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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Figure 1: data analysis was performed with Axon pCLAMP 10 Software Suite | Molecular Devices Figure 3, Supplementary figures 5-7: data collection was performed with Axon pCLAMP 10 Software Suite Figure 3, Supplementary figure 5,7: images collection was performed with LAS AF 2.7
Data analysis	Figure 1: Clampfit 10, Molecular Devices and Origin 7 Figure 2: LSM510 Meta system and the images were analyzed and prepared using ImageJ software. Figure 3, Supplementary figures 5-7: data analysis was performed with Axon pCLAMP 10 Software Suite | Molecular Devices, Origin9.1 | OriginLab and GraphPad Prism7 SFig.8: GraphPad PRISM version 5.0, GraphPad, San Diego, CA

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	Figure 1- Patch clamp on cell cultures: we use as a sample size, usually between 6-10 cells for patch experiments. These numbers are adequate for the kind of measurements, based on the fact that the signal to noise ratio is very high (at least >4). Figure 2 ans Suppl. Figure 2: Staining of hippocampal primary cultures: No statistical analyses have been performed. With regards to the quantitative analysis of surface/total staining, cells were chosen randomly from four to eight different coverslips (two to three independent experiments) and representative images are shown. Figure 3, Supplementary Figures 5-7: Patch clamp experiments in DRN brain slices- Sample size is chosen according to the "sample size calculator" implemented in the software Sigma-plot 12.0 taking into account the minimal detectable difference in means, the expected standard deviation, the desired power (0.80), the alpha value (0.05) and the statistic test applied (t-student test or 1WAY ANOVA). Estimated values used in the calculator were taken from a previous study on pharmacogenetic manipulations of serotonergic neurons: "D.J. Urban et al. Neuropsychopharmacology (2016) doi:10.1038/npp.2015.293" Figure 4: Sample size is chosen according to our experience with behavioral experiments. Because genotyping was not known before behavioral testing we tested enough larvae to have at least n>10 for each condition. Figure 5: For in vivo pain studies, We used n=6 samples based on power analysis and previous experience. Supplementary Figure 8: In each experiment, fertilized eggs were collected and randomly distributed into several subgroups, to be injected with either GFP RNA or wt and mutated forms of BLINK RNAs. Sample size was thus varying depending on clutches of eggs (usually 30-60 per group). Sample size was defined based on our experience in order to have statistically relevant numbers of embryos, but also to avoid overcrowded Petri dishes for escape response assays in order to better detect individual responses.
Data exclusions	Figure 1: Patch experiments on cell cultures: The criteria for discarding cells was if seal resistance remained > 1 gigaOhm throughout the experiment or not. Figure 2: Staining of hippocampal primary cultures: we have excluded dead neuronal cells and cells showing a certain suffering due to the transfection. Figure 3, Supplementary figures 5-7: cells were excluded based when the access resistance changed >20%, as described in the "Materials and Methods" or when defined as statistical outliers according to the "Identify Outliers" implemented in Prism7.0 using the ROUT method. Figure 4, 5: No data exclusion Supplementary Figure 8: Embryos showing grossly abnormal morphology, due to unspecific developmental problems/poor egg quality were excluded from touch-response assays. GFP-injected embryos were used as a control for unspecific developmental problems: when control embryos were massively not responding to mechanical stimuli, the entire experiment was discarded.
Replication	Figure 1: Patch experiments on cell cultures were performed once or twice in a week, cells were patched 12-24h after (transient) transfection. Each condition/protocol was tested at least in 3 independent experiments, each time the number of cells tested was > 5, usually 10-15.

Staining of hippocampal primary cultures: not relevant to our study

Figure 3, Supplementary figures 5-7: data were collected from 3 to 8 animals for each condition to ensure biological reproducibility.

Figure 4: data were reproduced in three independent experiments giving always comparable results. Results of one experiments are reported.

Supplementary Figure 8: In vitro transcribed RNAs from each construct were injected several times in independent clutches of eggs. Inhibitory effect of blue light exposure on touch-evoked escape response of BLINK-injected embryos, as well as reversibility in the dark, were thus verified in several individuals derived from several clutches of eggs, injected with several batches of in vitro transcribed RNAs.

Randomization

Figure 1: Patch clamp experiments on cell cultures: not relevant as the operator cannot influence the outcome of the measurement
Figure 3, Supplementary figures 5-7: not relevant
Figure 4: not relevant to our study
Figure 5: For in vivo pain studies, rats were ramdomly assigned to treatments and groups

Supplementary Figure 8: Fertilized eggs were randomly distributed into groups prior to microinjection

Blinding

Figure 1:Patch clamp experiments on cell cultures: not relevant as the operator cannot influence the outcome of the measurement
Figure 2:Staining of hippocampal primary cultures: not relevant to our study
Figure 3, Supplementary figures 5-7: not relevant
Figure 4: The genotyping of each larva was determined after behavioral response was recorded. Genotyping and beahvioral recording were performed by two independent investigators
Figure 5: For in vivo pain studies, experimenter was blinded to the groups and treatments.

Supplementary Figure 8: Investigators were not blinded to group allocation. Assays were always performed first on control group (GFP-injected embryos), to monitor the quality of the clutches.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used

Figure 2-5: anti-BLINK2: 8D6, custom made monoclonal antibody
Figure3, Supplementary figure 5,7, anti-eGFP: antibody name ab13970, chicken polyclonal antibody anti eGFP
Figure 5: anti-GFP (Cat# AB3080, Millipore, Billerica, MA), anti-PGP9.5 (Cat# NB600-1160, Novus Biologicals, Littleton, CO)

Validation

8D6 was validated for the Kcv channel expressed in several organisms and on the native Kcv in the PBCV-1 virus (Romani et al, 2013 J Gen Virol. 2013 Nov; 94(Pt 11): 2549–2556. doi: 10.1099/vir.0.055251-0); 8D6 was further validated against BLINK1 expressed in HEK 293T cells (Cosentino et al., 2015, Science 348(6235):707-10. doi: 10.1126/science.aaa2787).
Other primary antibodies used in Fig.2 are commercial antibodies frequently used in the literature (e.g. GM130 see Saraceno et al. 2014, PSD-95 see Marcello et al., 2007)

anti-eGFP: commonly used antibody, used in more than 1000 published scientific journals (https://www.abcam.com/gfp-antibody-ab13970-references.html).

Anti-GFP and anti PGP9.5 were validated by the company and widely used in the litterature.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK 293 T: obtained from ATCC (RRID:CVCL_0063)

Cell line source(s)	COS7, obtained from Gerhard Thiel lab, TU-Darmstadt, originally bought by ATCC
Authentication	both lines were authenticated by ATCC
Mycoplasma contamination	HEK 293T : Tested negative for mycoplasma COS7: not tested
Commonly misidentified lines (See ICLAC register)	HEK293 cells (but not HEK293T) are listed in the ICLAC database for possible contamination by HeLa cells. We think that for our purposes, i.e. virus amplification and heterologous expression of a synthetic light-activated channel conductance, such a contamination should not matter.

Animals and other organisms

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

Laboratory animals	Figure 2: E18 embryos from Sprague–Dawley rats for primary hippocampal neuron cultures were used. All the experiments were approved by the Institutional Animal Care and Use Committee of University of Milan and by the Italian Ministry of Health (#326/2015). Figure 3, Supplementary figures 5-7: Mus Musculus, C57BL/6J, males, P45-P70 Figure 4: Experiments with zebrafish embryos/larvae were conducted within the first 5 days post fertilization, when zebrafish are not considered as animals yet and are thus not subject to the European or local directives on animal research. Figure 5: adult male Sprague–Dawley rats (250 g; Envigo)
Wild animals	No wild animals were used in this study
Field-collected samples	No field-collected samplese were used in this study