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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data were collected using Sutterpatch v1.1.2 - 2.0.2 (Sutter Instrument, CA, USA), Olympus Fluoview v4.2.3.6 (Olympus Scientific Solutions), in vivo ERG data collected with Espion v6 (Diagnosys LLC, Lowell, MA, USA)
Data analysis	Analyses were carried out using Origin 2018 (OriginLab, MA, USA), image analyses were done in FIJI (ImageJ v1.53c), CMP registration was carried out using ir-tweak v1.4 (https://www.sci.utah.edu/download/ncrtoolset.html)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data that support the findings of this study are available at https://doi.org/10.12751/g-node.sayvud

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical sample size calculation was performed. Sample sizes were based on prior experience of suitable n numbers for ERG recordings in mice and a minimum of n of 3 animals for each condition (Vinberg et al. 2017, doi:10.7554/eLife.24550, Leinonen et al. 2020 eLife DOI: 10.7554/eLife.59422). CMP analyses sample size was a minimum of 3 animals for each condition based on prior experience (Marc et al. 1995, J Neurosci 15, 5106-5129). Human donor retina and macaque experiment sample sizes could not be predetermined and were based upon tissue and donor availability. We had 17 research donors, 3 donations after cardiac death, 5 donations after brain death, and 3 macaques included in this study.
Data exclusions	For human research donor maculae that did not respond, Rmax was set to 0 uV for analysis. When analyzing phototransduction parameters (e.g. time-to-peak and amplification constant) based on ex vivo ERG data, samples with Rmax < ~5uV were excluded due to low signal-to-noise-ratio. Two I1/2 outlier values from human retina samples (Supplementary Table 2) are reported but were excluded from mean calculations.
Replication	Retinal responses to different intensities of light were replicated by taking multiple responses from each retina to each light intensity measured while minimizing photoreceptor photopigment bleaching (minimum 3 repeats taken for the brightest intensity used, maximum of 10 for dim flash intensities). Responses from each retina replicated successfully and the average of these replicated responses was used for analyses. Each ex vivo ERG experiment was repeated in multiple animals (n numbers reported in figures, minimum of 3) across multiple different experimental days (between 6 - 11 days). IHC analyses was performed on multiple retinas (n >3) prepared on multiple days (2). Replication of results was successful for all mouse experiments. Almost every biological replicate in human experiments had a unique postmortem enculeation delay, i.e. we did not have biological replicates for every enucleation delay time point. For analysis of dominant times constant, kinetic and sensitivity parameters from organ donations after brain death we had at minimum of 2 biological replicates and minimum of 5 technical replicates (different tissue samples) for both macula and periphery.
Randomization	For analyses of pH and hypoxia effects an eye from each animal, of random sex, used was randomly allocated to the control or condition groups, the order each was recorded was alternated so that recording order was not a factor. For analyses of enucleation delay, each eye from each animal was allocated two time conditions randomly ranging between 0 min to 3h. For practical reasons the first eye recorded from each animal necessarily was assigned to the shorter enucleation delay.
Blinding	It was not possible to completely blind researchers to research donor enucleation delays since this information was provided by eye bank prior to acceptance of tissues. For experimental reasons it was not possible to blind investigators to postmortem delay time, pH or hypoxia condition or control groups during mouse ex and in vivo ERG experiments. However, investigators were blinded during analyses until summary statistics/condition group comparisons were carried out. For IHC and CMP acquisition and analyses investigators were blinded until summary statistics/condition group comparisons.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Anti-L-glutamate IgG (cat. no. E100R), anti-taurine IgG (cat. no. TT100R), anti-L-glutamine IgG (cat. no. Q100R), anti-GABA IgG (cat. no. YY100R), anti-glycine IgG (cat. no. G100R), Anti-glutathione IgG (cat. no. J100R) , Anti-L-aspartate IgG (cat. no. D100R), Anti-L-

arginine IgG (cat. no. R100R) from Signature immunologics. Anti-CRALBP IgG (RRID: AB_2314227) gift from Dr. Jack Saar, anti-rod opsin 1d4 IgG (RRID: AB_2315015) Gift of Dr. Robert Molday. Anti-GFAP IgG (cat. no. Z0334) Dako. Anti-Cone red/green Opsin IgG (cat. no. AB5405) EMD Millipore . Anti activated caspase 3 (cat. no. 559565) from BD Pharmingen.

Validation

CMP antibodies Anti-L-glutamate IgG (cat. no. E100R), anti-taurine IgG (cat. no. TT100R), anti-L-glutamine IgG (cat. no. Q100R), anti-GABA IgG (cat. no. YY100R), anti-glycine IgG (cat. no. G100R), Anti-glutathione IgG (cat. no. J100R) , Anti-L-aspartate IgG (cat. no. D100R), Anti-L-arginine IgG (cat. no. R100R) from Signature immunologics:
All antihapten IgGs from Signature Immunologics (Table) have been extensively characterized in prior publications (Marc et al., 1995; Marc, 1999; Marc and Cameron, 2002; Marc and Jones, 2002). Each IgG is an isotype (determined by affinity chromatography and immunoblotting) that are produced in rabbit hosts immunized with glutaraldehyde–amino acid conjugates to bovine serum albumin (BSA) as described in Marc et al. (1995). Five separate analysis characterized the specificity and detectability of each anti-hapten IgG: 1) dependence on target molecule trapping with glutaraldehyde; 2) immunodot assays against the cognate of small molecule–proteins; 3) binding curves on quantitative artificial antigen stacks; and 4) cluster analysis (Marc et al., 1995).

CMP antibodies Anti-CRALBP IgG (RRID: AB_2314227) gift from Dr. Jack Saar, anti-rod opsin 1d4 IgG (RRID: AB_2315015) Gift of Dr. Robert Molday. Anti-GFAP IgG (cat. no. Z0334) Dako. Anti-Cone red/green Opsin IgG (cat. no. AB5405) EMD Millipore:

Anti activated caspase 3 (cat. no. 559565) from BD Pharmingen:
Tested in mouse and human cells by Western blot and flow cytometry by manufacturer.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

C57Bl/6J males and females were obtained at >2 months from JAX laboratories. Gnat1-/- mice were bred in house on C57Bl6J background, males and females, >2 months. Mice were kept in standard housing with litter mates, provided with food and water ad libitum and maintained on a 12:12 (light-dark) cycle. Ambient temperature was maintained at 21 degrees celcius and humidity maintained at approximately 20%.

Wild animals

No wild animals were used in this study.

Field-collected samples

No field-collected sampled were used in this study.

Ethics oversight

Animal protocols were approved by the University of Utah Institutional Animal Care and Use Committee. All procedures related to human tissue procurement were carried out by representatives from the organ donor society Lifesharing, the Utah Lions Eye bank or the San Diego Eye bank, which are fully accredited for donor eligibility determination, recovery and distribution by the FDA, the Association of Organ Procurement Organizations (AOPO) and the Eye Bank of America Association. For these reasons, research was provided IRB exempt status by the University of Utah and Scripps Health (IRB #00106658 and #16-6781).

Note that full information on the approval of the study protocol must also be provided in the manuscript.