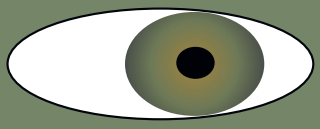
The retinal origin of congenital nystagmus

Hölzel, M.-B.

Publication date
2024

Chapter 6



General Discussion

In this thesis, we describe a revolutionary retinal mechanism leading to nystagmus in mouse models for CSNB (**Chapters 2 and 3**). **Fig. 6.1** shows an overview of this mechanism. In patients with CSNB and nystagmus, mutations were found that are specifically present in the photoreceptor to ON-bipolar cell synapse, both pre- and postsynaptically. Here, we studied one presynaptic (*$Ca_v1.4$*) and two postsynaptic mutations (*$mGluR6^{nob3}$*, *Nyx^{nob}*) that cause CSNB. We found that all three mouse models show nystagmus and oscillating RGCs, but that they varied phenotypically between the three genotypes. All three mutations lead to an altered membrane potential of the ON-BCs and consequently of the A_{II} ACs as well. The latter are then outside their normal working range and start to intrinsically oscillate. These oscillations are passed on to the RGCs, which also start to oscillate. This includes the ON-DSRGCs, that send an oscillatory retinal slip signal to the AOS, which consequently leads to compensatory, oscillatory eye movements, the nystagmus. The different phenotypes can thereby be explained by the difference in depolarization levels of the ON-BCs due to the different mutations. In mGluR6 mice, ON-BCs are the least depolarized and so in the dark the A_{II} AC membrane potential sits below the threshold required for intrinsic oscillations to occur. However, when stimulated by light, the A_{II} AC membrane potential depolarises enough to pass this threshold and start oscillating. In *Nyx^{nob}*, oscillations are already present in the dark since the ON-BCs, and hence the A_{II} ACs, are more depolarized than for mGluR6. Finally, in $Ca_v1.4$-KO mice the A_{II} ACs are depolarized the strongest compared to the other two mouse models due to the lack of PR input, leading to light independent oscillations with the highest frequency.

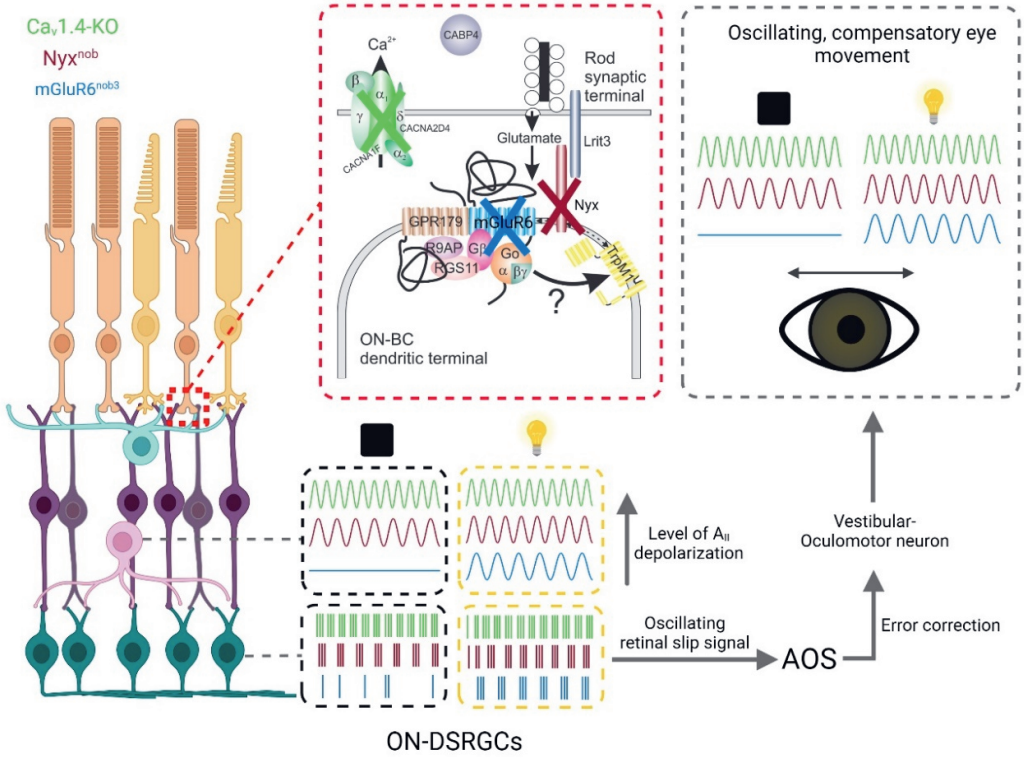


Figure 6.1: Schematic overview of the mechanism underlying nystagmus in CSNB.

Since CSNB and congenital nystagmus are associated with low visual acuity, in the **Chapters 5 and 6** we investigated, whether the receptive field size of RGC had changed in CSNB mice (*Nyx*^{nob}, *Lrit3*$^{-/-}$). We found subtle changes in the receptive field size of G-OFF RGCs, which could be virally restored in the *Lrit3*$^{-/-}$ mouse by expressing the WT form of *LRIT3* in rods but not in cones. Therefore, our data indicates, that the increase in receptive field size of *Lrit3*$^{-/-}$ G-OFF RGCs is not developmental but is a reversible network alteration.

Overall, this thesis shows the causal relation between RGC oscillations and the visual problems in congenital nystagmus.

Novel mechanism leading to congenital nystagmus

With this novel retinal mechanism, we replace the historical opinion that nystagmus originates in the vestibulo-oculomotor system. Recently, a group using the mutant-*FRMD7* mouse model proposed another retinal mechanism leading to congenital nystagmus (Yonehara et al., 2016). Mutations in *FRMD7* are know to cause congenital nystagmus in human patients (Thomas et al., 2008). Yonehara and colleagues suggested that the absence of a signal coding for horizontal direction selectivity leads to nystagmus. However, they did not find oscillating eye movements, i.e. nystagmus, in their *FRMD7* mouse model. Furthermore, it is likely that the absence of *FRMD7* in SACs will reduce their inhibition on ON-BCs and therefore lead to the depolarization of the A_{II} ACs and consequently to nystagmus. Therefore, the mechanism described in this thesis may underly the nystagmus in patients with mutations in *FRMD7* as well.

So far, long-lasting or permanent solutions to prevent or eliminate the eye movement oscillations of nystagmus have not been developed. In **Chapters 2 and 3**, clear evidence is presented that the oscillatory eye movements in mouse models for congenital nystagmus associated with CSNB have a retinal origin. Firstly, all the mutations studied were located in the PR to ON-BC synapse, and affect the signal transmission of ON-BCs. Secondly, we found a linear relationship between the oscillation frequency of the RGCs and the eye movements in the various mouse models (**Fig. 3.5E**). Furthermore, blocking the RGC oscillations or shifting the RGC oscillation frequency pharmacologically in the *Nyx*^{nob} mouse, had the corresponding effect on the eye-movement oscillations (**Fig. 2.4**). Based on these datasets, the origin of nystagmus can be clearly localized to the retina and not to higher-order processes. Knowing the real origin of the eye movement oscillations reveals a target for therapeutical approaches in the future.

A_{II} ACs as the oscillator

We identified the A_{II} ACs as the oscillator in the CSNB mouse model for the following reasons. In all three mouse models, the signal processing at the PR to ON-BC synapse is impacted, leading to alterations in the modulation of the TRPM1 channel. This will alter the membrane potential of the ON-BCs, and consequentially of the A_{II} ACs as well. Choi et al. found that A_{II} ACs start intrinsically oscillating when their membrane potential is outside their normal working range due to altered

input from the ON-BCs, which is also the case for all three CSNB mouse models (Choi et al., 2014). We found that the oscillations are broadly distributed across the retina, which indicates the origin is presynaptic of the RGCs and not generated by the RGCs themselves. Furthermore, RGC oscillations stop when gap junctions are blocked with MFA and when Cx36 is knocked out. As A_{II} ACs are strongly coupled electrically with BCs via gap junctions, this result is again suggestive of the role played by the A_{II} ACs. Moreover, we found that ON- and OFF- RGCs oscillations are in antiphase for *Nyx*^{nob} and *mGluR6*^{nob3} mice, which is again consistent with the A_{II} ACs being the oscillator. Finally, our computational model confirms our hypothesis. When changing the membrane potential of the ON-BCs, A_{II} ACs and consequently RGCs start oscillating. Depending on the level of depolarization, the model can account for all three genotypes (**Chapter 2**).

How general is the retinal mechanism leading to nystagmus?

We showed that the mechanism we propose can account for the nystagmus in *Nyx*^{nob}, *mGluR6*^{nob3} and *Cav1.4*-KO mice (**Chapters 1 and 2**) and therefore for CSNB mutations located pre- and postsynaptically in the PR to ON-BC synapse. There are many more impacted proteins located in the PR to ON-BC synapse that are associated with CSNB (Zeitz et al., 2015). Each one of these will have a slightly different influence on the membrane potential of the A_{II} ACs, which in turn leads to somewhat different nystagmus phenotypes. Consistent with this idea of a more general mechanism, RGCs of *TRPM1*$^{-/-}$ and *Lrit3*$^{-/-}$ mice were found to oscillate as well (Hasan et al., 2019; Takeuchi et al., 2018).

The mechanism leading to nystagmus can also account for other retinal diseases. Intrinsic A_{II} AC oscillations were originally described as the cause of RGC oscillations in a mouse model for retina pigmentosa (RP, Choi et al., 2014). However, to the best of our knowledge, it has not yet been determined if the RP mouse models also show oscillating eye movements. There are studies describing human RP patients with nystagmus (Chiew, 2023; Verbakel et al., 2018), which potentially could be explained with the mechanism described in this thesis. However, there is one important aspect that needs to be considered: Unlike CSNB, RP is associated with retinal degeneration and the progressive loss of PRs. The loss of PRs causes several morphological changes in the retina (Strettoi et al., 2002) and following our hypothesis these changes may in themselves influence the A_{II} AC oscillations. Alternatively, the absence of nystagmus in RP mice does not automatically act as evidence against our hypothesised mechanism. The patchy loss of light sensitivity across the RP retina may prevent the global synchronisation of the A_{II} AC/RGC oscillations and so the mice may not exhibit oscillatory eye movements.

Nystagmus was also found to be caused by a specific mutation in Munc 18-1 (Li et al., 2020). Munc 18-1 is involved in the docking of synaptic vesicles to the membrane. The mutation leading to nystagmus enhances the binding of Munc 18-1 with syntaxin 3B, which is specifically expressed in PRs and BCs (Curtis et al., 2008; Li et al., 2020; Sherry et al., 2006) and most likely leads to a reduction in PR glutamate release. This would result in depolarization of the A_{II} AC and therefore to intrinsic oscillations, oscillating RGCs and nystagmus.

The mechanism proposed in this thesis is, however, not limited to mutations in the PR to ON-BC synapse. A_{II} ACs are strongly connected and receive various inputs. Any depolarization sufficiently strong enough to move the A_{II} ACs membrane potential past its oscillation threshold can potentially lead to intrinsic oscillations and consequently nystagmus.

Taken together, there is potentially a wide variety of mutations that cause A_{II} ACs to be out of their normal working range and consequently lead to nystagmus. Establishing that one mechanism underlies several subtypes of congenital nystagmus, will allow to limit future therapeutic strategies to one common approach.

Prevalence of oscillations depends on the area of the mouse retina

One particularly intriguing finding is that UV-preferring RGCs may oscillate less than green-preferring RGCs (**Chapter 4**). To verify that this is indeed the case, RGC activity needs to be recorded separately from the ventral and dorsal parts of the *Nyx*^{nob} retina and the spectral specificity determined by green and UV light flashes. However, this experiment was outside the scope of this thesis project. The hypothesis that retinal areas containing mainly UV-RGCs are less affected by oscillations is consistent with the receptive field sizes of UV-RGCs in *Nyx*^{nob} and *Lrit3*$^{-/-}$ being comparable with WT, while G-RGCs showed altered receptive field sizes (**Chapters 4 and 5**).

How can oscillating RGCs in only part of the retina lead to nystagmus? UV-RGCs are mainly located in the ventral retina and G-RGCs in the dorsal part. The density of ON-DSRGCs, which project to the AOS and are important for the OKR loop, is highest in the dorso-temporal retina and less so in the ventral region (Yonehara et al., 2008). Furthermore, we found that in the *Nyx*^{nob} retina, ON-DSRGCs are oscillating (**Fig. 2.2D**). Combining their location in the retina and their oscillatory behaviour, ON-DSRGCs are likely G-RGCs. Their oscillatory input drives the oscillating compensatory eye-movements without the need for input from the UV-RGCs in the ventral part of the retina.

Translation of our results to human patients

Here we studied the origin of nystagmus in three afoveate mouse models. How do our findings translate to the foveate human CSNB patients? There is excellent conformance between the two species. Firstly, the oscillation frequency of patients with the NYX mutation is similar to the *Nyx*^{nob} mice (**Chapter 1**). Secondly, the models used in this thesis have mutations in murine orthologue genes of those impacted in human patients in the highly conserved PR-ON-BC synapse. Furthermore, similar to the mouse, the primate retina comprises ON-DSRGCs that project to the AOS, an important element of the mechanism leading to nystagmus described in this thesis (Patterson et al., 2022; Wang et al., 2023). Considering the similarities in both the visual systems as well as the results of *Nyx*^{nob} mice and patients, the mechanism described in this thesis is most likely underlying the nystagmus in the human patients as well.

The *$Ca_V1.4$-KO* mouse, however, doesn't fully represent the human phenotype. This mouse is blind and therefore has a more severe phenotype than occurs in the human CSNB patients. Currently, the frequency of nystagmus is rarely measured/ registered, but instead is generally estimated. For a better comparison between the mouse models and human nystagmus patients, it will be very important to include the precise determination of the nystagmus frequency in the medical examination routines. In this regard, it is also important to perform gene sequencing as a standard part of the medical examination routines to be able to correlate the oscillation frequency of the nystagmus to a specific genetic mutation.

Clinical relevance

Knowing the mechanism underlying congenital nystagmus allows for the development of therapeutical approaches for human patients. One possible approach is targeting the A_{II} ACs such that their membrane potentials are returned back to their normal working range. One way to do so would be to develop a drug that hyperpolarizes A_{II} ACs according to the genotype underlying the nystagmus. In **Chapter 2**, we showed that linopirdine, which blocks the M-type potassium current in the A_{II} ACs, reduces the oscillation frequency of both RGCs and eye movements. Linopirdine was tested as a potential treatment in Alzheimer's patients, but did not improve cognitive function and might not be entirely safe to use (Rockwood et al., 1997). Therefore, more research is needed to develop a suitable, safe drug that targets the A_{II} ACs to treat nystagmus. Also important will be to develop delivery methods such that the drugs will be applied locally in the eye to avoid spreading into other regions of the human body and causing potential side effects.

Repairing the original defect via gene therapy is an option as well, but the therapy needs to be developed for each of the different CSNB associated genes separately, which is not very cost effective. Therefore, a more general approach would be preferrable. Optogenetic targeting of the A_{II} ACs is one such approach. Kahbou and colleagues recently reported that they restored ON- and OFF-responses in the rd1 retina by optogenetic stimulation of the A_{II} ACs (Khabou et al., 2023). This could be a promising therapeutic approach.

One aspect for developing treatments is the differentiation of developmental and acute effects. Gene therapy should for example be used early in the life of the patient to not only cure the nystagmus but also prevent other developmental effects, like the low visual acuity and the desegregation of the projections to the dLGN.

Future outlook

Even though we present indirect proof via blocking and knockout experiments as well as our modelling work, it would be very interesting to record directly from the A_{II} ACs in the various mouse models to have direct proof of our hypothesis. Our preliminary data indicates that A_{II} ACs are indeed oscillating in the *Nyx^{nob}* mouse, but our sample size is too small to be definitive.

As described in **Chapter 3**, in vivo and in vitro conditions are leading to differences in eye movement, and RGC oscillation frequencies. This is most likely due to small variations in recording conditions. It would therefore be interesting to measure both eye movements and RGC oscillations simultaneously. Hong et al. developed a mesh recording device that can be implanted underneath the retina and record the RGC condition in vivo (Hong et al., 2018). If technically possible, this would allow the simultaneous recording of RGC and eye-movements and therefore show a clearer correlation in recordings from the same animal at the same time. It would be interesting in this context to also test the effects of pharmacology and developmental effects in the combined recording approach.

References

Chiew, Y. R. (2023). See-saw nystagmus associated with retinitis pigmentosa. *QJM: An International Journal of Medicine*, *116*(7), 572–573. https://doi.org/10.1093/qjmed/hcad052

Choi, H., Zhang, L., Cembrowski, M. S., Sabottke, C. F., Markowitz, A. L., Butts, D. A., Kath, W. L., Singer, J. H., & Riecke, H. (2014). Intrinsic bursting of AII amacrine cells underlies oscillations in the rd1 mouse retina. *Journal of Neurophysiology*, *112*(6), 1491–1504. https://doi.org/10.1152/jn.00437.2014

Curtis, L. B., Doneske, B., Liu, X., Thaller, C., McNew, J. A., & Janz, R. (2008). Syntaxin 3b is a t-SNARE specific for ribbon synapses of the retina. *Journal of Comparative Neurology*, *510*(5), 550–559. https://doi.org/10.1002/cne.21806

Hasan, N., Pangeni, G., Cobb, C. A., Ray, T. A., Nettesheim, E. R., Ertel, K. J., Lipinski, D. M., McCall, M. A., & Gregg, R. G. (2019). Presynaptic Expression of LRIT3 Transsynaptically Organizes the Postsynaptic Glutamate Signaling Complex Containing TRPM1. *Cell Reports*, *27*(11), 3107-3116.e3. https://doi.org/10.1016/j.celrep.2019.05.056

Hong, G., Fu, T.-M., Qiao, M., Viveros, R. D., Yang, X., Zhou, T., Lee, J. M., Park, H.-G., Sanes, J. R., & Lieber, C. M. (2018). A method for single-neuron chronic recording from the retina in awake mice. *Science*, *360*(6396), 1447–1451. https://doi.org/10.1126/science.aas9160

Khabou, H., Orendorff, E., Trapani, F., Rucli, M., Desrosiers, M., Yger, P., Dalkara, D., & Marre., O. (2023). Optogenetic Targeting of AII Amacrine Cells Restores Retinal Computations performed by the inner retina. *Molecular Therapy - Methods & Clinical Development*. https://doi.org/10.1016/j.omtm.2023.09.003

Li, Y., Jiang, L., Wang, L., Wang, C., Liu, C., Guo, A., Liu, M., Zhang, L., Ma, C., Zhang, X., Gao, S., & Liu, J. Y. (2020). p.His16Arg of STXBP1 (MUNC18-1) Associated With Syntaxin 3B Causes Autosomal Dominant Congenital Nystagmus. *Frontiers in Cell and Developmental Biology*, *8*. https://doi.org/10.3389/fcell.2020.591781

Patterson, S. S., Bembry, B. N., Mazzaferri, M. A., Neitz, M., Rieke, F., Soetedjo, R., & Neitz, J. (2022). Conserved circuits for direction selectivity in the primate retina. *Current Biology*, *32*(11), 2529-2538.e4. https://doi.org/10.1016/j.cub.2022.04.056

Rockwood, K., Beattie, B. L., Eastwood, M. R., Feldman, H., Mohr, E., Pryse-Phillips, W., & Gauthier, S. (1997). A Randomized, Controlled Trial of Linopirdine in the Treatment of Alzheimer's Disease. *Canadian Journal of Neurological Sciences / Journal Canadien Des Sciences Neurologiques*, *24*(2), 140–145. https://doi.org/10.1017/S031716710002148X

Sherry, D. M., Mitchell, R., Standifer, K. M., & du Plessis, B. (2006). Distribution of plasma membrane-associated syntaxins 1 through 4 indicates distinct trafficking functions in the synaptic layers of the mouse retina. *BMC Neuroscience*, *7*(1), 54. https://doi.org/10.1186/1471-2202-7-54

Strettoi, E., Porciatti, V., Falsini, B., Pignatelli, V., & Rossi, C. (2002). Morphological and Functional Abnormalities in the Inner Retina of the rd/rd Mouse. *The Journal of Neuroscience*, *22*(13), 5492–5504. https://doi.org/10.1523/JNEUROSCI.22-13-05492.2002

Takeuchi, H., Horie, S., Moritoh, S., Matsushima, H., Hori, T., Kimori, Y., Kitano, K., Tsubo, Y., Tachibana, M., & Koike, C. (2018). Different Activity Patterns in Retinal Ganglion Cells of TRPM1 and mGluR6 Knockout Mice. *BioMed Research International*, *2018*, 1–6. https://doi.org/10.1155/2018/2963232

Thomas, S., Proudlock, F. A., Sarvananthan, N., Roberts, E. O., Awan, M., McLean, R., Surendran, M., Anil Kumar, A. S., Farooq, S. J., Degg, C., Gale, R. P., Reinecke, R. D., Woodruff, G., Langmann, A., Lindner, S.,

Jain, S., Tarpey, P., Raymond, F. L., & Gottlob, I. (2008). Phenotypical characteristics of idiopathic infantile nystagmus with and without mutations in FRMD7. *Brain*, *131*(5), 1259–1267. https://doi.org/10.1093/brain/awn046

Verbakel, S. K., van Huet, R. A. C., Boon, C. J. F., den Hollander, A. I., Collin, R. W. J., Klaver, C. C. W., Hoyng, C. B., Roepman, R., & Klevering, B. J. (2018). Non-syndromic retinitis pigmentosa. *Progress in Retinal and Eye Research*, *66*, 157–186. https://doi.org/10.1016/j.preteyeres.2018.03.005

Wang, A. Y. M., Kulkarni, M. M., McLaughlin, A. J., Gayet, J., Smith, B. E., Hauptschein, M., McHugh, C. F., Yao, Y. Y., & Puthussery, T. (2023). An ON-type direction-selective ganglion cell in primate retina. *Nature*. https://doi.org/10.1038/s41586-023-06659-4

Yonehara, K., Fiscella, M., Drinnenberg, A., Esposti, F., Trenholm, S., Krol, J., Franke, F., Scherf, B. G., Kusnyerik, A., Müller, J., Szabo, A., Jüttner, J., Cordoba, F., Reddy, A. P., Németh, J., Nagy, Z. Z., Munier, F., Hierlemann, A., & Roska, B. (2016). Congenital Nystagmus Gene FRMD7 Is Necessary for Establishing a Neuronal Circuit Asymmetry for Direction Selectivity. *Neuron*, *89*(1), 177–193. https://doi.org/10.1016/j.neuron.2015.11.032

Yonehara, K., Shintani, T., Suzuki, R., Sakuta, H., Takeuchi, Y., Nakamura-Yonehara, K., & Noda, M. (2008). Expression of SPIG1 reveals development of a retinal ganglion cell subtype projecting to the medial terminal nucleus in the mouse. *PloS One*, *3*(2), e1533. https://doi.org/10.1371/journal.pone.0001533

Zeitz, C., Robson, A. G., & Audo, I. (2015). Congenital stationary night blindness: an analysis and update of genotype-phenotype correlations and pathogenic mechanisms. *Progress in Retinal and Eye Research*, *45*, 58–110. https://doi.org/10.1016/j.preteyeres.2014.09.001