre+/−/C0 retina expressing shCtrl/DsRed (top panel, red) or shDync1h1/EGFP (bottom panel, green). Virus administered at P1.
(B) PKCα (green/red) in 3-week-old retina with virally transduced rods expressing shCtrl/DsRed (red) or shDync1h1/EGFP (green).
(C) Representative vertical intensity line profile (position indicated by white arrowheads in B).
(D) Number of displaced rod PR nuclei per 1,000 μm² of retina.
(E) ONL thickness.

(legend continued on next page)
nuclei per mm² of retina (as viewed from the apico-basal retinal axis; Mann-Whitney test, p = 0.0006; Figure 6D). No significant reductions in ONL thickness were seen over the time frame examined (shCtrl/DsRed: 57.3 ± 4.8 μm versus shDync1h1/EGFP: 55.0 ± 7.2 μm; unpaired t test, p = 0.730), indicating that displacement does not bring about widespread cell death (Figure 6E).

We sought to formally exclude the possibility that failure to reposition within the nascent ONL may result from a loss of apical attachment and delamination rather than nuclear motility defects. Differences in the levels of GFP and DsRed mean that often fluorescence signal is detectable only in the soma; whether this reflects a lack of an apical process or sub-threshold fluorescence levels was therefore not distinguishable (Figures 5A, 5D, and 5E). However, 3D reconstruction and apical process tracing of individual shCtrl/DsRed+ve and shDync1h1/EGFP+ve cells at 4 DIV post-electroporation at P1 showed that at least a proportion of cells exhibited apical processes, regardless of displacement status (Figures 6F and 6G). Looking only at those shDync1h1/EGFP+ve cells that were basally displaced beyond the ONL, 16% ± 2% presented a clearly visible apical process (Figure 6H, top panel). This is similar to the proportion of total apical process bearing shDync1h1/EGFP+ve cells (20% ± 9%; Figure 6H, bottom panel); unlike the presence/absence of a detectable apical process did not correlate with basal displacement status, making it unlikely that apical detachment is required for basal displacement. Similarly, in 3-week-old mice receiving AAV2/8 shDync1h1/EGFP at P1, most (71% ± 13%) analyzed basally displaced shDync1h1/EGFP+ve cells retained an apical process (Figures 6I and 6J; N = 3 experimental repeats, n = 93 cells). Thus, although we cannot completely rule out the possibility that some of either shDync1h1/EGFP+ve or shCtrl/DsRed+ve cells lose their apical process, this is not a prerequisite for basal displacement.

**Dynein 1 loss of function in rod PRs impairs correct synapse formation**

We next considered whether rods whose nuclei were basally displaced into and beyond the OPL following Dyn1h1 knockdown also exhibited synaptic abnormalities. First, we stained AAV2/8 shRNA-treated retinae for the pre-synaptic ribbon synapse marker ribeye and the rod BC marker PKCa. As expected, in AAV2/8 shCtrl-treated retinae, ribeye expression is confined to a band at the level of the OPL (Figure 7A), presenting as a single horseshoe-shaped ribeye structure per reporter-labeled pre-synaptic bouton (N = 3 retinae, n = 93 cells; Figures 7B and 7E–7H; Video S10). In contrast, in retinae treated with AAV2/8 shDync1h1/EGFP, ribeye staining was frequently displaced into the INL (Figure 7C). Of note, the number of ribeye structures per labeled cell significantly increased, from 1.0 ± 0.1 in shCtrl/DsRed+ve controls to 3.1 ± 2.2 in basally displaced shDync1h1/EGFP+ve cells (N = 3 retinae, n = 40 cells; Mann-Whitney test, p < 0.0001; Figures 7D–7F; Video S11), although some displaced cells lacked ribeye structures altogether (Figure 7G; Video S12). In shCtrl/DsRed+ve rods, 100% ± 0.0% ribeye staining presented as the classic “horseshoe” shape (Schmitz et al., 1996) versus 31.3% ± 29.5% in shDync1h1/EGFP+ve cells, with the remainder being punctate in appearance (Mann-Whitney test, p < 0.0001; Figure 7G).

Rod displacement into the OPL/INL showed heterogeneity with respect to their basal process; many lacked any discernible basal process (Figure 7D), whereas others extended processes laterally or even basally, into the INL (Figures 7K, 7L, and 5S5). In keeping with these observations, the cellular location of ribeye was shifted from inside a clearly defined pre-synaptic bouton in shCtrl/DsRed+ve cells (bouton/process: 96.8% ± 17.8%; soma: 3.2% ± 17.8%) to a predominantly somatic location in shDync1h1/EGFP+ve cells (bouton/process: 15.6% ± 27.8%; soma: 84.4% ± 27.8%; Mann-Whitney test, p < 0.0001; Figure 7H).

We next investigated whether these ectopic rod pre-synaptic structures retained the ability to associate with post-synaptic BC dendrites by staining retinae for the post-synaptic marker mGluR6, as well as ribeye. This process should typically result in a 1:1 apposition between horseshoe-shaped ribeye staining and punctate mGluR6 staining with a mean distance of 0.51 μm between these markers (Akiba et al., 2013). This finding was indeed the case for shCtrl/DsRed+ve rods (Figures 7I and 7O). However, the probability of ribeye/mGluR6 apposition significantly decreased, from 1.0 ± 0 in shCtrl/DsRed+ve rods to 0.5 ± 0.4 in basally displaced shDync1h1/EGFP+ve cells (one-way ANOVA, p < 0.0011; Figures 7J–7O). Furthermore, within shDync1h1/EGFP+ve cells, punctate-shaped ribeye structures had a significantly lower mGluR6 apposition probability (0.4 ± 0.5) than those of horseshoe-shaped ribeye (0.7 ± 0.4) (one-way ANOVA, p < 0.01; Figure 7O).

Given dynine’s role in numerous trafficking events, it is possible that the loss of ribeye localization relates to some other dynine-dependent process, rather than nuclear basal displacement per se. We thus also examined ribeye/mGluR6 distribution in those shDync1h1/EGFP+ve cells that remained within the ONL. Indeed, 96% ± 0% of shDync1h1/EGFP+ve rods retained within the ONL and extending a visible basal protrusion also exhibited normal synapses in terms of ribeye foci location, shape, and number, as well as mGluR6 expression (N = 3 retinae, n = 137 cells; Figures 7P and 7Q); this value is virtually identical to that of shCtrl/DsRed+ve-expressing rod cells, which exhibited 100% ± 0% correct synapses (N = 3 retinae, n = 93 cells) (Figures 7F–7I and 7Q). These results confirm that nuclear displacement beyond the ONL due to Dyn1h1 knockdown is likely to lead to significant perturbations in synaptic organization.
DISCUSSION

The PR layer of the mouse retina is many nuclei deep, and its correct development facilitates the formation of correctly located synaptic contacts with the inner retina, which in turn ensures proper retinal function (Dick et al., 2003; Haeseleer et al., 2004; Maddox et al., 2015; Mansergh et al., 2005). Here, we show that ONL lamination requires PR motility featuring repeated, apically directed movements to retain PRs within the ONL. Rapid apical nuclear translocation is an active process, driven by the MT-associated dynein 1 motor protein. Dynein 1 disruption in rod PRs impaired apical translocation and displaced many nuclei beyond the normal limits of the ONL, into the OPL and INL. For those basally displaced cells, this was also associated with impaired synapse formation. This study thus identifies repeated rapid apical nuclear translocation as a mechanism important for correct stratification of post-mitotic PR neurons within the ONL of the mammalian retina.

There are notable similarities in the nuclear kinetic movement profiles of post-mitotic PRs and neuroepithelial progenitor cells undergoing interkinetic nuclear migration (INM), for which nuclei translocate within the elongated progenitor cell in sync with the cell cycle (Kosodo et al., 2011; Leung et al., 2011; Norden et al., 2009; Strzyz et al., 2015; Tsai et al., 2010). Both undergo repeated cycles of rapid, apical translocations and slower, basally directed movements, with the obvious distinction that PR nuclear movement is not linked to the cell cycle. Whether repeated rapid apical nuclear translocation, with or without cell division, is common to other apically located cell populations in stratified epithelia is yet to be explored. Nevertheless, our study identifies repeated apically directed translocations as a novel pattern of somal translocation in post-mitotic neurons (for comprehensive reviews of neuronal migration see Cooper, 2013; Marin et al., 2010).

Apically directed nuclear translocations have been ascribed to different molecular mechanisms, depending on the species studied (reviewed in Kosodo, 2012). In the relatively short (apico-basal length) neuroepithelia of the zebrafish, actomyosin contractile mechanisms drive apical translocation of RPCs (Norden et al., 2009; Strzyz et al., 2016). However, in the thicker neuroepithelium of the developing mammalian cortex, MTs and associated protein motors (dynein 1 and kinesins) are more important (Baffet et al., 2015; Hu et al., 2013; Kosodo et al., 2011; Tsai et al., 2005, 2010). Here, we find that post-mitotic PRs use MT-associated dynein 1 to power rapid apical nuclear translocation. This result is in keeping with findings from genetic studies in zebrafish and Drosophila in which the nuclei of PRs defective in dctn1 (a dynein 1 co-factor) (Tsujikawa et al., 2007; Whited et al., 2004) or dynactin1 (Insinna et al., 2010) were also displaced toward the basal edge of the ONL. Similarly, mammalian cone nuclei failed to migrate apically in mice that overexpressed KASH, which disrupts LINC complexes and may uncouple dynein from the nuclear envelope (Razafsky et al., 2012).

We hypothesize that PR nuclear oscillations are a consequence, at least partially, of proximal RPC INM motions. The purpose behind the apical translocation of G2-phase progenitor cell nuclei has been a matter of considerable debate but appears to be connected to correct lamination and epithelial integrity (Spear and Erickson, 2012; Strzyz et al., 2015). However, in a spatially constrained sheet with an apical limit, it also basally displaces the nuclei of neighboring cells due to steric crowding at the apical margin. In silico simulations of progenitor cell INM to demonstrate that all basal displacements can be exclusively caused by apically directed nuclear translocations support this notion (Kosodo et al., 2011). Razafsky and colleagues speculated that the basal displacement of cones observed following disruption of LINC complexes resulted either from an atypical kinesin-based, LINC-independent mechanism or was the result of passive basal...
displacement by neighboring cells (Razafsky et al., 2012). Our data are consistent with the latter. With respect to frequency, it is reasonable to posit that rod oscillations are time locked with RPC cell cycle length, which lengthens during development (30 h at P1, ~40 h by P5) (Young, 1985). The declining mitotic rates with progressing retinogenesis may reduce the requirement for rod apical translocations until both ultimately come to a halt around P10. In keeping with these time frames, we determined that rod PR nuclei undergo a rapid apical translocation event at least once every 50 h at P3–P4. Indirect support for this model comes from our data showing that blocking actomyosin constrictions with blebbistatin, an established inhibitor of mitotic cytokinesis (Straight et al., 2003), attenuated the average travel distance of basally directed rod nuclear translocation events. However, further studies are required to fully define the extent to which dividing RPCs exert basally displacing forces on PRs.

Conversely, we identified dynein 1 to be a key driver of PR nuclear apical translocation. Short-term Dync1h1 knockdown led to impaired rapid apical translocation events and a shift of affected rod nuclei to more basal locations. Long-term Dync1h1 knockdown resulted in significant basal displacement of rod PRs, of which many accumulated basal to the ONL, leading to disrupted stratification. Importantly, basally displaced cells typically retain their apical attachment, supporting the hypothesis that displacement arises from a failure to migrate apically rather than from apical detachment. We can only speculate why displaced PRs were not displaced even further into the retina but suggest that it may be due to the network of horizontal cell neurites that already exists in the presumptive OPL (Huckfeldt et al., 2009).

Basal displacement was accompanied by impaired synapse formation, as seen by mis-localization of pre-synaptic ribeye and a reduction of correctly apposed post-synaptic mGlur6. Conversely, those shDync1h1-EGFP-ve cells remaining in the ONL exhibited normal synaptic labeling. This finding suggests that rod PR synaptic architecture is affected not because of dynein disruption per se but because of nuclear displacement beyond the ONL. We cannot completely exclude the possibility that these synaptic abnormalities arise from other dynein-dependent processes and/or differences in the extent of dynein knockdown in individual cells, and this will be an important area of future study. It will also be important to ascertain the effect of these atypical synaptic contacts on visual function.

Another key area for future study will be to determine whether and how cell extrinsic cues from neighboring cells or the extracellular matrix interact with the intrinsic mechanisms of nuclear movement. Our data show that the onset of apical movement is not predetermined to occur at a certain depth within the NBL. Interactions between PRs and their local surroundings may provide a combination of cues that are integrated into the decision to initiate nuclear translocation, as suggested in other systems (Elias et al., 2007; Famulski et al., 2010; Marin et al., 2010). Rod BCs could provide such instructive signals for rod PRs, at least at later stages of development. Sarin and colleagues showed that the OPL manifests within the extent of the nascent ONL in the developing mouse retina, initially resulting in a number of displaced rod PRs (Sarin et al., 2018). They proposed that BC-derived Wnt5a/5b correctly guides rod PR nuclei and axons to the correct location. This idea would be consistent with our findings and those reported in other systems (Witze et al., 2008). Dopaminergic signaling represents another instructive signal. In response to light, intrinsically photosensitive RGCs stimulate dopaminergic amacrine cells to engage in dopaminergic signaling with cone PRs, promoting correct cone nuclear enrichment at the apical limit of the ONL (Tufford et al., 2018) presumably, based on our findings, in a dynein-1-dependent manner. These and other, currently undescribed, signaling mechanisms may jointly provide the cues necessary for PR lamination.

In conclusion, we report a previously undescribed pattern of movement for post-mitotic neurons in stratified epithelia, namely, repeated, rapid apically directed nuclear translocation. We propose that this movement serves to enrich and retain neurons within a given layer and, in the case of the mammalian retina, ensure correct stratification of the ONL.

**STAR METHODS**

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**SUPPLEMENTAL INFORMATION**

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AUTHOR CONTRIBUTIONS

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DECLARATIONS OF INTERESTS

The authors declare no competing interests

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## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**       |        |            |
| Rabbit polyclonal α-tubulin | Abcam | Cat#ab24246, RRID:AB_447954 |
| Goat polyclonal IFT88 | Abcam | Cat#ab42497, RRID:AB_778681 |
| Sheep mGlur6         | Kind gift from K. Martemyanov | N/A |
| Rabbit polyclonal PH3 | Millipore | Cat#06-570, RRID:AB_310177 |
| Rabbit polyclonal PKCα | Sigmaaldrich | Cat#P4334, RRID:AB_477345 |
| Mouse monoclonal ribeye | BD Biosciences | Cat#612044, RRID:AB_399431 |
| Mouse monoclonal γ-tubulin | Abcam | Cat#ab11316, RRID:AB_297920 |
| Alexa Fluor® 488 goat anti-rabbit | ThermoFisher Scientific | Cat#A-11001, RRID:AB_2534069 |
| Alexa Fluor® 546 goat anti-rabbit | ThermoFisher Scientific | Cat#A-11018, RRID:AB_2534085 |
| Alexa Fluor® 546 donkey anti-mouse | ThermoFisher Scientific | Cat#A-11056, RRID:AB_2534103 |
| Alexa Fluor® 488 donkey anti-sheep | ThermoFisher Scientific | Cat#A-11015, RRID:AB_2534082 |
| Alexa Fluor® 546 donkey anti-sheep | ThermoFisher Scientific | Cat#A-21098, RRID:AB_2535752 |
| **Bacterial and virus strains** |        |            |
| β-Select Gold Competent _E. coli_ | Bioline | Cat#BIO-85027 |
| AAV2/8 shDync1h1/EGFP | This paper | N/A |
| AAV2/8 shCtrl/DsRed | This paper | N/A |
| **Chemicals, peptides, and recombinant proteins** |        |            |
| Blebbistatin | Sigmaaldrich | Cat#B0560 |
| Cillobrevin D | Millipore | Cat#250401 |
| Demecolcine | Sigmaaldrich | Cat#D7385 |
| DMSO | Sigmaaldrich | Cat#D8418 |
| Taurine | Sigmaaldrich | Cat#T4571 |
| **Critical commercial assays** |        |            |
| Papain Dissociation System | Worthington | Cat#LK003153 |
| RNeasy Micro Kit | QIAGEN | Cat#74004 |
| QuantiTect Reverse Transcription Kit | QIAGEN | Cat#205311 |
| **Experimental models: Cell lines** |        |            |
| HEK293T cells | RRID:CVCL_0063 | |
| **Experimental models: Organisms/strains** |        |            |
| Nrl.GFP*+* mice (B6.Cg-Tg(Nrl-EGFP)1Asw/J) | kind gift of A. Swaroop | RRID:IMSR_JAX:02 1232 |
| Nrl.Cre+/− mice (C57BL/6J-Tg(Nrl-cre)1Smgc/J) | The Jackson Laboratory | RRID:IMSR_JAX:02 8941 |
| Aβ9 mice (B6:129S6-Gf(ROSA)26Sortm1Crl/+/+;CAGtdTomato[Hze/J]) | The Jackson Laboratory | RRID:IMSR_JAX:007905 |
| Chrm1B.4EGFP mice (Tg(Chrm1B4EGFP)CL200Gsat/Mmnc) | MMRRC | RRID:MMRRC_0002 59-UNC |
| C57BL/6J mice | Harlan Laboratories | N/A |
| **Oligonucleotides** |        |            |
| Actb F primer (AAGGCCAACCAGGAAAAGAT) | Sigmaaldrich | N/A |
| Actb R primer (GTGTTAGCGACCCAGGAGTAC) | Sigmaaldrich | N/A |
| Dync1h1 F primer (ATGAGACCCTCCTGCTCTTTG) | Sigmaaldrich | N/A |
| Dync1h1 R primer (GTCATGGTTTCTGCTCAGTCG) | Sigmaaldrich | N/A |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Rachael Pearson (rachael.pearson@kcl.ac.uk).

Materials availability
All unique/stable reagents generated in this study will be made available on request but may require a payment and/or a completed Materials Transfer Agreement if there is potential for commercial application.

Data and code availability
The imaging data reported in this study cannot be deposited in a public repository because they do not comprise a standardized datatype. Moreover, the authors are undertaking further analysis and any outputs arising from these will be published in due course. To request access, contact the lead author.

All original code has been deposited in the ‘Photoreceptor_tracking’ Github repository and is publicly available as of the date of publication. URL is listed in the Key resources table.

Any additional information required to re-analyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice

Nrl.GFP+/+ mice (2.5kb upstream segment of Nrl gene drives EGFP expression; kind gift of A. Swaroop, University of Michigan, USA; bred in-house; RRID:IMSR_JAX:021232) (Akimoto et al., 2006), Nrl.Cre+/– mice (1.7kb mouse Nrl promoter drives Cre recombinase...
expression; kind gift of S. Chen; University of Washington, USA; bred in house as hemizygotes; RRID:IMSR_JAX:028941) (Brightman et al., 2016), Ai9 mice (RRID:IMSR_JAX:007905) (Madsen et al., 2010), Chrnb4.EGFP mice (RRID:MMRRC_000259-UNC) (Gong et al., 2003), and wild-type C57BL/6J mice (Harlan Laboratories) were used, according to the NC3R ARRIVE guidelines, between embryonic day (E) 16 and P14. Adult mice were 6-8wks of age. Both male and female mice were used in this study without discrimination.

Male and female mice were group housed in the animal facility at University College London on a standard 12-hour light/dark cycle at the same light levels throughout the experimental period. Animals were kept in individually ventilated cages on animal grade wood chip and given access to nesting material and food and water ad libitum.

All animal studies were carried out under the Animals (Scientific Procedures) Act 1986 under a project license PPL 70/8120 issued by the UK Government Home Office and conducted in accordance with protocols approved by the Animal Welfare and Ethics Committee of the UCL Institute of Ophthalmology. All animals were killed by cervical dislocation performed by trained personnel (approved under Schedule 1 as a method of humane killing). All efforts were made to minimize the number and suffering of animals used in these experiments.

Cell lines
HEK293T cells (RRID:CVCL_0063; Sex: female) were used for the production of AAV vectors. They were maintained as adherent cell cultures in 15 cm Petri dishes in 20 mL maintenance medium (DMEM (GIBCO, ThermoFisher Scientific) supplemented with 10% fetal bovine serum (GIBCO, ThermoFisher Scientific)) at 37°C and 5% CO2 in humidified incubators. For passaging, 80% confluent cells were incubated in 0.05% trypsin solution (GIBCO, ThermoFisher Scientific) for 5 min at 37°C and 5% CO2. Trypsin was subsequently inactivated by the addition of maintenance medium, followed by cell splitting as appropriate.

METHOD DETAILS

Molecular reagents and plasmid design
Nrl promoter-driven expression constructs
EGFP, Cent2-DsRed (Addgene plasmid #29523) (Tanaka et al., 2004), EB3-ttdTomato (Addgene plasmid # 50708) (Merriam et al., 2013), and myr/palm-mCherry (Zacharias et al., 2002) were each subcloned into a pD10 expression and AAV packaging-compatible construct downstream of the NRL promoter region. A 2.5 kb segment upstream of the Nrl gene was cloned from mouse genome and used as the NRL promoter in this study, as per (West et al., 2012).

shDync1h1/EGFP and shCtrl/DsRed RNAi constructs
To clone the conditional shDync1h1 RNAi construct pD10 mU6.TL-shCtrl-TL.shDync1h1 / CMV.FL-pA-FL.EGFP (abbreviated to shDync1h1/EGFP), a scrambled control short hairpin (target sequence: 5'-GATCGGACACTCCTCATAA-3') flanked by TATA-lox (TL) sites designed for conditional shRNA expression from the mU6 promoter (Ventura et al., 2004) was placed between mU6 promoter and a short hairpin sequence against Dync1h1 (target sequence: 5'-AGGGTTAACCAGCAGATAA-3'; based on findings by Tsai et al. (2007) who reduced protein levels by 50% in cultured rat neurons) (Figure 6A). This entire conditional RNAi module against Dync1h1 was synthesized (GeneArt Gene Synthesis, ThermoFisher Scientific). A separate conditional reporter module was cloned into the same plasmid by placing a poly(A) sequence flanked by loxP sites between CMV promoter and EGFP open reading frame. Cre enzyme mediates independent recombination events at shRNA and reporter modules. The RNAi control construct pD10 mU6.shCtrl / CMV.FL-pA-FL.DsRed (abbreviated to shCtrl/DsRed) provides constitutive shCtrl expression by placing the scrambled control short hairpin sequence immediately downstream of the mU6 promoter and conditional reporter expression by placing a poly(A) sequence flanked by loxP sites between CMV promoter and DsRed open reading frame.

Both constructs were cloned into a pD10 plasmid backbone
All plasmids were transformed into $\alpha$-Select Gold Competent E. coli (Bioline) and subsequently purified using the QIAGEN plasmid mega kit (QIAGEN).

Recombinant adeno-associated virus (AAV) production
The conditional RNAi (shDync1h1/EGFP) and control constructs (shCtrl/DsRed), containing AAV-2 inverted terminal repeats (ITRs), were encapsidated into recombinant AAV particles of serotype 8 to produce AAV2/8 shDync1h1/EGFP and AAV2/8 shCtrl/DsRed. This was achieved using a tri-partite plasmid transfection system on HEK293T cells, as previously described (Gao et al., 2002). Briefly, DNA mix consisting of the three DNA plasmids shDync1h1/EGFP or shCtrl/DsRed, AAV8 capsid and pHGTi helper plasmid were mixed at a molar ratio of 1:1:3 with PEI transfection reagent (Polysciences; 2.25 mg PEI per 1 mg DNA) in DMEM. The transfection mix was added to 80% confluent HEK293T cells at 50 μg DNA per 15 cm cell culture plate. Three days post transfection, HEK293T cells were harvested in harvesting buffer (140 mM NaCl / 5 mM KCl / 0.7 mM K2HPO4 / 3.5 mM MgCl2 / 25 mM Tris base in H2O, pH 7.5). Cells were subsequently lysed by four freeze/thaw/vortex cycles. Viral particles were purified by affinity chromatography on an AVB Sepharose column (GE Healthcare). The eluate was concentrated to a volume of 200 μl using Vivaspin columns (Sartorius AG) to achieve a titer of 5 × 1013 vector genomes (vg)/ml. Viral titers were determined by real-time quantitative PCR using primers specific for the ITRs, as described previously (Kruczek et al., 2017).
AAV sub-retinal injection
For in vivo administration, 0.4 µl of viral preparation (2 × 10^10 vg/eye) were sub-retinally injected into P0-P1.5 Nrl.Cre+/− mice anaesthetized on ice prior to injections. Eye lids were surgically opened, pupils were dilated using 1% tropicamide and treated with topical anesthetic (amethocaine). Eyes were protected from dehydration with Viscotears™ (Novartis). Sub-retinal injections were administered under direct visual control through an operating microscope (Zeiss) using a sterile syringe (Hamilton) fitted with a 34 gauge hypodermic bevel-edged needle placed between neural retina and RPE. Mice were subsequently allowed to recover on heat mat before being returned to parent mice.

Ex vivo retinal electroporation
DNA plasmids were transfected by electroporation (Hsiau et al., 2007; Matsuda and Cepko, 2004). For in vitro electroporation, retinas from P0 - 2.5 mice were transferred into a 2-mm gap size electroporation cuvette (BTX) containing 1 µg/µl plasmid DNA in PBS. Using a pulse generator (model ECM 830, BTX), the retinae were electroporated with 5 × 30 V square pulses of 50 ms duration and with 950 ms intervals. The retinae were allowed to recover for 5 min each in serum free and subsequently in 5% fetal calf serum (Thermo-Fisher Scientific) containing media (1:1 DMEM/F-12 with L-glutamine and 15 mM HEPES (ThermoFisher Scientific) supplemented with1 mM Taurine (Sigma)).

Ex vivo retinal explant culture
Following the protocol by Donovan and Dyer (2006) explanted retinae were placed vitread side down on 0.2 µm polycarbonate membranes (Whatman) and cultured at 37°C / 5% CO₂ in a sitting drop of 5% fetal calf serum (ThermoFisher Scientific) for four to ten days. To counteract evaporation, explant culture-containing sitting drops were re-supplied with 50-100 µl fresh media every day.

Retinal dissociation and FACS
Neural retinae were harvested from Nrl.Cre+/− mice 10 days post injection at P0-P1.5 with AAV2/8 shDync1h1/EGFP or AAV2/8 shCtrl/DsRed by dissection and dissociated using the papain dissociation system (Worthington) according to the manufacturer’s instructions. Briefly, retinae were enzymatically dissociated in EBSS / 20 U/ml papain / 1:100 v/v antibiotic/antimycotic at 37°C / 5% CO₂ for 45 mins. The cell suspension was gently triturated with a 200 µL pipette tip, passed through a 70 µm strainer and spun down at 200 g for 5 mins. Cell pellets were resuspended in EBSS / 1 mg/ml ovomucoid protease inhibitor / 100 U/mL DNase I and incubated for 5-10 mins at 37°C / 5% CO₂. The suspension was subsequently layered over an EBSS / 10 mg/ml ovomucoid protease inhibitor solution and centrifuged at 100 g for 5 mins. Finally, cell pellets were resuspended in FACS buffer (EBSS / 1% FCS) for sorting on a special order 5-laser BD Influx Cell Sorter (BD Biosciences). FACS sorted GFP+ or DsRed+ cells were collected in EBSS / 50% FCS.

qRT-PCR
RNA was extracted from FACS sorted GFP+ or DsRed+ cells using the RNeasy Micro Kit (QIAGEN) and reverse-transcribed using the Quantitect Reverse Transcription Kit (QIAGEN). qPCR assays were performed using the 2x FastStart TaqMan® Probe Mastermix (Roche) in conjunction with the Universal ProbeLibrary system technology (Roche). Primers for the target (Dync1h1; F: ATGAAGCCCTCCGTCTCTTC, R: GTCAATGTTTTCGTCAGTCCAG) and endogenous reference control markers (Actb; F: AAGGCCAACCGTGAAAAGAT, R: GTGGTACGACCAGGACATAC) were designed and probes were chosen (probe # 88 and 56 respectively) according to recommendations by the Universal Probe Library Design Center (Roche). Reaction mixes were prepared according to the table below:

| Reagent          | Stock concentration | Volume (µl) | Final concentration |
|------------------|---------------------|------------|---------------------|
| Forward primer   | 20 µM               | 0.2 µl     | 200 nM              |
| Reverse primer   | 20 µM               | 0.2 µl     | 200 nM              |
| Probe            | 10 µM               | 0.2 µl     | 100 nM              |
| cDNA             | 1.5 x dilution from reverse transcription reaction | 5 µl | variable |
| PerfeCTa® qPCR FastMix® II, Low ROX™ | 2 x | 10 | 1 x |
| ddH₂O            | -                   | 4.4 µl     | -                   |
| Final volume     | 20 µl               |            |                     |

Reaction mixes were loaded onto MicroAmp® Optical 96-Well Reaction Plates (Applied Biosystems). qRT-PCR was performed on an ABI Prism 7900HT Fast Real-Time PCR Sequence Detection System (Applied Biosystems) set to perform the following program:
### Immunohistochemistry

For immunohistochemistry, eyes or retinal tissue were fixed in 4% (wt/vol) paraformaldehyde (Sigma) for at least 30 mins prior to cryopreservation in 20% (wt/vol) sucrose (Sigma) overnight (o/n). After embedding in OCT (Pyramid Innovation), tissues were sectioned at 18 μm thickness on a Bright OTF5000 cryostat (Bright Instruments Co Ltd). Tissue sections were washed with PBS (pH 7.4), blocked in PBS supplemented with 5% (vol/vol) goat or donkey serum (Bio-Rad), 1% (wt/vol) BSA (Sigma) and 0.1% (vol/vol) Triton X-100 (Sigma). Primary antibodies were applied to sections over night at 4°C, followed by washes in PBS and subsequent application of secondary antibodies for 2–4 hr at room temperature (goat/donkey Alexa Fluor antibodies with 488, 546 or 633 fluorophores as appropriate (Thermofisher). Nuclei were counterstained with 4',6-Diamidino-2-phenylindol (DAPI; Sigma; shown in blue in all confocal images) at 1 μg/ml. Primary antibodies used in this study were: α-tubulin (Abcam, ab24246, RRID:AB_447954, 1:500), mGluR6 (kind gift from K. Martemyanov, 1:200) PKCα (Sigma, P4334, RRID:AB_477345, 1:10,000), PH3 (Millipore, 06-570, RRID:AB_310177, 1:250), ribeye (BD Biosciences, 612044, RRID:AB_399431, 1:100) and γ-tubulin (Abcam, ab11316, RRID:AB_297920, 1:100). Negative controls omitted the primary antibody.

### Microscopy

**Live imaging by time lapse 2-photon microscopy**

Live retinae were flattened and whole mounted with the PR side up onto a 0.45 μm MF-Millipore nitrocellulose membrane (Millipore). Placed in DMEM supplemented with 4x 30 min washes in imaging medium at 37°C 5% CO2. For image acquisition, xyz image series were captured at a resolution of 512x512, at a step size of 1 μm and at 15 s (EB3-tdTomato experiments) or 10 min intervals (nuclear motility experiments). For pharmacological investigations, retinae were treated with 25 μM Blebbistatin (Sigma), 25 μM Cilobrevin D (Millipore) or 45 nM Demecolcine (Sigma) after a 2-hour control period. 0.1% DMSO was used as vehicle control. For the Cilobrevin D washout experiment, explanted retinae were drug-treated for 30 min at 37°C 5% CO2 followed by 4x 30 min washes in imaging medium at 37°C 5% CO2 prior to imaging. xyz image series were processed and registered in Fiji/ImageJ (Schindelin et al., 2012).

**Confocal microscopy of fixed specimens**

Fixed tissues were imaged using a Leica TCS SPE confocal laser scanning microscope fitted with 40x (NA = 1.15) and 63x (NA = 1.3) objectives and photomultiplier tubes to detect fluorescence emission. For image acquisition, xyz confocal stacks were captured at a resolution of 1024 × 1024 pixels and at a step size of 0.25 - 1 μm, as appropriate. Selected images were deconvolved using Huygens Deconvolution software (Scientific Volume Imaging). For image presentation in figures, color labels were placed on top of black background surface for increased readability for Figures 6A, 6F, 7 (all image panels), S5F, and S5G.

### Analysis of displaced nuclei

For the analysis of rod nuclear displacement on confocal micrographs of fixed retinae in the shRNA expression experiments (shCtrl/DsRed or shDync1h1/EGFP), the apico-basal position of the centers of transfected/transduced rod cell nuclei was assessed, using Fiji/ImageJ. To quantify the position of each individual rod soma relative to the ONL, we defined the ONL as the radial width (based on DAPI labeling) from the apical-most to the basal-most DAPI labeling at the exact tangential position of each rod cell within its associated field of view and retina. Apico-basal positions between apical and basal ONL margins were classified as correctly localized, whereas positions more basal than the basal ONL margin were classified as basally-displaced. This methodology was applied to both short term (electroporation into P0 retinal explants and tissue harvest after 4 days of in vitro culture) and long term shRNA expression experiments (in vivo AAV injection at P0 and tissue harvest at 3 weeks post injection).

### Analysis of synapses

For the analysis of synapses between shCtrl/DsRed or shDync1h1/EGFP-expressing rods and bipolar cells, retinae were immunolabelled for ribeye (pre-synaptic) and mGluR6 (post-synaptic). Image analysis was performed in Fiji/ImageJ. For shCtrl/DsRed+ PRs, analysis was restricted to cells with clearly traceable and connected soma, axon, and synaptic bouton, while only those shDync1h1/EGFP+ rod cells with a basally displaced nucleus were considered.

The ribeye confocal signal was processed to increase the signal:noise ratio and to clearly segment true pre-synaptic structures (usually horseshoe-shaped), similarly to Akiba et al. (2019). This was achieved by using the “enhance contrast” function, applying...
a bandpass filter to remove small particle noise, followed by the “smooth” function to obtain a more homogeneous ribeye signal within each region and finally by applying a size filter on manually thresholded images within the “analyse particles” function to further remove small particle background noise. Ribeye structures clearly located within shCtrl/DsRed or shDyn1h1/EGFP-expressing rods were counted and classified according to shape (horseshoe versus punctated) and subcellular location (synaptic bouton/process versus soma).

The mGluR6 confocal signal was similarly processed by utilizing the “enhance contrast” function, followed by applying a size filter on manually thresholded images within the “analyse particles” function to remove small particle background noise; finally, the “smooth” function was used to obtain a more homogeneous mGluR6 signal within each region. Opposition between pre- and post-synaptic termini was evident when pre-synaptic ribeye and post-synaptic mGluR6 structures were within a distance of 0.51 μm (Akiba et al., 2019).

Cell tracking methodology
To track the movement of rod cell bodies in time lapse recordings, the spot tracking tool within IMARIS software (Bitplane) was applied to the xyzt time-lapse image series and set to identify the following traceable features of immature rod somata: i) GFP+ve, ii) ellipsoid in shape, iii) ellipsoids of dimensions 7.5 μm. Computer-generated positional information over time (x, y, z, and t) was manually verified.

To track location and dimension parameters of the apical processes of rod PRs in confocal micrographs from fixed retinae the Fiji/ImageJ plug-in NeuronJ was used (Meijering et al., 2004).

Analyses of nuclear motion
Kinetic analyses on nuclear trajectories were performed in Microsoft Excel (Microsoft) and with custom routines in MATLAB (Mathworks). We restricted our analyses to one-dimensional nuclear trajectories along the apico-basal tissue axes (zt), since motility along this dimension was predominantly observed compared with two-dimensional lateral motility (xyt) (see Figure S1B). The following velocity criteria were used for the different movement types observed in this study: x ≤ −10 μm in 30 min (rapid apical), x ≥ 15 μm in ≥2hrs (basally-directed). The criterion for rapid apical movement was formed to reflect the rapid apical translocations observed in this study. To distinguish between persistent basally-directed movement and other periods of no net movement (stochastic), we used a threshold of movement greater than 2x apico-basal rod somal lengths, typically at 7.5 μm (15 μm) in a 2 hr period. To compare oscillation, rapid apical, and basal event frequency, event counts were normalized by the cumulative recording minutes (sum of all trajectory durations (mins) within a given retina). This was necessary due to differences in the number and duration of trajectories between retinae. Absolute event counts were normalized as follows: normalized event count = absolute event count / cumulative recording minutes x 1000 recording minutes.

Instantaneous velocity calculations: average velocities were first obtained by determining nuclear positional changes relative to the apical tissue margin in consecutive time frames. These were subsequently transformed into instantaneous velocity measurements in the ith frame by dividing by the recording interval between frames (Δt = 10 min) according to:

\[ v(iΔt) = \frac{Δz}{Δt} = \frac{z((i+1)Δt) - z(iΔt)}{Δt}, \]

where \( z(iΔt) \) and \( z((i+1)Δt) \) denote the z position of a given nucleus at time frames \( iΔt \) and the consecutive time frame \( (i+1)Δt \).

Mean squared displacement: we used the mean squared displacement (MSD) as a function of elapsed time as a measure of the average distance traveled by PR nuclei (Ruthardt et al., 2011). MSD analysis was analogously used previously when describing nuclear motilities of dividing epithelial progenitor cells undergoing INM (Leung et al., 2011; Norden et al., 2009). MSD values were calculated by taking the average of squared displacements displayed by a nucleus within a given trajectory over successively increasing time-intervals. This was followed by further averaging the trajectories of populations of cells. To this end, the following equation was applied:

\[ \text{MSD}(Δt) = \frac{1}{N-n} \sum_{i=1}^{N-n} [z((i+n)Δt) - z(iΔt)]^2 \]

where \( z(iΔt) \) and \( z((i+n)Δt) \) are the z positions of a given nucleus at time frames \( iΔt \) and \( (i+n)Δt \) respectively, \( n \) is an integer representing the time interval between those positions and \( N \) is the total number of time points within the time-lapse recording. To quantify changes in the MSD, the MSD data points were subjected to curve fitting. For particles subject to non-directional motion, the MSD is a linear function of elapsed time \( Δt \),

\[ \text{MSD} = 2RDΔt \]

with a one-dimensional slope of \( 2RD = 2D \), where \( R \) is the dimensionality (in the present study, \( R = 1 \)) and where \( D \) is the coefficient of movement. The coefficient \( D \) was used to quantitively compare MSDs of non-directional and total rod nuclear translocations (since total rod nuclear translocations were predominantly non-directional, they were also subjected to linear function curve fitting). For rapid apically- and basally-directed movements, the MSD displays a quadratic dependence on elapsed time, which is indicative...
of active and/or directed movement (Berg, 1993; Ruthardt et al., 2011). The MSD profiles of rapid apical and basally-directed translocations were curve fitted in GraphPad Prism® software (GraphPad Software Inc., RRID:SCR_002798) with the quadratic function

\[ y = ax^2 \]

to obtain the quadratic factor \( a \) as numerical representative of curve steepness, which was used to quantitatively compare MSDs of rapid apical and basally-directed rod nuclear translocations.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All means are stated ± standard deviation, unless otherwise specified. \( N = \) number of eyes and \( n = \) number of cells analyzed. For qualitative and quantitative histological assessments, at least 3 eyes from independent animals were used per group. For time lapse studies, given the required duration of the live imaging experiments, only one retina from a given animal was imaged in any one experimental run and is considered an independent sample. We used GraphPad Prism® software (GraphPad Software Inc.) and custom routines in MATLAB (Mathworks) for statistical analyses. D’Agostino and Pearson test was used to assess the normality of datasets. For statistical tests involving one independent variable to be compared between 2 groups we used the unpaired t test and Mann-Whitney test for normally and non-normally distributed datasets respectively. For the comparison of one independent variable between > 2 groups, we used 1-Way ANOVA with Tukey’s multiple comparison test. For statistical tests involving two independent variables we used two-way ANOVA with a post hoc permutation test including Monte Carlo randomization (Anderson and Ter Braak, 2003). Significance was accepted at \( p \leq 0.05 \).