We shall see?

An optogenetic approach to restore vision

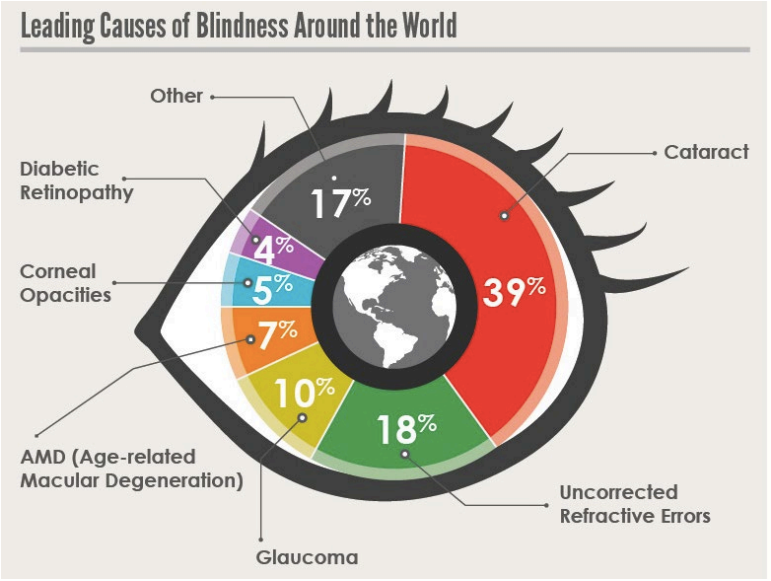
“The window to the soul”

Matthew 6:22-23

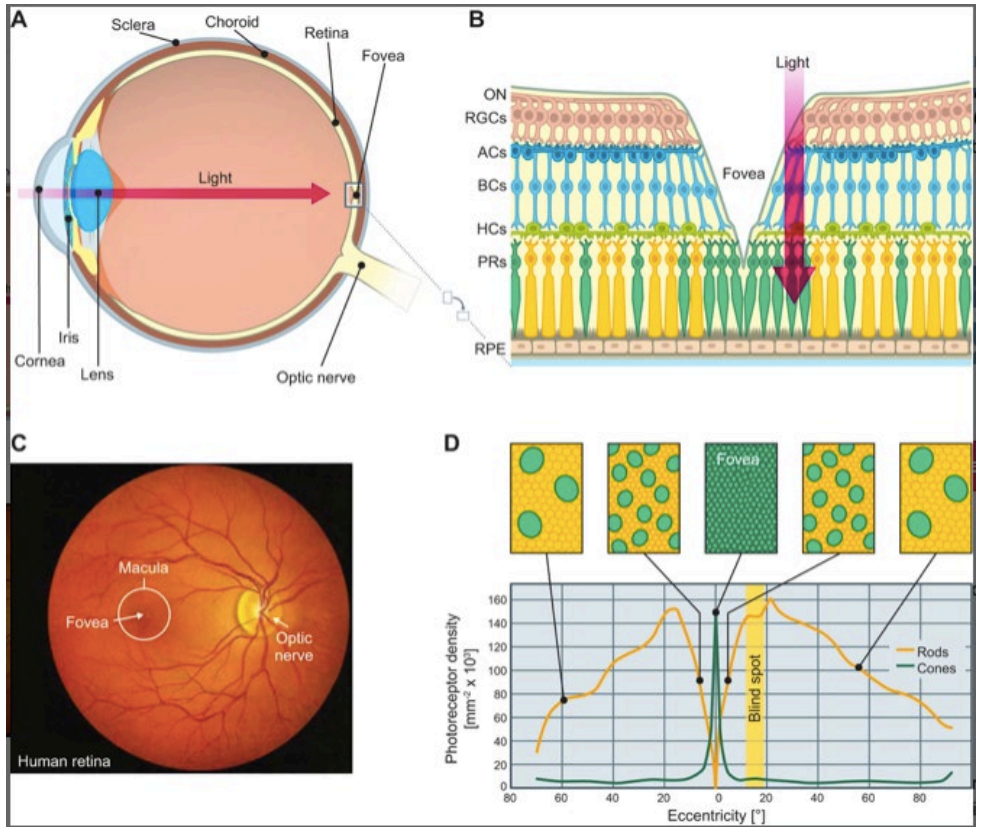
“The lamp of the body is the eye. If therefore your eye is sound, your whole body will be full of light. But if your eye is evil, your whole body will be full of darkness. ”

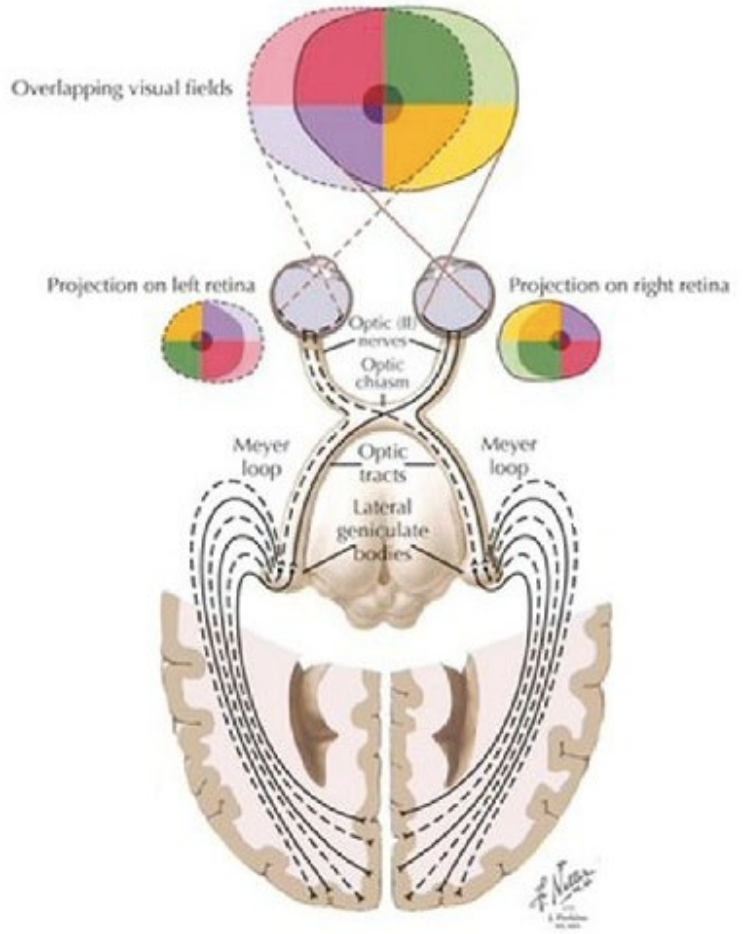
Vision impairment and blindness

- **Prevalence**: 2.2 billion people with vision impairment worldwide; >50% older than 50 yrs
- 50% of vision impairment treatable/preventable
- Leading causes of vision impairment globally:
  - **uncorrected refractive errors**
  - **cataract**
  - age-related macular degeneration
  - glaucoma
  - diabetic retinopathy
  - Trachoma



The visual system



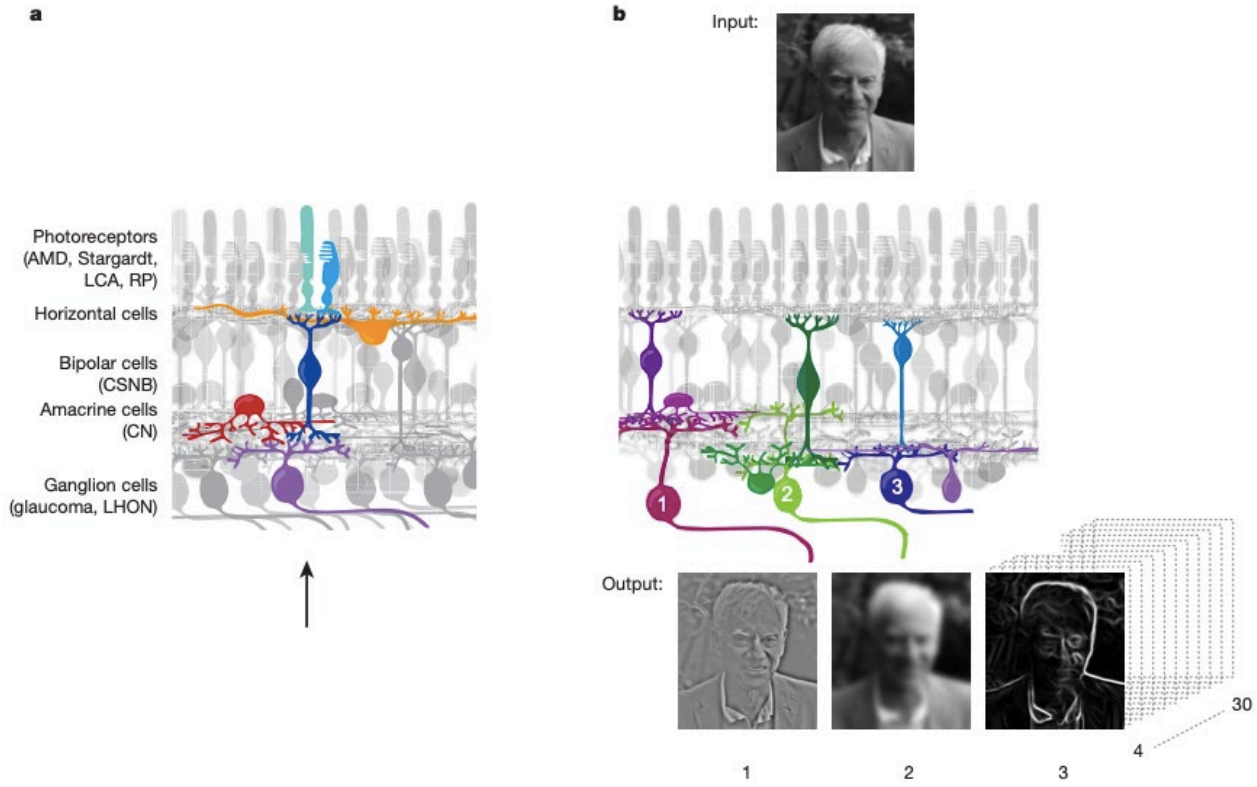


~55% of the cortex specialized for visual processing
(3% for auditory, 11% somatosensory processing)

Kleinlogel S, Physiol Rev. 2020

The human retina

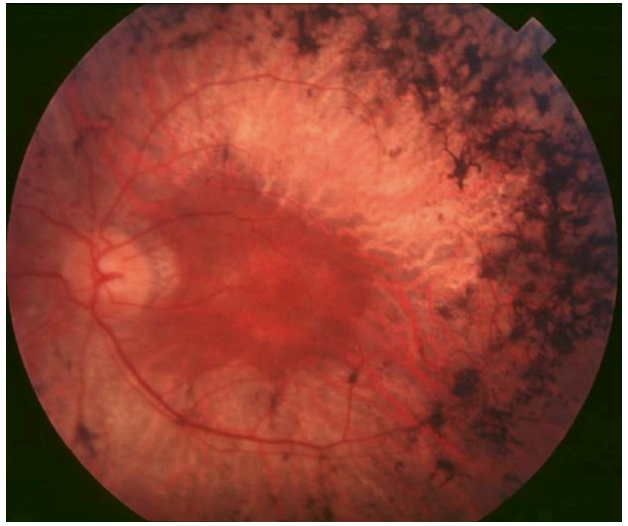
- broad spectral (400–700 nm) and light sensitivity (10^4 to 10^{16} photons $cm^{-2}\,s^{-1}$), high temporal resolution (up to 60 Hz)
- Massive parallel information processing (inhibitory horizontal signaling)
- Around 30 image representations of the visual scene processed in parallel



Roska B. Restoring vision. Nature 2018

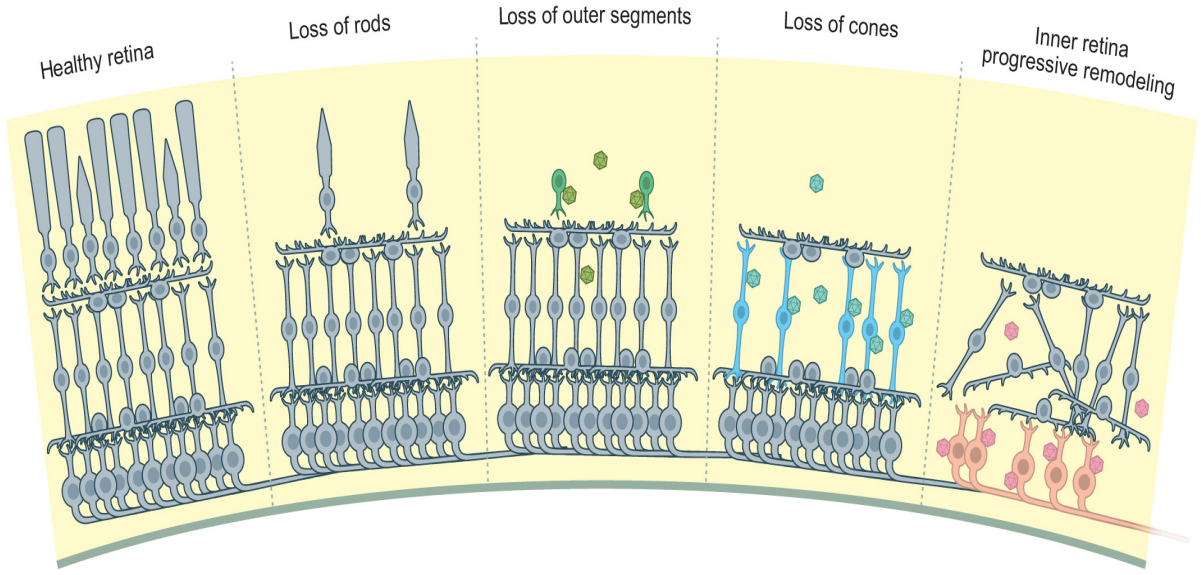
Retinal diseases

- Monogenic or multifactorial
- 2 mio people affected worldwide
- Hereditary **retinitis pigmentosa**
  - 1 in 3,500 people in US and Europe
  - 50% of inherited retinal diseases
  - 71 causative genes (AR, AD, X-linked)
  - Loss of rods, secondary loss of cones
  - Nyctalopia followed by tunnel vision
  - gene replacement therapy for early-onset RP caused by mutation in *RPE65* (around 1,000 – 2,000 people affected in US)



”bone spicules” in the fundus

Retinal degeneration in rod dystrophies

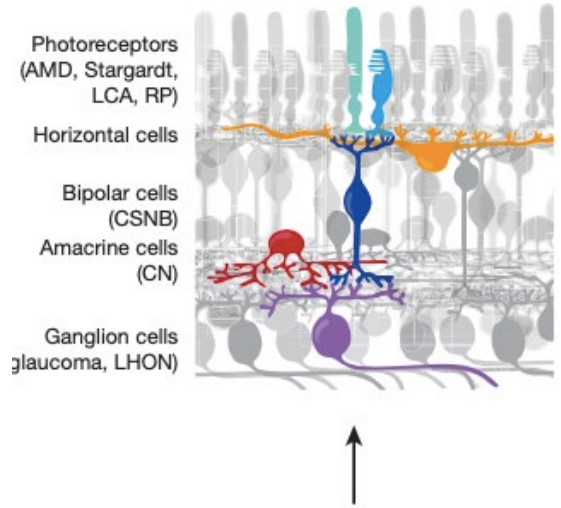


DOI: (10.1152/physrev.00035.2019)

- Loss of rods followed by secondary loss of cones renders retina light-insensitive
- Bipolar cells and eventually retinal ganglion cells target of optogentic treatment
- RGCs connect to thalamus (CGL)

The human retina

- Cell-type specific intervention preferred in order **to restore high resolution vision**
- Which cells to target?
  - **Bipolar cells**
    - Contrast discrimination
    - Edge detection and foveal tracking
    - Small risk of immunogenic response beyond eye (terminate in retina)
  - **Retinal ganglion cells**
    - Relevant for patients with late stage degeneration
    - Massive information processing takes place upstream of RGCs, compromising quality of restored vision
    - Project to the brain: immunogenicity?

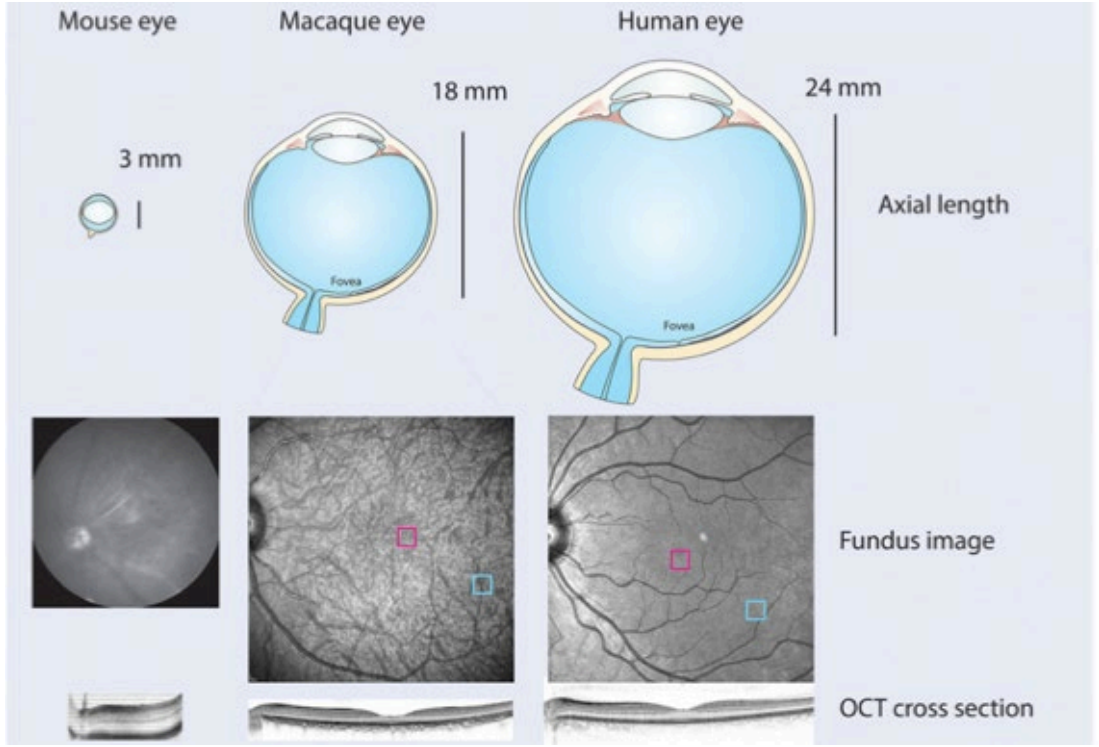


How to achieve cell type specificity

- **Promotor choice**: cell specificity and expression, long-term expression stability
- **AAV serotype**
- Additional influential factors: species, route of virus administration, state of the tissue (healthy vs degenerated), viral dose

Restoring vision?

- **Intrinsic regeneration** of mammalian retina weak or absent
- Many diseases **cell-type specific**
- **Inner limiting membrane** between the retina and the vitreous
  - Limited diffusion and restricted efficacy of intravitreally delivered gene therapy
- **large surface area** of the human retina
- **Differences between mice and men**
  - Primates the only mammals with a fovea
  - Different cell tropism of AAVs
  - Differences in cell type specific gene expression (e.g. Usher I genes)
  - Certain cell types missing in mice (e.g. midget ganglion cells important for high-resolution image-formation vision)



New model systems

- Human **retinal organoids**
  - Different cell types, can be engineered to harbor specific mutations, allows control of growth medium
- **Post-mortem human retinas**
  - Can be kept in culture for weeks, can be dissected into many smaller pieces
- **Non-human primates** (e.g. marmosets)
  - Have a fovea

Things to consider

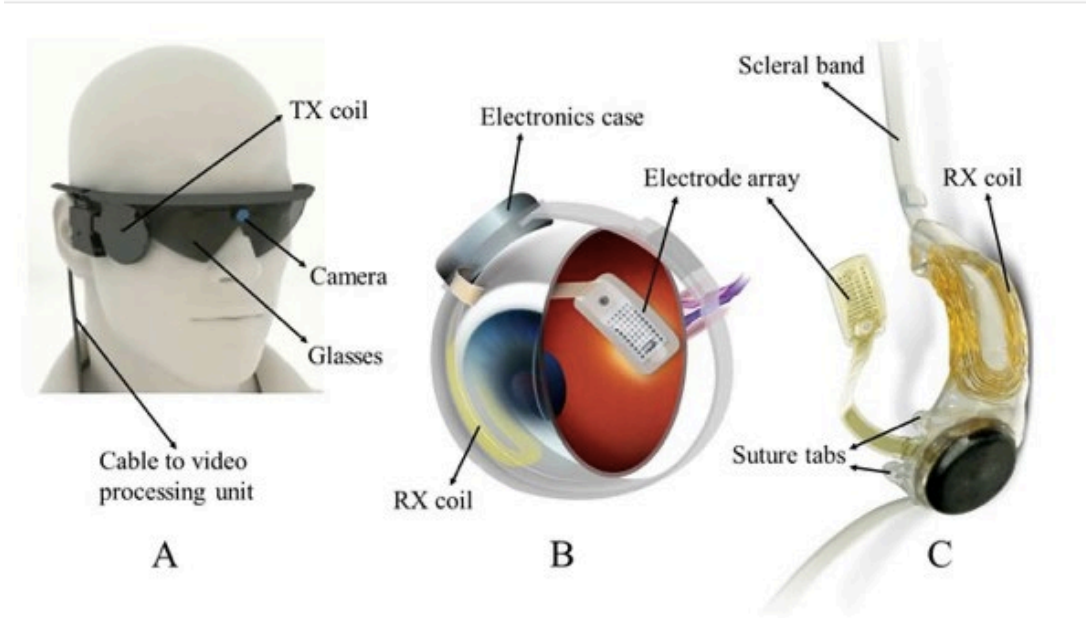
- Vision is lost vs useful vision remains
  - Vision restoration vs prevention or slowing of vision loss
  - Critical period of restoring vision in congenitally blind people
- **Outcome evaluation**
  - Different ways of evaluation (imaging, psychophysical tests, real-life performance), different time points after treatment, differences in gene therapy vectors and mode of delivery, differences in disease stage, learning capacity of patient
- Technologies complementing vision restoration
  - GPS-linked talking maps, voice-written emailing, word processing, web browsing

Approaches to restore vision

- **Gene therapy**
  - Gene replacement or substitution
  - AAVs as vectors to deliver genes of interest to retinal cells
- **Cell therapy**
  - Ectopic cell transplantation (embryonic or induced pluripotent stem cells)
- Induced **retinal regeneration**
  - Yamanaka factors (OCT4, SOX2, and KLF4) expression in retinal ganglion cells
- Artificial **retinal stimulation**
  - Electronic implants, optogenetics, photoswitches

Where we are today

- Gene therapy for a form of Leber congenital amaurosis (Luxturna; *RPE65*); FDA approval 2018
- Electric stimulation of the retina in adult patients with photoreceptor degeneration (Argus II retinal prosthesis); FDA approval in 2013
- Transplantation of retinal pigment epithelial cells behind the retina for age-related macular degeneration and Stargardt disease; Phase 1/2 in 2017



nature medicine

ARTICLES

https://doi.org/10.1038/s41591-021-01351-4

Check for updates

Partial recovery of visual function in a blind patient after optogenetic therapy

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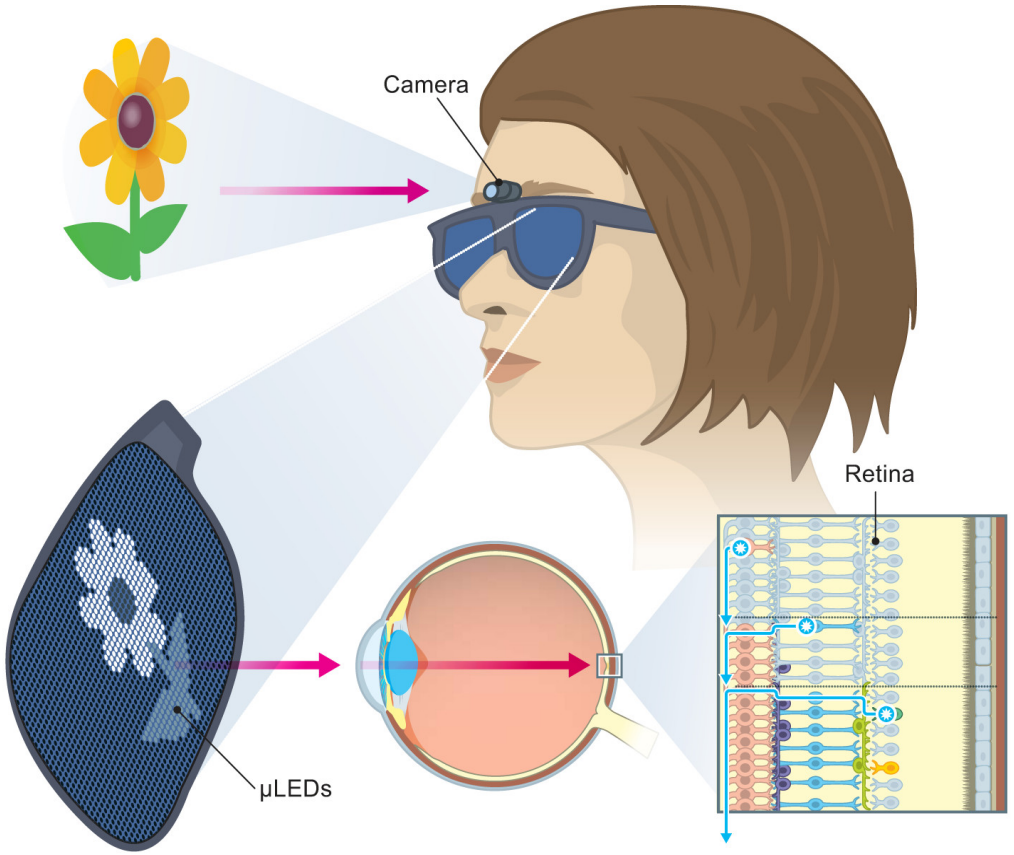
Study design

- investigational treatment for patients with advanced nonsyndromic RP
- combines injection of an **optogenetic vector with wearing light-stimulating goggles**
- adeno-associated viral vector encoding the light-sensing channelrhodopsin protein (**ChrimsonR**) fused to tdTomato
- **single intravitreal injection** into the worse-seeing eye
- Target: mainly foveal **retinal ganglion cells**

Study design II

- multicenter, phase 1/2a, nonrandomized, dose-escalation study to evaluate **safety and tolerability** of an adeno-associated viral vector
- evaluate visual and visuomotor function with and without light-stimulating goggles
- 3 dose-escalation cohorts (**5.0×10^{10}, 1.5×10^{11} and 5.0×10^{11} viral genomes per eye**) of 3 participants each and an extension cohort treated at the highest tolerated dose
- because of COVID-19, **only one patient** from the first cohort could perform sustained (*n* = 15) postinjection training sessions

The principle



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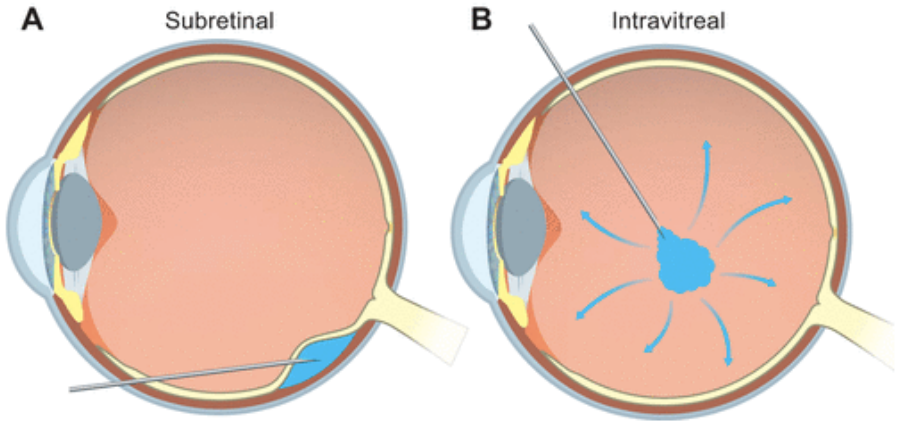
Route of application



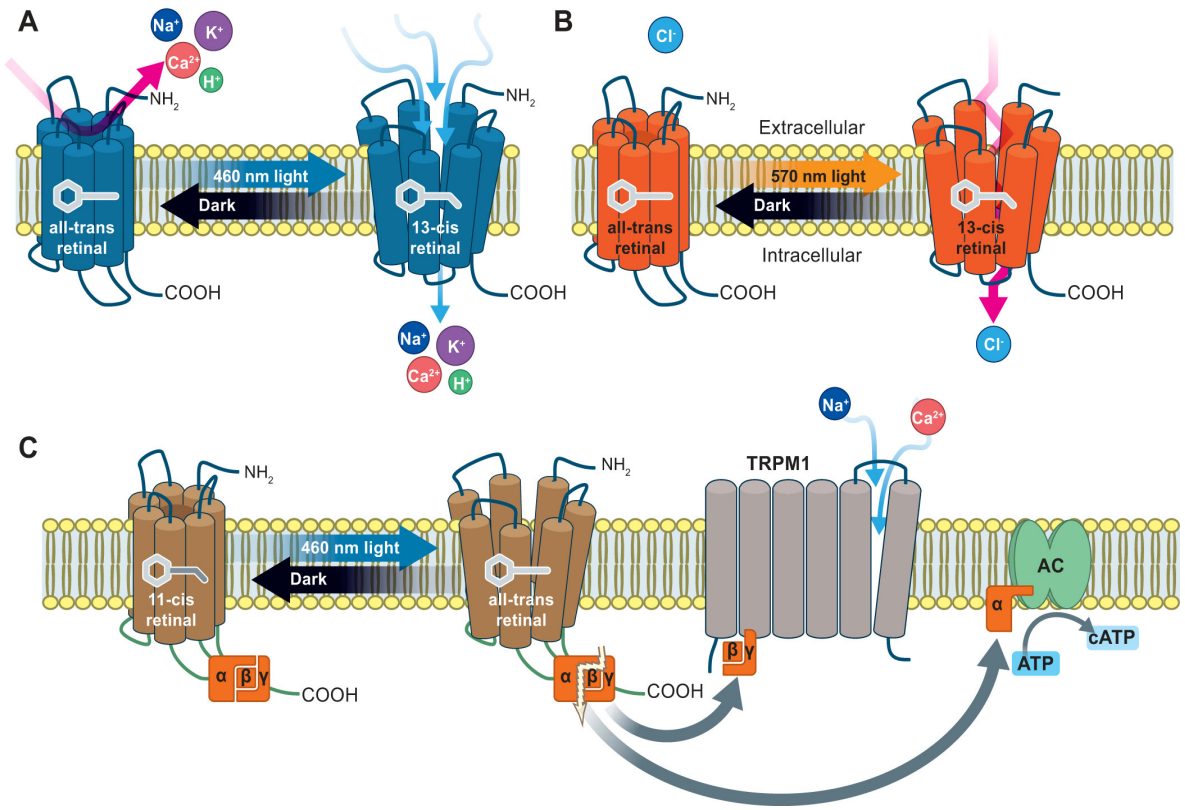
- **Subretinal**: concentrates vector in close proximity to retinal cells, risk of retinal detachment/damage
- **Intravitreal**: technically less difficult, higher doses required, immunogenic response and vector toxicity

Optogenetics

- Transgenic expression of light-sensitive proteins (**opsins**) to render sensory neurons light controllable
- spatiotemporal control of neuronal activity through light application
- Research tool to study neural circuits as well as therapeutic to restore vision
- Advantage: **mutation-independent**, circuit-specific restoration of neuronal function

Opsins

- **Microbial opsins**: ion channels or pumps, high temporal but low light sensitivity (>10^{15} photons cm^{-2} s^{-1})
- **GPCRs**: low temporal resolution, compared to rod photoreceptors, rhodopsin activation in ganglion cells/bipolar cells much slower (rod with discs containing all phototransduction cascade proteins)

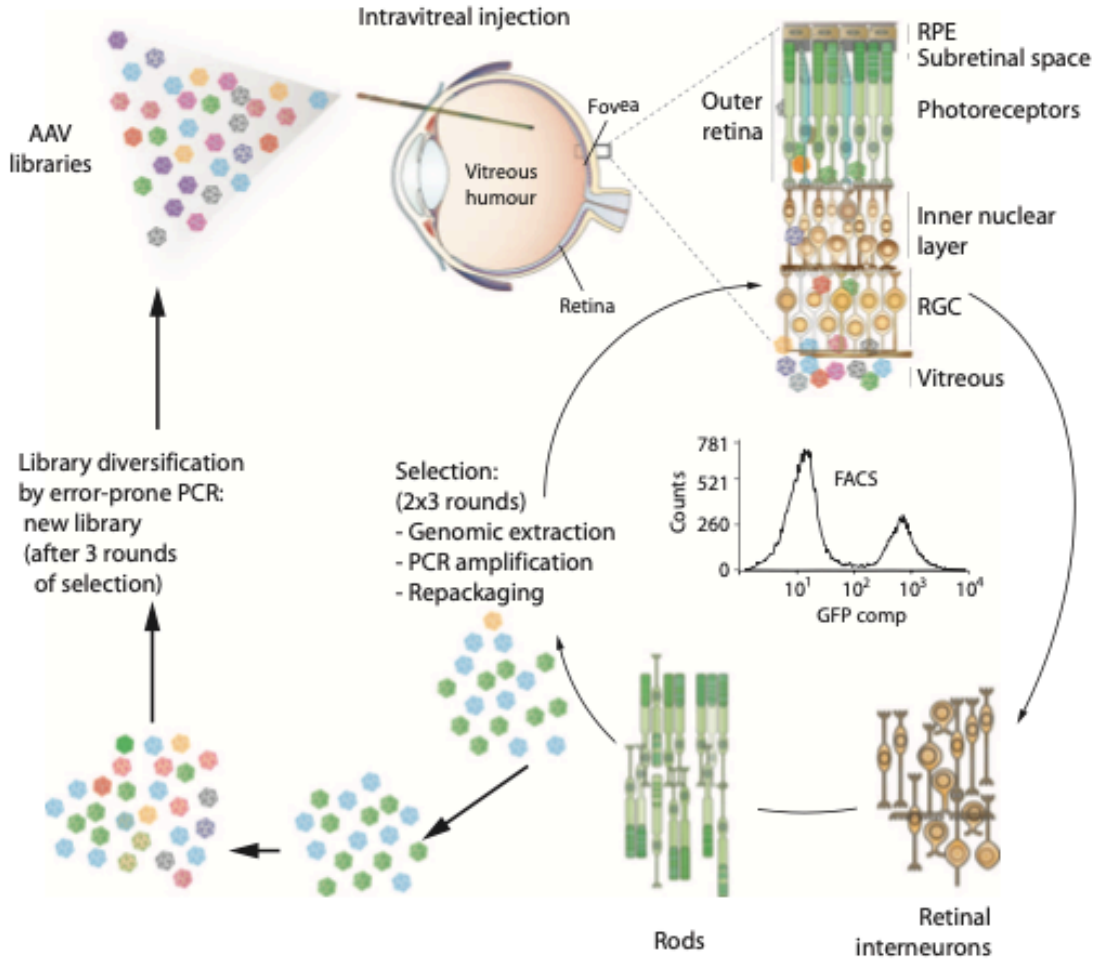


ChrimsonR-tdTomato

- **ChrimsonR**: light-gated cation channel, mammalian codon-optimized version of channel derived from green algae
  - peak sensitivity: 590 nm (amber color)
  - red-shifted spectra
    - less pupil constriction
    - safer than highly phototoxic blue-light wavelengths, expose retina to higher light intensities
- **tdTomato**: increases expression of ChrimsonR in the cell membrane

Viral vector
AAV2.7m8-CAG-ChrimsonR-tdTomato

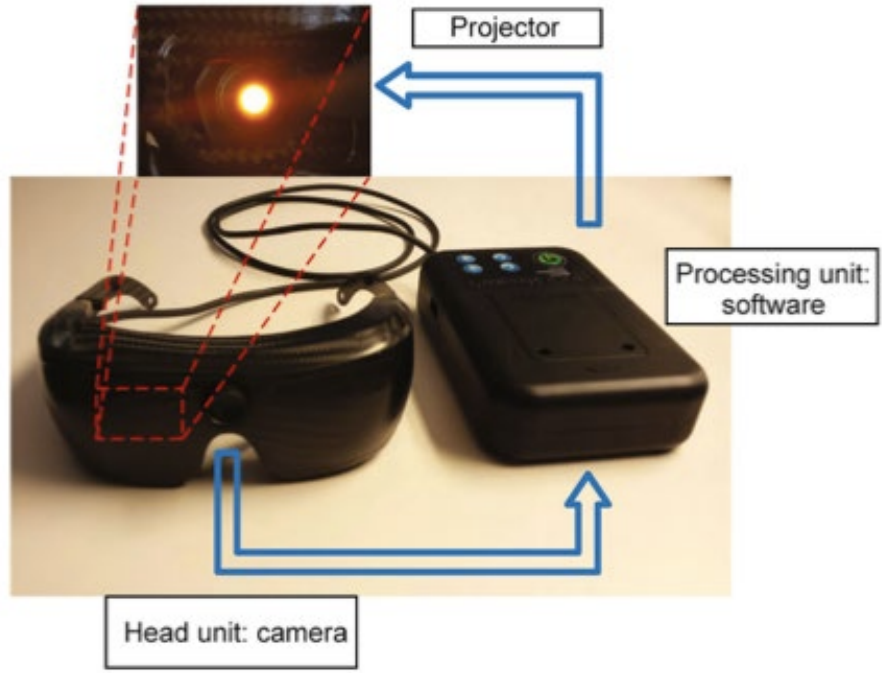
- Identified through **in vivo–directed evolution** of AAV2 for therapeutic outer retinal gene delivery from the vitreous
- heptamer insertion disrupts binding to heparan sulfate proteoglycan, facilitates ILM penetration
- use of relatively low dosages, immune reactions to the 7m8 capsid upon vector readministration

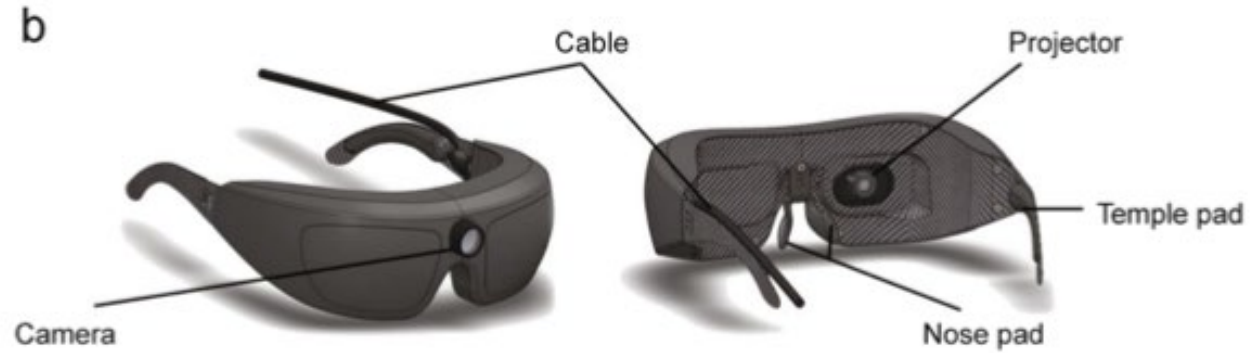


Dalkara D et al. Sci Transl Med. 201

Light-stimulating goggles

- Camera: pixel by pixel (304x240) detection of changes in local relative light intensity as distinct events
- Transformation of events into **monochromatic images**, real time projection as local 595-nm light pulses onto the retina via micromirrors (**binary images**: individual pixel either ON or OFF)
- Temporal redundancy suppression to reduce data volume at sensor output





Safety of the optogenetic vector and light-stimulating goggles

- 58-year-old male, diagnosed with RP at age 18, visual acuity limited to light perception
- worse-seeing eye treated with 5.0×10^{10} vector genomes of optogenetic vector

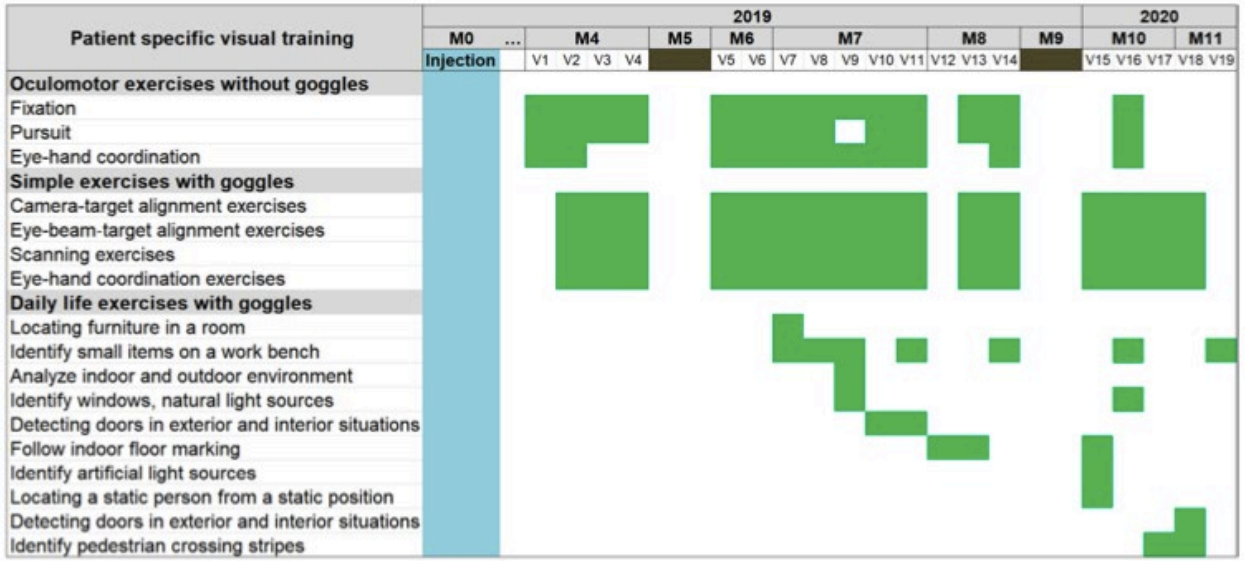
Safety of the optogenetic vector and light-stimulating goggles

- Both before and after the injection
  - ocular examinations
  - optical coherence tomography images, color fundus photographs and fundus autofluorescence images taken on several occasions over **15 visits spanning 84 weeks**
  - monitoring for signs of intraocular inflammation (guidelines of the Standardization of Uveitis Nomenclature Working group)
  - vital signs at each visit, general examination and electrocardiogram before and after the injection

- no intraocular inflammation, no changes in the anatomy of the retina and no ocular or systemic adverse events over the follow-up period
- treated eye retained light perception over the 84 weeks of testing

- light-stimulating goggles tested on patient three times before vector injection
  - no change of vision or photophobia
- **4.5 months after injection**: start of systematic visual training using the light-stimulating goggles
  (expression of ChrimsonR-tdTomato in foveal ganglion cells stabilizes between 2-6 months after injection in nonhuman primates)
- **7 months after the start of visual training**: patient reports signs of visual improvement when using the goggles

Visual training program



M: month
V: training visit

- teach patient to
  - become aware of the direction of his gaze
  - control his eye movements
    - camera–target alignment exercises
    - eye–beam– target alignment exercises
    - scanning exercises
    - eye–hand coordination exercises
- daily life exercises

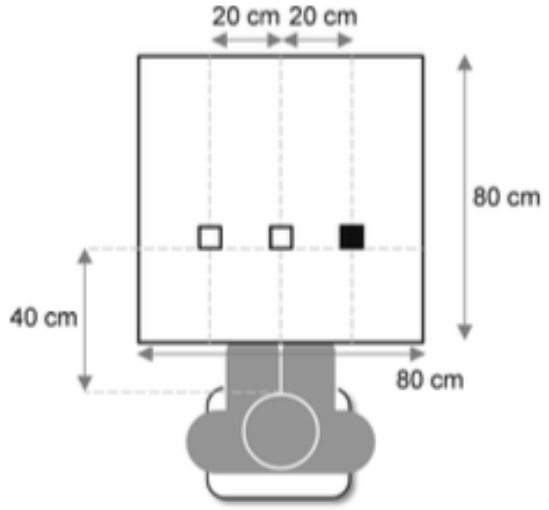
Partial recovery of visual function

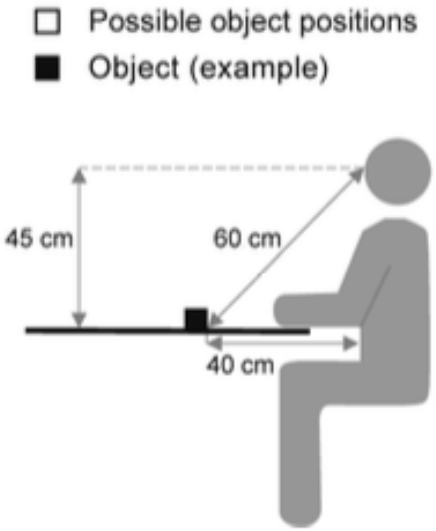
- Testing of visual improvement under three conditions with three psychophysical tests

- conditions :
  - (1) both eyes open without the light-stimulating goggles (**natural binocular**)
  - (2) untreated eye covered, treated eye open without the goggles (**natural monocular**)
  - (3) untreated eye covered, treated eye open and stimulated with the goggles (**stimulated monocular**)

First test

- perceiving, locating and touching a single object placed on a white table
- Objects:
  - large (12.5 × 17.5 cm2; **notebook**) vs
  - small (3 × 5.5 cm2; **staple box**)
- presented one by one in three different grayscale contrasts in random order





First test: Results

Table 1 | First test: finding the notebook or staple box

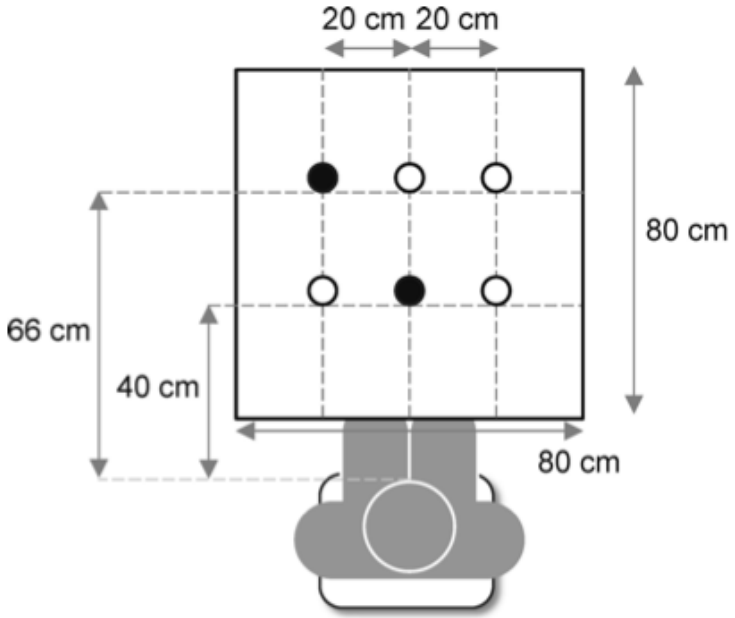
Stimulus	Natural binocular: both eyes open without the light-stimulating goggles			Natural monocular: untreated eye covered, treated eye open without the light-stimulating goggles			Stimulated monocular: untreated eye covered, treated eye open and stimulated with the light-stimulating goggles		
	Perceive	Locate	Touch	Perceive	Locate	Touch	Perceive	Locate	Touch
Notebook, contrast = 40%	0/1	0/1	0/1	0/1	0/1	0/1	4/4	4/4	4/4
Notebook, contrast = 55%	0/1	0/1	0/1	0/1	0/1	0/1	4/5	4/5	4/5
Notebook, Contrast = 100%	0/1	0/1	0/1	0/1	0/1	0/1	4/4	4/4	4/4
Staple box, contrast = 40%	0/1	0/1	0/1	0/1	0/1	0/1	3/6	3/6	2/6
Staple box, contrast = 55%	0/1	0/1	0/1	0/1	0/1	0/1	2/5	2/5	1/5
Staple box, contrast = 100%	0/1	0/1	0/1	0/1	0/1	0/1	1/4	1/4	1/4

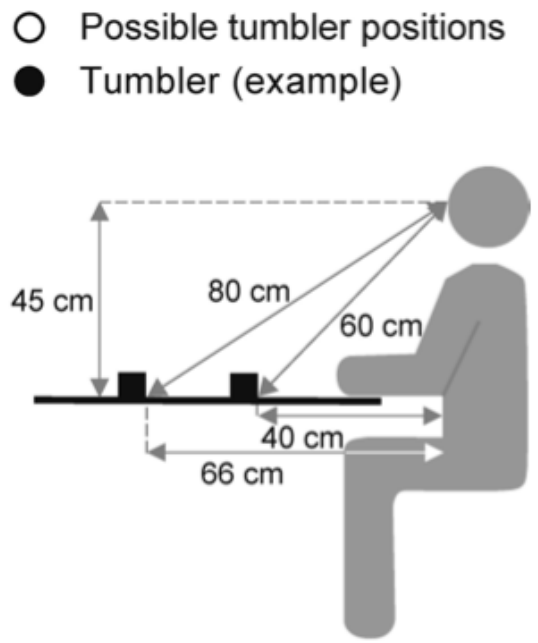
No test repetition was performed because the patient was unable to complete the task. He could not see anything and did not want to try again.

- patient unable to perceive any object under natural binocular or natural monocular conditions
- stimulated monocular: perceived presence of, located and touched the larger object in 92% (36/39); smaller object in 36% (16/45)
- **multivariate logistic regression analysis** for success
  (contrast, object size and task as dependent variables)
  - success rate depends on **object size**, with significantly higher rate of successful trials with the larger object ($P < 0.001$)
  - success rate similar for objects at different contrasts ($P = 0.29$)
  - success rate similar for different tasks, suggesting **coordination of motor system with percept** ($P = 0.79$)

Second test

- perceiving, counting and locating more than one object (two or three tumblers); determine number of objects placed on the table and point to them without touching
- objects shown at three contrasts





Second test: Results

Table 2 | Second test: counting and locating tumblers

Stimulus	Natural binocular: both eyes open without the light-stimulating goggles			Natural monocular: untreated eye covered, treated eye open without the light-stimulating goggles			Stimulated monocular: untreated eye covered, treated eye open and stimulated with the light-stimulating goggles		
	Perceive	Count	Locate	Perceive	Count	Locate	Perceive	Count	Locate
Tumblers, contrast = 40%	0/1	0/1	0/1	0/1	0/1	0/1	4/6	4/6	4/6
Tumblers, contrast = 55%	0/1	0/1	0/1	0/1	0/1	0/1	5/7	5/7	5/7
Tumblers, contrast = 100%	0/1	0/1	0/1	0/1	0/1	0/1	3/6	3/6	2/6

No test repetition was performed because the patient was unable to complete the task. He could not see anything and did not want to try again.

- patient unable to perceive objects under natural binocular or natural monocular conditions
- stimulated monocular condition: objects perceived in 63% of the trials (12/19), counted in 63% (12/19), located in 58% (11/19))
- success rate similar for objects at different contrasts (low=67% (12/18); medium=71% (15/21); high=**44%** (8/18)

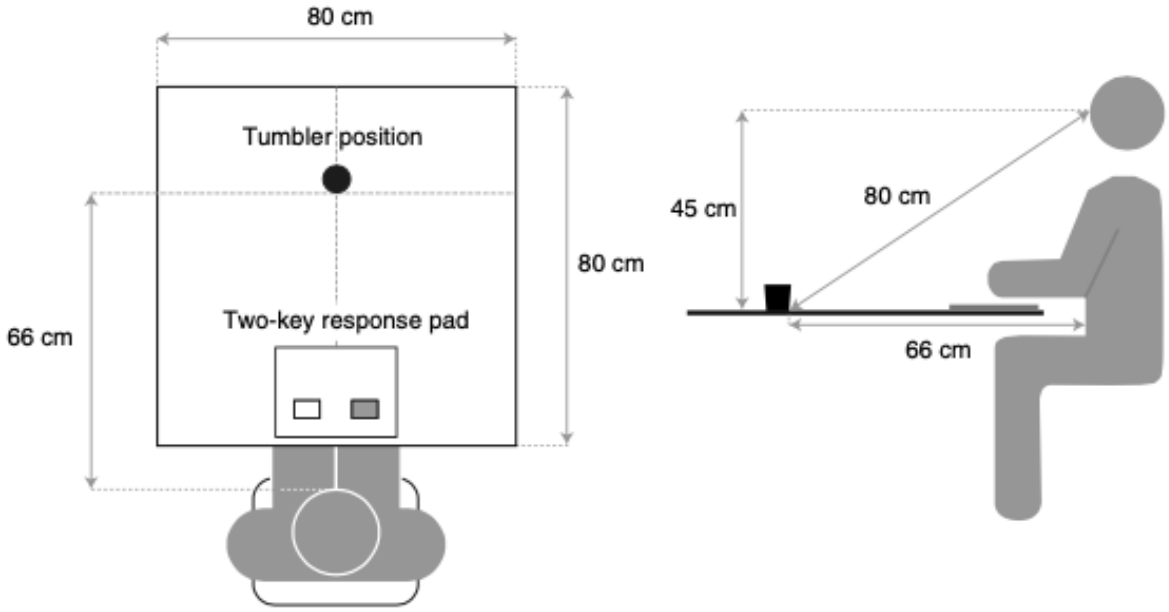
Neural correlates of vision recovery

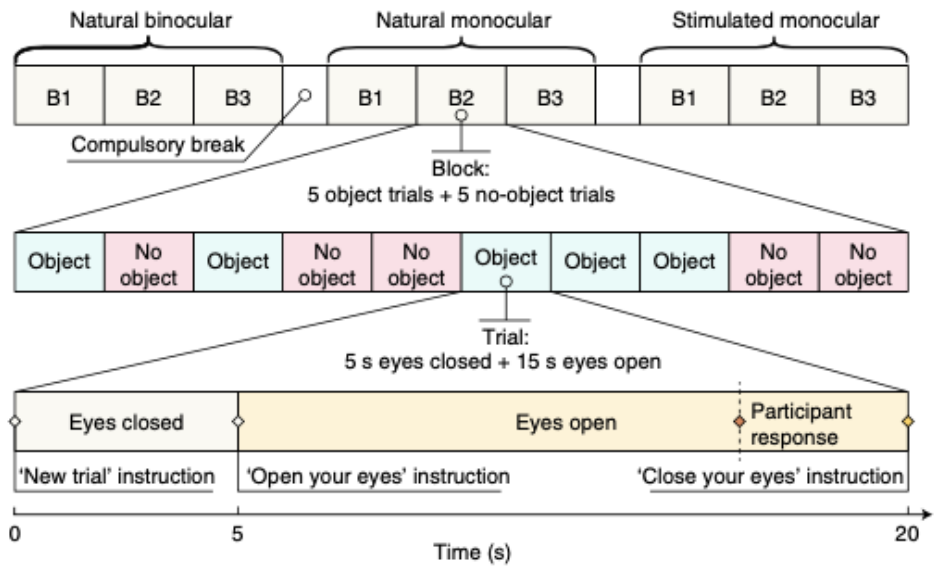
investigate link between partial vision recovery and neuronal activity: combine assessment of vision with extracranial multichannel electroencephalography (EEG)

fMRI impossible (metallic components of the goggles)

Third test

- EEG traces analyzed in eyes-open and eyes-closed states separately for 3 conditions (natural binocular, natural monocular and stimulated monocular)
- tumbler (6-cm diameter and 6-cm height) placed or not placed on a white table
- assess presence or absence (tumbler always placed at the same position)




Natural binocular
Natural monocular
Stimulated monocular
B1
B2
B3
B1
B2
B3
B1
B2
B3
Compulsory break
Block:
5 object trials + 5 no-object trials
Object
No object
Object
No object
No object
Object
Object
Object
No object
No object
Trial:
5 s eyes closed + 15 s eyes open
Eyes closed
Eyes open
Participant response
'New trial' instruction
'Open your eyes' instruction
'Close your eyes' instruction
0
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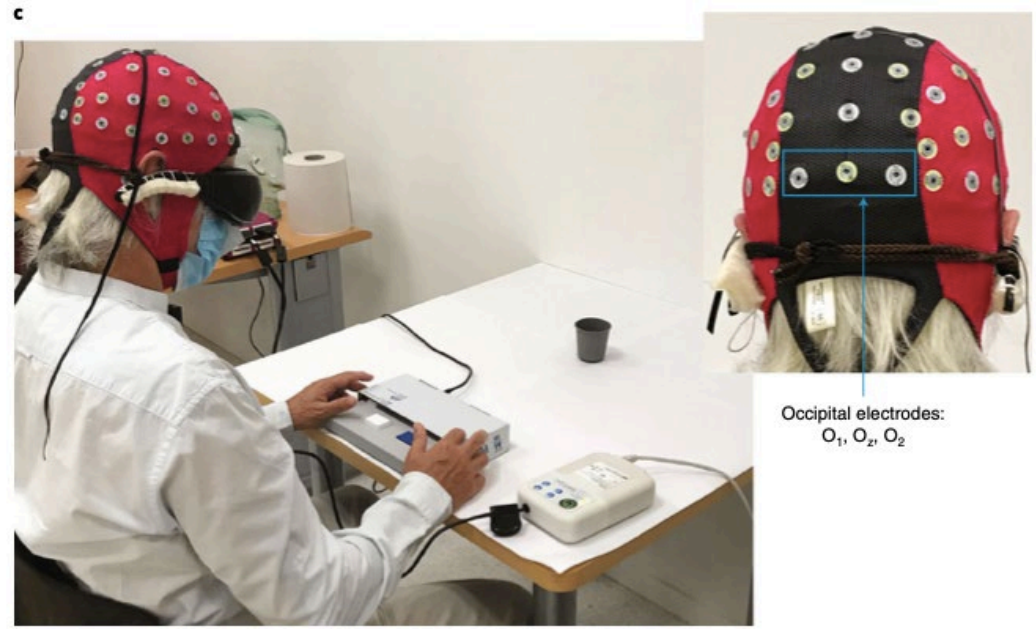
Time (s)


c
Occipital electrodes:
O_1, O_z, O_2

Third test: results

Table 3 | Third test: visual detection task (coupled with EEG recordings)

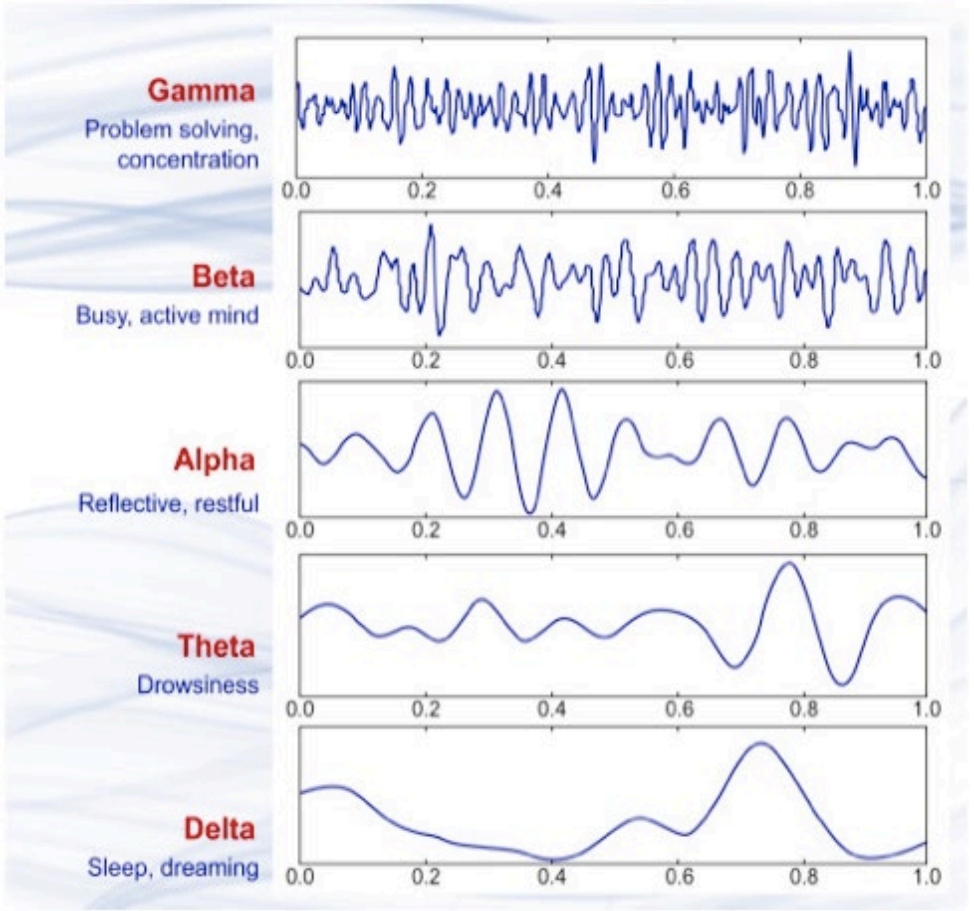
Trial	Natural binocular: both eyes open without the light-stimulating goggles			Natural monocular: untreated eye covered, treated eye open without the light-stimulating goggles			Stimulated monocular: untreated eye covered, treated eye open and stimulated with the light-stimulating goggles		
	Answer: yes object	Answer: no object	No answer	Answer: yes object	Answer: no object	No answer	Answer: yes object	Answer: no object	No answer
Object trial	3/30	0/30	27/30	2/30	2/30	26/30	21/32	2/32	9/32
No-object trial	3/30	1/30	26/30	2/30	1/30	27/30	3/31	5/31	23/31

multivariable logistic regression analysis for correct assessments
condition (stimulated versus natural) and object presence (yes or no) as the explanatory variables

- correct assessments significantly higher under stimulated monocular (**41% (26/63)**) than natural binocular or monocular conditions (**5.8% (7/120**) for both conditions; $P < 0.001$

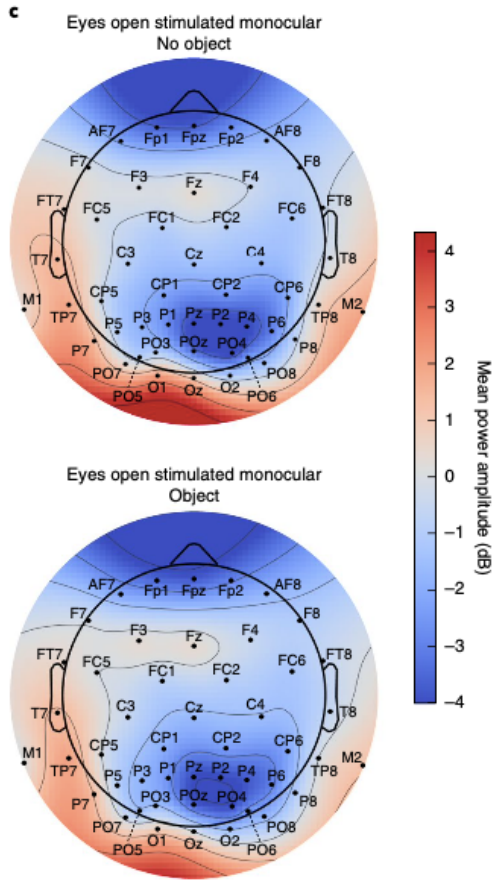
EEG recording

- localize the neuronal activity with the highest information content about the visual object across the cortex
  - Spectral analysis of recorded signals across 48 EEG channels in the **alpha-band (8–14Hz)** in the eyes-open stimulated monocular condition
- **Alpha waves** mainly in eyes closed state, relaxed, passive attention; associated with intensity of visual processing in the occipital region



EEG recording

- localize the neuronal activity with the highest information content about the visual object across the cortex
  - highest discriminant power for the object/no-object trial: **occipital cortex contralateral to monocular stimulation** (channels O1 and Oz at 14Hz)

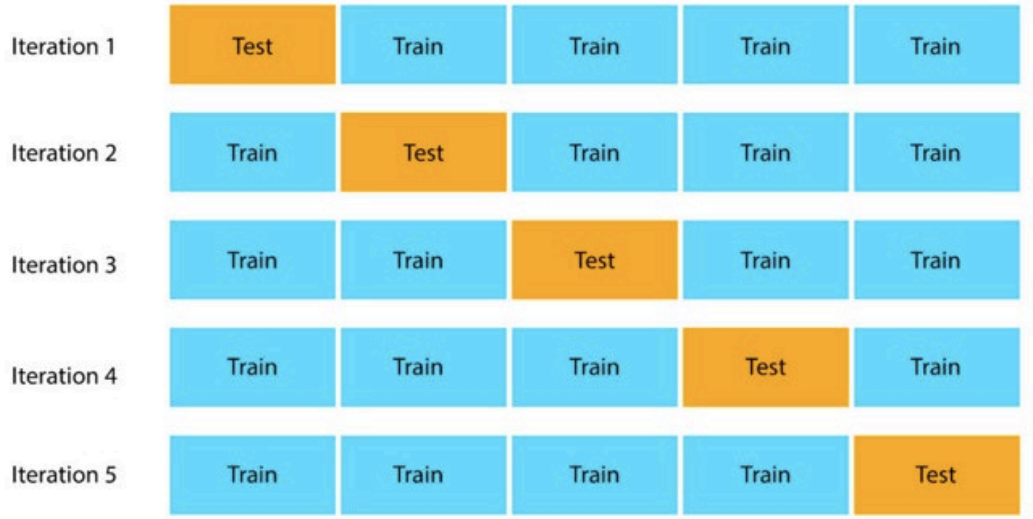


K **fold** cross validation

- statistical method to estimate the performance (accuracy) of machine learning models, protects against overfitting, especially when data is limited
- fixed number of folds (or partitions) of the data, run the analysis on each fold, average the overall error estimate
- data split into training data and testing data
- model should be tested on data it has not seen before
- guarantees that accuracy does not depend on the way the training and test set was picked

K **fold** cross validation

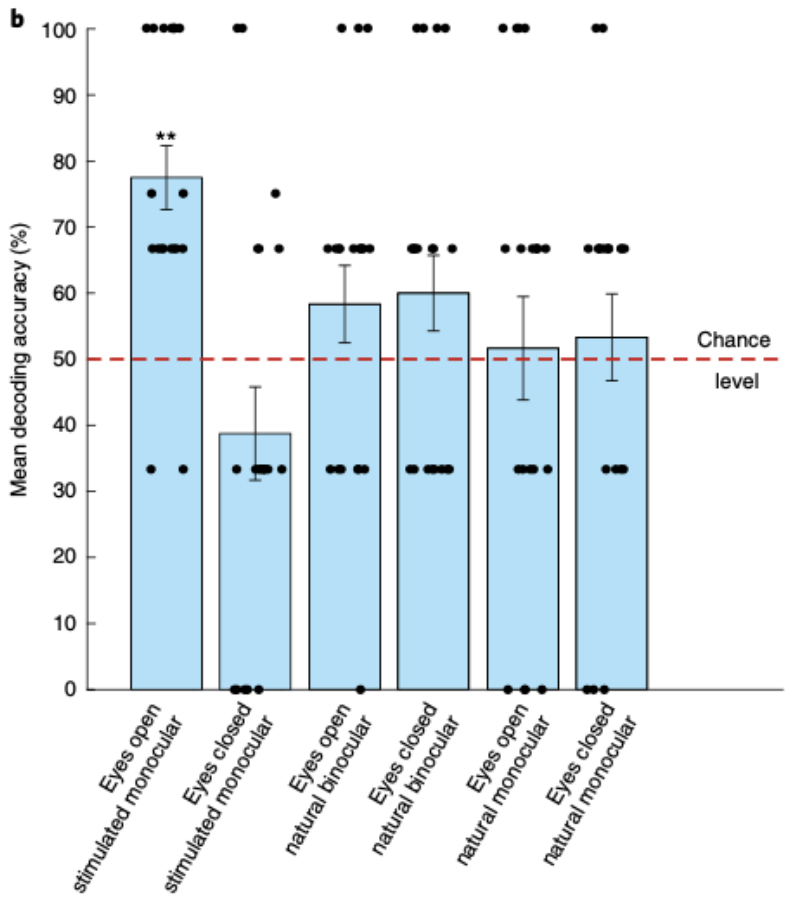
- dataset split into k number of folds (subsets)
- model built on k – 1 folds of the dataset, test the model to check the effectiveness for kth **fold**
- Repeated until each of the k-folds has served as the test set
- average of k recorded accuracy = cross-validation accuracy, performance metric for the model.
- every observation from the original dataset has the chance of appearing in training and test set
  - less biased model compare to other method, especially for limited input data
  - Disadvantage: training algorithm needs to be rerun k times (computational load)



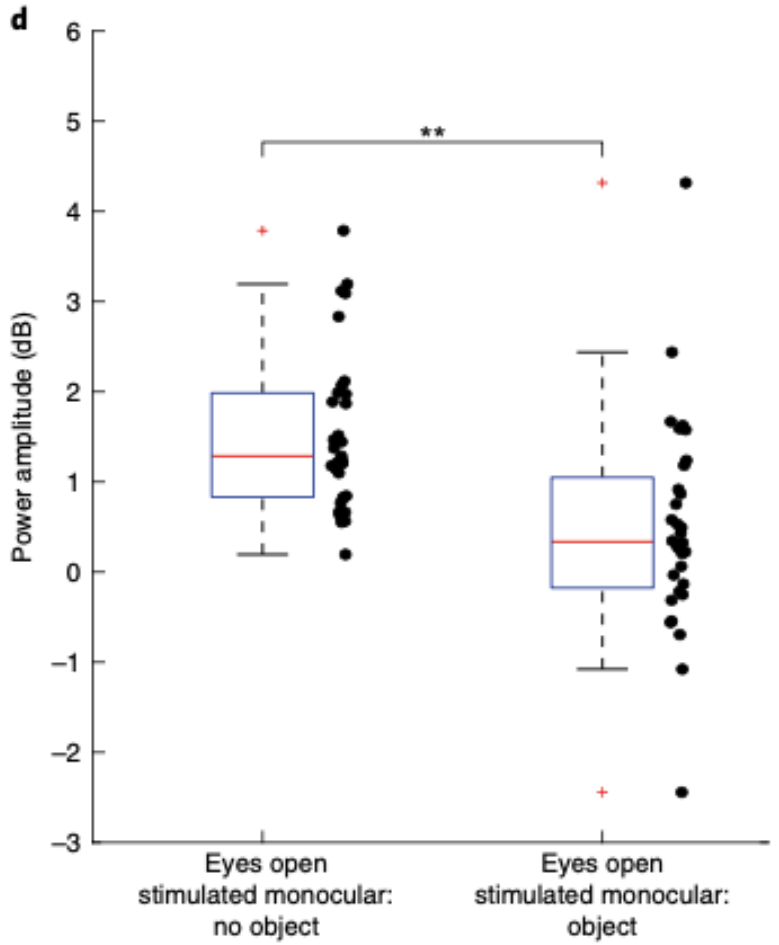
https://www.mygreatlearning.com/blog/cross-validation/?highlight=k%20fold

K **fold** cross validation

- algorithm trained with mean alpha-power amplitudes of occipital channels to discriminate object versus no-object trials
  - stimulated monocular mean accuracy: **78%** (±4.8)
  - at chance level when trained under eyes-closed state of stimulated monocular condition, both eyes-open and eyes-closed states (natural binocular and natural monocular conditions)



Object-triggered optogenetic stimulation: significant power **decrease (desynchronization) of occipital 14-Hz alpha oscillations**



Discussion

Main conclusions

- first evidence that injection of an optogenetic sensor-expressing gene therapy vector combined with wearing of light-stimulating goggles can partially restore visual function in a patient with RP with a visual acuity of only light perception
- visual process leading to the percept effective enough to enable orientation toward the object and reaching for it
- gain in visual function stable over a period of 5 months (interval between test 1/2 and 3)
- EEG recording of occipital cortex signals modulated by presence/absence of a visual object
  (neurophysiological confirmation of the individual's partially recovered visual perception?)

Observations

Head-scanning strategy

- patient adopted a **head-scanning strategy** when wearing the goggles to detect the presence of objects during the visual tests
  - field of optogenetic activation too small to detect objects not aligned with the camera center
  - small area of field of optogenetic stimulation (region of optogenetic protein expression in human retina estimated to be 2.5 mm diameter based on studies in NHPs; surface of human retina: 1,094 mm^2)

Vertical vibrations

- patient reported '**vertical vibrations**' when perceiving an object (not reported before the injection with goggles); optogenetic activation likely responsible for phenomenon
- Hypothesis: vibrations due to event-based camera (localized light pulses at each pixel where changes in contrast are detected)
- synchronized light pulses sent to the eye, might be perceived as vibrations
- why vertical?

Benefit in daily life?

- tests performed in an indoor laboratory
- locomotion outside on the street: patient spontaneously reported identifying crosswalks, could count the number of white stripes
- improvement in daily visual activities (detecting a plate, mug or phone, finding a piece of furniture in a room, detecting a door in a corridor; **only** when using the goggles)

"treatment led to a level of visual recovery likely to be of meaningful benefit in daily life"

https://static-content.springer.com/esm/art%3A10.1038%2Fs41591-021-01351-4/MediaObjects/41591_2021_1351_MOESM4_ESM.mp4

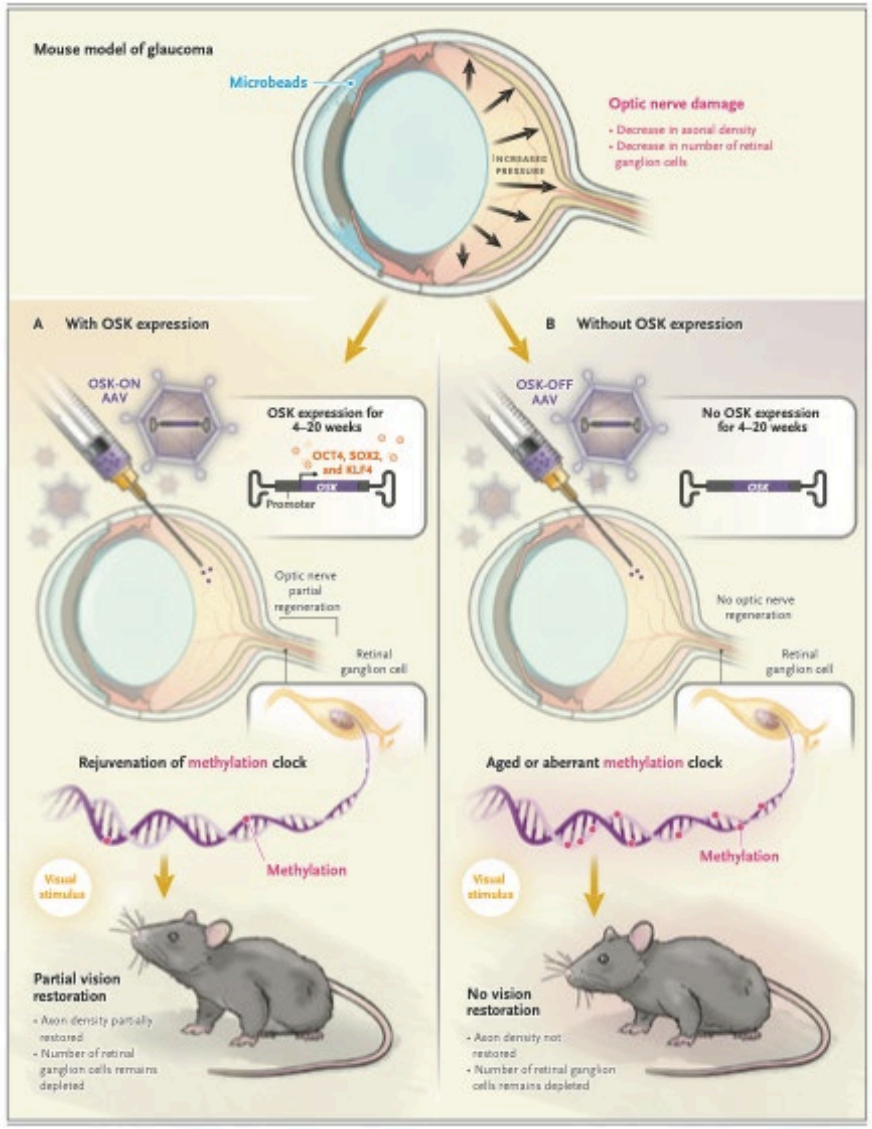
Outlook

- tdTomato encoded by the injected vector could be visualized by a scanning laser ophthalmoscope
- not yet approved for clinical use
- In case of approval: direct visualization of cells expressing ChrimsonR-tdTomato
  - useful to monitor vector transduction and individually tailor size and location of the light beam projected by the device
- Dose escalation

Thank you for your attention!

Questions?

Alternative approaches



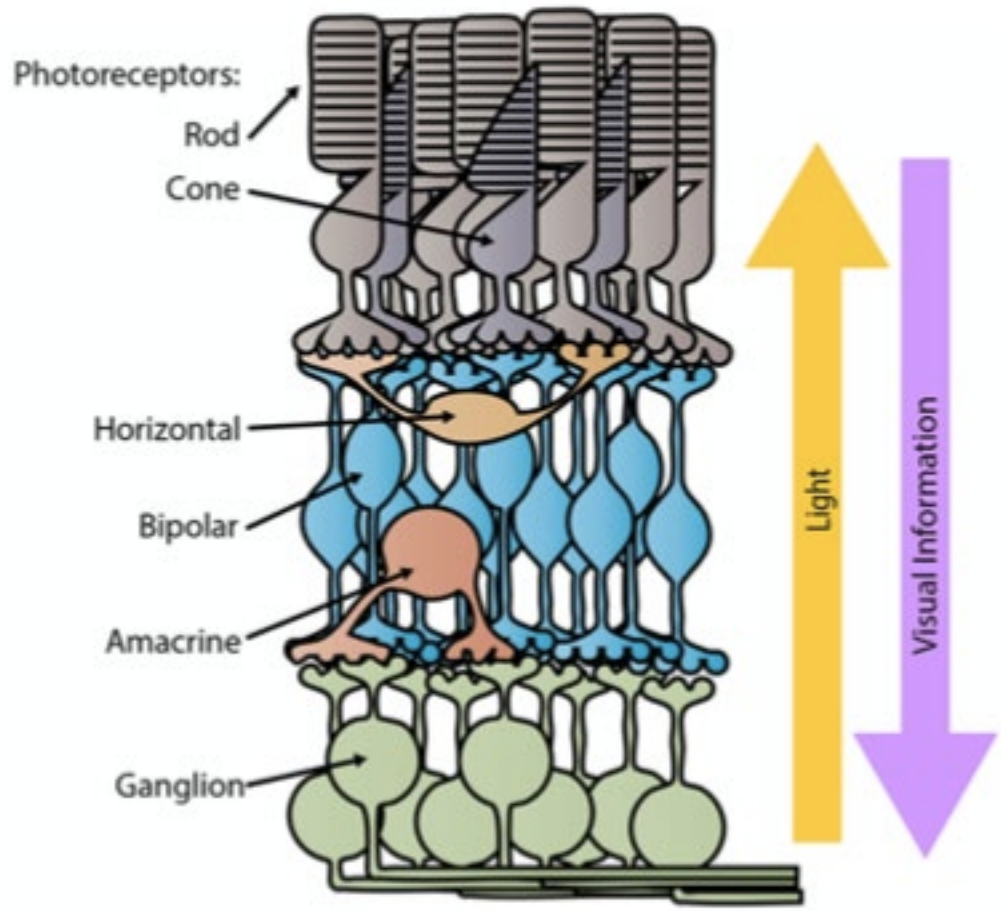
Contrast

- **Michelson contrast**:
  $(I_{max} - I_{min})/(I_{max} + I_{min})$,
  where I_{max} is the luminance intensity of the table and I_{min} that of the object.

- **Luminance** is a photometric measure of the luminous intensity (in Watt) per unit area of light travelling in a given direction

Visus			Winkel in Minuten	Besonderheit
Metrisch	Dezimal	Snellen		
6/3	2,0	20/10	0,5′	
6/4,5	1,33	20/15	0,75′	
6/6	**1,0**	**20/20**	1′	Norm-Wert
6/7,5	0,8	20/25	1,25′	
6/9	0,67	20/30	1,5′	
6/12	0,5	20/40	2′	
6/15	0,4	20/50	2,5′	
6/30	0,2	20/100	5′	
6/60	0,1	20/200	10′	
6/120	**0,05**	**20/400**	**20′**	blind laut WHO
6/300	0,02	20/1000	50'	blind laut SGB

Im Zähler steht die Ist-Entfernung, also die Entfernung, aus der der Untersuchte das Sehzeichen erkennt. Im Nenner steht die Normentfernung, die Entfernung, bei der ein Mensch mit einer Sehschärfe von 1,0 dasselbe Sehzeichen erkennen könnte. Gemessen wird die Entfernung entweder im metrischen System oder bei Snellen im englischen *Foot*-Maß, die ineinander umgerechnet werden können. Häufig erfolgt die Angabe der Sehschärfe als Dezimalzahl.


Photoreceptors:
Rod
Cone
Horizontal
Bipolar
Amacrine
Ganglion
Light
Visual Information