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TITLE: Repair of the Traumatically Injured Central Visual System by Interneuron Transplantation

PRINCIPAL INVESTIGATOR: Robert Hunt, PhD

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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Regents of the University
of California, Irvine
141 Innovation, Suite 250
Irvine, CA 92697-7600

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Fort Detrick, Maryland 21702-5012

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12. DISTRIBUTION / AVAILABILITY STATEMENT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In the first year of the award, we performed a comprehensive analysis of the anatomical and in vivo electrophysiological responses of V1 to central visual system TBI. These studies were published (Frankowski, Foik, et al., 2021) and form the basis from which we will test an interneuron cell therapy. Toward this goal, we performed initial cell transplants into V1 of control animals and 1 week after TBI. Transplanted cells display the expected characteristics, and we are currently evaluating their electrophysiological incorporation into the injured brain. During the next two years, we plan to continue progress toward developing interneuron cell therapy for V1 TBI. This work will provide new information about the plasticity of the injured visual system and advance our long-term goal of developing an interneuron cell therapy for TBI.

15. SUBJECT TERMS

Traumatic brain injury, vision, central visual system, interneuron, cell therapy, transplantation, circuit plasticity

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	24	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Traumatic injuries, such as those that occur on the battlefield, can result in permanent visual impairment, because neurons that die from brain injury cannot be regenerated. We propose studies to restore vision following traumatic brain injury (TBI) using a cell therapy approach. Based on findings from our lab and others that inhibitory interneurons are particularly vulnerable to TBI and play critical roles in visual circuit function, the planned cell therapy product is a progenitor population that develops into physiologically mature inhibitory neurons. This approach is builds off our recent work demonstrating that progenitors of inhibitory interneurons derived from the embryonic medial ganglionic eminence (MGE) migrate, integrate and restore inhibition in the injured adult brain. This is a robust property of MGE cells that makes them an ideal candidate for use in cell therapy. The proposed research will address the Vision Research Program Investigator-Initiated Research Award Focus Area of "Restoration of visual function after trauma-related vision loss or severe visual impairment." We will test the hypothesis that MGE cell transplantation results in widespread synaptic incorporation of functionally mature GABAergic interneurons that reconstruct visually-relevant circuits and restore long-lasting impairments in vision after visual cortex injury. Our aims are to (1) examine the integration of MGE cells into brain injured visual cortex and (2) assess the therapeutic potential of MGE cells in a mouse model of visual cortex TBI. We will transplant MGE progenitors into a mouse model of visual cortex injury at acute and chronic stages post-injury. In Aim 1, we will determine precisely where these cells integrate within brain injured visual circuits. Our approach includes (1) immunostaining to evaluate the survival, migration and cell types generated by cell grafts into V1; (2) viral tracing, iDISCO tissue clearing and whole brain light-sheet imaging to visualize the pre- and post-synaptic targets of grafted neurons and (3) a combination of whole-cell patch-clamp recordings and optogenetics in acute brain slices to determine input-output patterns of these cells within recipient brain circuits. In Aim 2, we will test whether MGE transplantation can correct long-lasting impairments in visual acuity or the responses of visual cortex neurons to a range of visual stimuli *in vivo*. Comparisons will be made between adult control and brain injured mice receiving cell grafts or media injections. Our work has the potential to allow injured soldiers to return to active service or return to normal civilian life.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Traumatic brain injury, vision, central visual system, interneuron, cell therapy, transplantation, circuit plasticity

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1: Immunohistochemical analysis of transplanted MGE cells	Months	% complete
Subtask 1: ACRURO and IACUC approvals	1	100%
Subtask 2: Immunostaining to evaluate MGE cell survival / migration in brain injured V1 - up to 1 year after transplantation	1-24	33%
Subtask 3: Immunostaining to evaluate markers expressed by transplanted MGE cells	1-24	33%
Major Task 2: Visualization of local and long-distance connections	6-24	
Subtask 1: Immunostaining to evaluate post-synaptic targets of transplanted interneurons in control and brain injured V1	6-24	0%
Subtask 2: Viral tracing to evaluate pre-synaptic targets of transplanted interneurons in control and brain injured V1	6-24	0%
Major Task 3: Electrophysiological analysis	9-24	
Subtask 1: Current-clamp recordings from transplanted interneurons	9-24	5%
Subtask 2: Voltage-clamp recordings from host neurons	9-24	5%
Subtask 3: Current-clamp recordings from transplanted interneurons; Voltage-clamp recordings from host neurons	9-24	5%

Major Task 4: Behavioral assessment of visual acuity **Hypothesis:** MGE-derived interneurons correct long-term visual deficits in adult mice following CCI injury	12-36	
Subtask 1: Behavioral assessment of visual acuity in MGE-grafted mice	12-36	10%
Subtask 2: Confirm the presence of GFP cells in MGE-grafted mice.	18-36	0%
Major Task 5: In vivo electrophysiology	12-36	
Subtask 1: Use *in vivo* electrophysiology to test if MGE transplantation restores responses to visual stimuli	12-36	20%
Subtask 2: Use *in vivo* electrophysiology to test effect of MGE transplantation on center-surround responses	12-36	0%
Subtask 3: Confirm the presence of GFP cells in MGE-grafted mice.	18-36	0%

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities completed during year 1:

1. All protocols necessary to carry on the proposed projects were approved

2. We optimized the cortical impact to produce only mild TBI in V1, ensuring reproducibility of lesion size, location, etc.

3. We performed transgenic animal croses and established breeding colonies for the proposed MGE transplant studies.

4. We purchased and assembled a visual acuity behavior assay to test vision after TBI and after MGE transplantation.

5. We performed immunostaining experiments to test survival, migration and cell phenotype of MGE grafted cells in brain injured mice (outlined in Aim 1 of the proposal).

6. We initiated patch-clamp electrophysiology studies (outlined in Aim 1 of the proposal) to test electrophysiologyical phenotype of the transplanted cells and to test whether MGE transplantation increases inhibition after V1 TBI.

7. We published a paper describing in detail the histological and in vivo electrophysiological repsonses of V1 to mild TBI (Frankowski, Foik, Communications Biology, 2021). Experiments were performed at acute (0.5 month) and chronic (3 month) time points.

2) Specific objectives

1. Determine if MGE cells survive, migrate and mature into inhibitory interneurons (Aim 1, Exp 1)
    a. This objective was accomplished for acute transplants, and we are analyzing the data. We have generated brain injured mice for transplants during the chronic period, but these experiments are still underway.
2. Evaluate the electrophyiological integration of MGE transplanted cells
    a. This objective has not been completed. Experiments have been initiated and are expected to take two years to complete.

3) Significant results or key outcomes

1. We used anatomy and in vivo electrophysiological recordings in adult mice to quantify neuron responses to visual stimuli two weeks and three months after mild controlled cortical impact injury to primary visual cortex (V1). We found that V1 remained largely intact in brain-injured mice, but there was ~35% reduction in the number of neurons after TBI. Inhibitory cells were more broadly impacted than excitatory neurons. V1 neurons showed dramatically reduced activity, impaired responses to visual stimuli and weaker size selectivity and orientation tuning in vivo. Our results show a single, mild contusion injury produces profound and long-lasting impairments in the way V1 neurons encode visual input. These findings provide initial insight into cortical circuit dysfunction following central visual system neurotrauma and form the basis from which we will now test the effect of MGE tranpslantation. This work was published in Communications Biology.

2. We performed initial MGE transplants into V1 of control animals and 1 week after TBI. MGE cells dispersed throughout V1, migrating ~1.5mm in all directions from the injection site. Survival is ~15% at 7 days after transplantation and ~10% at 30 days after transplantation. MGE grafted cells expressed PV and SST, consistent with a MGE cell phyenotype, and did not express VIP (CGE phenotype).

4) Other achievements

1. Results from this award were presented at the following conferences:
    a. Tierno A, Frankowski JC, Foik AT, Machhor JR, Lyon DC, Hunt RF (2021) Traumatic brain injury to primary visual cortex produces long-lasting circuit dysfunction. **Society for Neuroscience Abstracts**

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

1. **Structured one-on-one training with collaborators at UCI:** Lab members recieved joint mentorship and training in the Hunt and Lyon labs through one-on-one mentorship, joint lab meetings, etc. These on-going interactions have been extremely valuable as they provide a forum for lab members to discuss project progress and exchange technical knowledge within our collaborative group at UCI.

2. **Seminars at UCI:** Our laboratories actively participate in the UCI Center for Translational Vision Research, Epilepsy Research Center as well as the Stem Cell Research Center. Each center hosts a seminar series featuring outside speakers. As a faculty member in each of these programs, I strongly encourage lab participation and attendance at these meetings. David Lyon presented our research as an invited talk in the 8th Annual Bench-to-Bedside Research Symposium for the Gavin Herbert Eye Institute (June, 2022). The Department of Anatomy & Neurobiology also hosts a seminar series and a monthly Progress in Neuroscience series in which lab members presented the funded research. In these meetings, postdocs and graduate students in the department present their unpublished research in a journal club format. These events have been an excellent opportunity to interact with other faculty / laboratories, present original data and discuss shared research interests.

3. **Responsible Conduct of Research Lecture:** All lab members attended our research ethics course sponsored by the UCI School of Medicine in the Spring Quarter. I participated as a faculty instructor in this lecture series (and every year).

4. **Conferences:** Alexa Tierno presented our initial results at the Society for Neuroscience annual meeting in Nov 2021.

5. **Individual Development Plans (IDPs):** All trainees in my laboratory (postdocs, graduate students, undergraduates and technicians) are required to complete an IDP based on the AAAS myIDP (http://myidp.sciencecareers.org/). I meet one-on-one with each member of the lab (from undergraduate to postdoc) in Septameber every year to discuss career/research progress and future goals. Graduate students also complete an annual IDP as part of their training in the Neuroscience Graduate Program.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

We presented our research to the broader neuroscience community through scientific publication (e.g., Frankowski, Foik, 2021) and national conferences (e.g., SfN). We also presented our research locally to neuroscientists, clinicians and the public through lectures (e.g., Bench-to-Bedside symposium, departmental talks, etc.)

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the next year, I plan to fully complete the following:
1. Major Task 1 – immunostainin analysis of cell phenotype, survival and migration after transplantation into brain injured animals at the acute and chronic phase after injury
2. Major Task 3, Subtask 1 & 2 – basic electrophysiological characterization of transplanted and host cells

I anticipate we will make progress on, but will not complete, the following:
3. Major Task 2 – circuit tracing. The mouse colonies have been established, but the analysis is complex. This will take two years to complete.
4. Major Task 3, Subtask 3 – optogenetics
5. Major Tasks 4 and 5 – in vivo analysis of MGE grafted animals. This is Aim 2 of the proposal. These experiments should be underway, but the analysis is complex and will take two years to complete these studies.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal

disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

This proposal directly addresses circuit plasticity of the central visual system after TBI. Our initial results from this work were recently published (Frankowski, Foik, Communications Biology, 2021). This is the first comprehensive report of acute and long-term deficits that emerge after brain injury to V1. As we are essentially the only group studying central visual system TBI and repair, we consider this work foundational to our understanding of how V1 neurotrauma affects visual function.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Long-term, our studies will directly test important hypotheses about the role of inhibition in visual system plasticity that have been proposed in the literature, but have not yet been tested. For example, one hypothesis suggests inhibitory interneuron transplantation works via activating plasticizer molecules in the host brain whereas other studies (including our own prior work) demonstrate electrophysiological integration of the new neurons into injured brain circuits is important.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects
NA

Significant changes in use or care of vertebrate animals
None

Significant changes in use of biohazards and/or select agents
None

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Frankowski JC, Foik AT, Tierno A, Machhor JR, Lyon DC, Hunt RF (2021) Traumatic brain injury to primary visual cortex produces long-lasting circuit dysfunction. **Communications Biology**. 4(1):1297.

  Yes – acknowledgement of federal support

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.Identify for each one-time publication: author(s); title; editor; title*

of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

None

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Tierno A, Frankowski JC, Foik AT, Machhor JR, Lyon DC, Hunt RF (2021) Traumatic brain injury to primary visual cortex produces long-lasting circuit dysfunction. **Society for Neuroscience Abstracts**

Yes – acknowledgement of federal support

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  NA

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  NA

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  NA

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*

- *other.*

NA

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*Robert Hunt, PhD*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-4490-8718*
Nearest person month worked:	*1.2*
Contribution to Project:	*Dr. Hunt has performed work in the area of MGE cell transplants, anatomy and electrophysiology.*

Name:	*David Lyon, PhD*
Project Role:	*Co-PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1.2*
Contribution to Project:	*Dr. Lyon has performed work in the area of electrophysiology and data analysis.*

Name:	*Jae Hyouk Choi, PhD*
Project Role:	*Postdoc*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3.0*
Contribution to Project:	*Dr. Choi has performed work in the area of TBI surgeries and slice electrophysiology.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were

involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx)should be updated and submitted with attachments.*

Nothing to report

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

communications biology

ARTICLE

https://doi.org/10.1038/s42003-021-02808-5 OPEN

Traumatic brain injury to primary visual cortex produces long-lasting circuit dysfunction

Jan C. Frankowski[1,3], Andrzej T. Foik[2,3], Alexa Tierno[1], Jiana R. Machhor[1], David C. Lyon[1] & Robert F. Hunt[1✉]

Primary sensory areas of the mammalian neocortex have a remarkable degree of plasticity, allowing neural circuits to adapt to dynamic environments. However, little is known about the effects of traumatic brain injury on visual circuit function. Here we used anatomy and in vivo electrophysiological recordings in adult mice to quantify neuron responses to visual stimuli two weeks and three months after mild controlled cortical impact injury to primary visual cortex (V1). We found that, although V1 remained largely intact in brain-injured mice, there was ~35% reduction in the number of neurons that affected inhibitory cells more broadly than excitatory neurons. V1 neurons showed dramatically reduced activity, impaired responses to visual stimuli and weaker size selectivity and orientation tuning in vivo. Our results show a single, mild contusion injury produces profound and long-lasting impairments in the way V1 neurons encode visual input. These findings provide initial insight into cortical circuit dysfunction following central visual system neurotrauma.

[1] Department of Anatomy & Neurobiology, University of California, Irvine, CA 92697, USA. [2] Ophthalmic Biology Group, International Centre for Translational Eye Research, Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland. [3] These authors contributed equally: Jan C. Frankowski, Andrzej T. Foik. ✉email: robert.hunt@uci.edu

Posterior impact injuries to the occipital cortex are extremely common in human. Traumatic brain injury (TBI) can lead to long-lasting visual impairments, such as visual acuity and field loss, binocular dysfunction, and spatial perceptual deficits[1–3], and as many as 75% of military Service members live with permanent visual dysfunction or cortical blindness resulting from a TBI[3]. Restrictive lesions applied to the visual cortex have been shown to trigger cortical plasticity and functional disturbances[4–6]. However, TBI involves mechanical brain damage and a wide range of cortical network abnormalities including cell death, inflammation, and synaptic circuit remodeling[7]. There is essentially nothing known about how visual circuit function is affected by TBI.

Following TBI in human, histological studies have documented a reduction in the number of neurons in the hippocampus[8] and neocortex[9]. In nonhuman animal models, TBI produces region- and subtype-specific reductions of neurons in various brain areas[10–21], dramatic circuit rewiring ([22–31]), and a loss of inhibition that does not recover with time[20,21,27,32–39]. However, nearly all of the information about neocortical responses to TBI comes from studies evaluating somatosensory, motor, or frontal cortex. Each of these areas receives numerous intra- and inter-hemispheric inputs from throughout the topographic map[40–42], whereas callosal connectivity of the visual cortex is limited to the vertical meridian representation along the V1 border[43,44]. Therefore, a deeper understanding of functional disturbances in the brain-injured visual cortex is important, because it has the potential to provide a rational basis for the development of circuit-level therapies for visual cortex injury.

To produce central visual system TBI in adult mice, we applied a focal controlled cortical impact (CCI) injury to the primary visual cortex (V1). We show that although mild contusion injury did not produce a sizable lesion, there was a subtype- and layer-specific loss of neurons in the brain-injured V1. Then, using in vivo electrophysiological recordings of visually evoked responses, we found that mild contusion injury chronically impairs the response of V1 neurons to a variety of visual stimuli. These findings suggest there are profound long-lasting impairments in visual circuit function that result from a single, mild contusive injury to the central visual system. As an initial characterization of central visual system neurotrauma, our results also lay the foundation for future mechanistic investigations of altered cortical network activity and preclinical studies to restore circuit function in the traumatically injured visual cortex.

Results

Occipital CCI produces a mild contusion in V1. To evaluate the effect of a single, mild contusion injury to the central visual system, we delivered mild CCI injury centered over the rostral end of V1 in young-adult mice at P60 (Supplementary Fig. 1). We selected CCI as a model, because the injury is highly reproducible from animal to animal, reliably recapitulates structural and functional deficits of TBI and focal contusion injuries are among the most common posterior impact injuries observed in human[1–3]. In all CCI-injured animals ($N = 7$ mice), the lesion consisted of mild tissue compression that was restricted to superficial layers of the cortex at the injury epicenter (Supplementary Fig. 2).

To define the lesion location, we examined glial responses in V1 following mild CCI injury (Fig. 1a). To do this, we performed an immunostaining analysis at 0.5 months and 3 months after injury for glial fibrillary acidic protein (GFAP), a marker of astrocytes, and ionizing calcium-binding adaptor molecule 1 (IBA1), a marker of activated microglia. In brain-injured animals, the impact site could be clearly identified by a dense pattern of GFAP and IBA1 staining in V1 ipsilateral to the injury. A significant increase in GFAP expression was found in V1 surrounding the injury at 0.5 months, as compared to uninjured controls, sham animals that received a craniotomy but no injury and contralateral tissue sections (Supplementary Fig. 3), and it remained significantly elevated 3 months post-CCI (Fig. 1b). IBA1 immunostaining was also significantly increased ipsilateral to the injury, but only at 0.5 months after injury (Fig. 1c). Uninjured and sham controls did not have an identifiable cortical lesion in any animal.

At 0.5 months post-CCI, a time point when lesion volume is considered to be largely stable[15,45], there was no significant difference in cortical volume between uninjured control and brain-injured littermates (TBI: 96 ± 3%, sham: 102 ± 2%, compared to 99 ± 1% in uninjured control; $P = 0.15$; one-way ANOVA; $N = 4$–6 mice per group; Fig. 1d). However, when we evaluated the thickness of cortical tissue remaining in the contused portion of the visual cortex, we found a 14% decrease in cortical thickness in brain-injured animals at the injury epicenter, as compared to controls (uninjured: 883 ± 25 µm, sham: 966 ± 58 µm, TBI: 760 ± 32 µm, $P = 0.048$; two-way rmANOVA; $N = 3$–4 mice per group; Fig. 1e). This difference was only observed at the injury epicenter (0 µm); no difference in cortical thickness was observed in tissue sections 300 and 600 µm caudal to the epicenter. We found a similar degree of mild tissue loss at 90d post-CCI (Fig. 1f, g). Thus, CCI produced a mild focal injury with minimal structural damage to V1.

Neuron loss after V1 injury. Next, we quantified neuron density in V1 using GAD67-GFP reporter mice that label nearly all GABAergic neurons[46]. Sections were immunostained for GFP to identify inhibitory interneurons and NEUN to identify putative excitatory neurons (i.e., NEUN-positive/GAD67-GFP-negative) (Fig. 2). At 0.5 months after TBI, we found a ~35% reduction in NEUN + /GAD67-GFP- cell density in V1 ipsilateral to the injury (Fig. 2a, b; Supplementary Data 1). The reduction in excitatory neurons was most profound at the injury epicenter (45% reduction after TBI) and rapidly decreased with distance away from the impact site (Fig. 2c). We also observed ~35% decrease in the overall density of GAD67-GFP+cells in V1 ipsilateral to the injury ($P = 1.07E-06$, TBI versus uninjured control, two-way ANOVA; Fig. 2d; Supplementary Data 1). However, unlike excitatory neurons, GFP + interneuron density was reduced by ~35% at each distance from the impact site (Fig. 2e). No change in cell density was observed in the contralateral hemisphere. These findings suggest mild contusion to the visual cortex produces substantial neuron loss in V1, and the loss of inhibitory neurons is more widespread than excitatory neurons.

To determine if post-traumatic neuron loss was layer-specific, we quantified neuron density in cortical layers I, II/III, IV, and V/VI of brain-injured and uninjured control littermates (Fig. 3; Supplementary Data 2). For this analysis, we fitted a random intercept mixed model for each cell type to account for the distance from the injury, layer, and treatment condition. We found that excitatory cell loss extended throughout the cortical column ipsilateral to the injury, with significant reductions in NEUN+/ GAD67-GFP- cells in cortical layers II/III, IV, and V/VI (Fig. 3b, f, j); no significant differences were found in layer I where excitatory neurons are rarely found. In contrast, GFP + inhibitory neuron density was most profoundly affected in superficial layers, with significant reductions in GAD67-GFP + neurons in layers I-IV (Fig. 3c, g, k). However, no change in inhibitory neuron density was observed in layers V/VI. Despite these cell-type specific changes in cell density, the ratio of excitatory to inhibitory neurons did not change in any layer of V1 (Fig. 3d, h, l). We conclude that there are subtype- and layer-

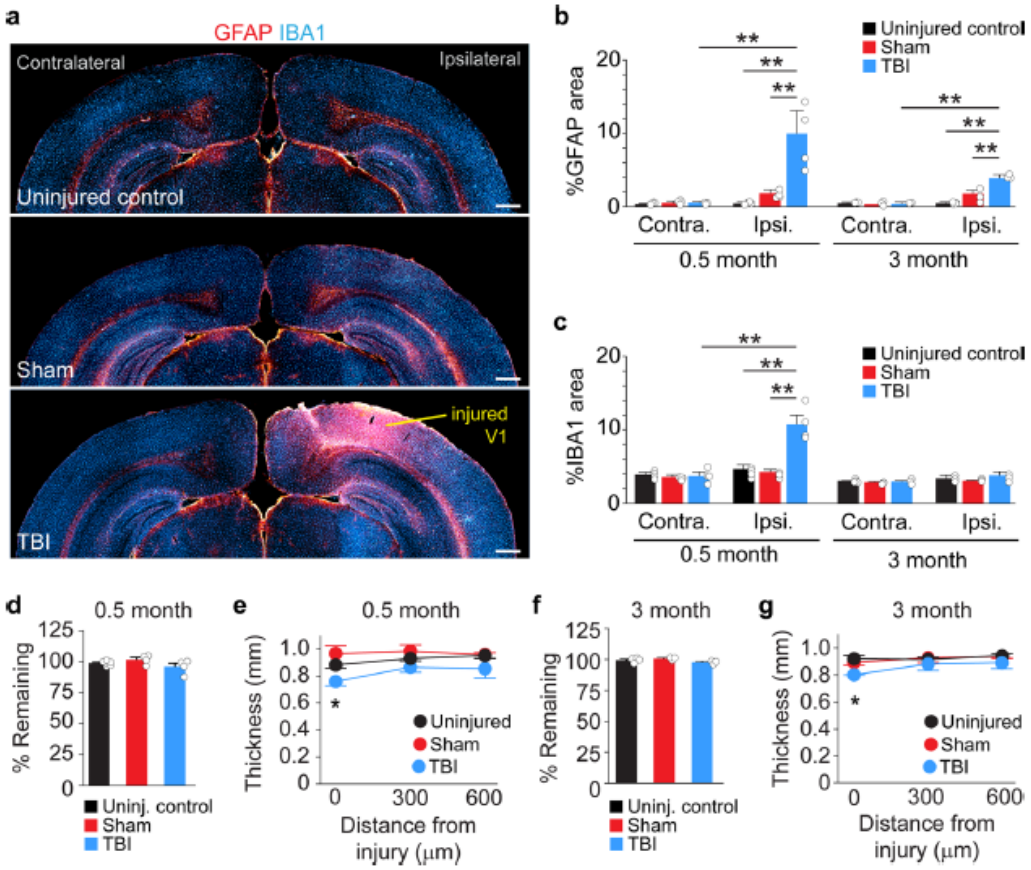

Fig. 1 Visual cortex TBI produces a mild cortical lesion. a Coronal sections of GFAP (red) and IBA1 (blue) labeling in a control animal and 0.5 months after sham or CCI injury. **b** Quantification of GFAP expression in V1 at 0.5 and 3 months postinjury. $^{**}P = 2.9E\text{-}07$, ipsilateral control versus ipsilateral TBI; $^{**}P = 1.3E\text{-}05$, ipsilateral sham versus ipsilateral TBI; $^{**}P = 1.3E\text{-}06$, ipsilateral TBI versus contralateral TBI at 0.5 months; $^{**}P = 1.3E\text{-}06$, ipsilateral control versus ipsilateral TBI; $^{**}P = 8.7E\text{-}05$, ipsilateral sham versus ipsilateral TBI; $^{**}P = 6.0E\text{-}06$, ipsilateral TBI versus contralateral TBI at 3 months two-way ANOVA with Tukey's post hoc test, $N = 3$–6 mice per group. **c** Quantification of IBA1 expression in V1 at 0.5 and 3 months postinjury. $^{**}P = 2.4E\text{-}08$, ipsilateral control versus ipsilateral TBI, $^{**}P = 2.9E\text{-}08$, ipsilateral sham versus ipsilateral TBI, $^{**}P = 2.3E\text{-}08$, ipsilateral TBI versus contralateral TBI at 0.5 months; two-way ANOVA with Tukey's post hoc test, $N = 3$–6 mice per group. **d** Quantification of cortical tissue volume in control, sham, and CCI-injured mice 0.5 months post-CCI. **e** Average thickness of cortex with distance from the injury 0.5 months post-CCI. $^{*}P = 0.048$, Control versus TBI, two-way repeated-measures ANOVA with Tukey's post hoc test, $N = 4$–6 mice per group. **f** Quantification of cortical tissue volume in control, sham, and CCI-injured mice 3 months post-CCI. **g** Average cortex thickness with distance from the injury 3 months post-CCI. $^{*}P = 0.023$, Uninjured versus TBI, two-way repeated-measures ANOVA with Tukey's post hoc test, $N = 3$–4 mice per group. Scale bars, 500 μm; error bars, SEM.

specific differences in the degree and extent of neuron loss after visual cortex injury.

We next asked whether the loss of neurons at the injury site persisted long term. At 3 months after injury, NEUN+/GAD67-GFP- cell density remained reduced by 32% ipsilateral to the injury (Fig. 4a, b; Supplementary Data 3). GAD67-GFP+ interneuron density was also reduced 3 months post-CCI by 32% (Fig. 4a, c; Supplementary Data 3). These data reproduce our observations at 0.5 months and are consistent with a chronic loss of excitatory and inhibitory neurons after mild contusion injury to the visual cortex.

Early and long-term disruption of visually evoked responses after TBI. To evaluate the in vivo functional state of the visual cortex following TBI, we measured visually evoked potentials (VEPs) and single-unit responses to a range of stimuli across a wide extent of injured V1 at 0.5 and 3 months after injury (Figs. 5–7). First, we recorded VEPs in response to brief flashes of light. These local field potential responses represent the electrical response of a population of V1 neurons to light stimuli. Representative examples of flash-evoked responses are shown in individual animals (Fig. 5a), along with group averages (Fig. 5b). Compared to uninjured controls, evoked VEP amplitudes were significantly reduced by more than 80% in brain-injured mice (control: 277 ± 39 μV, 0.5 months after TBI: 24 ± 4 μV, 3 months after TBI: 53 ± 7 μV; $P = 9.95E\text{-}09$, Kruskal–Wallis H test; Fig. 5c), and response latencies rose to more than 60% longer (control: 88 ± 6 ms, 0.5 months after TBI: 146 ± 17 ms, 3 months after TBI: 100 ± 6 ms; $P = 0.02$, Kruskal–Wallis H test; Fig. 5d). Of note, response latencies between light flash and maximal response were longer only at 0.5 months after injury and were similar to controls at 3 months. At both time points, we found that wave profiles in the injured brain lacked a negative wave component normally present in deeper cortical layers (Supplementary Fig. 4).

Single-neuron responses to the same flashes of light were also measured (Fig. 6a; Supplementary Fig. 5). Average response profiles showed moderate to negligible activity at both 0.5 and

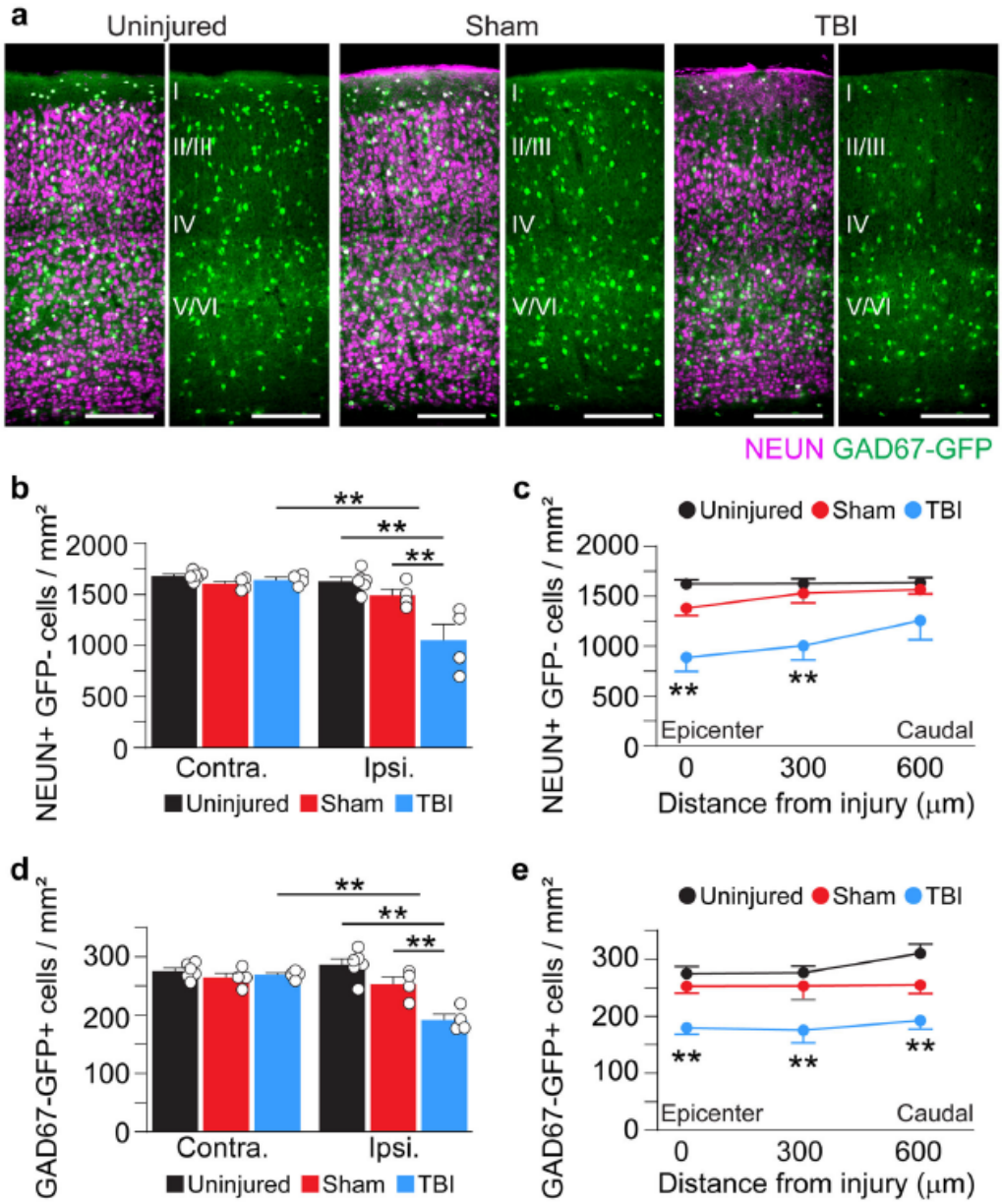

Fig. 2 Neuron loss in V1 0.5 months after TBI. a Coronal images of control, sham, and CCI-injured V1 labeled for NEUN (magenta) and GAD67-GFP (green). **b** Quantification of NEUN+/GFP- cell density in uninjured control, sham, and brain-injured mice 0.5 months after CCI. $^{**}P = 1.79E\text{-}05$, ipsilateral control versus ipsilateral TBI, $^{**}P = 1.77E\text{-}03$, ipsilateral sham versus ipsilateral TBI, $^{**}P = 4.56E\text{-}05$, ipsilateral TBI versus contralateral TBI; two-way ANOVA with Tukey's post hoc test, $N = 4$–6 mice per group. **c** NEUN+/GFP- cell density at 0–600 μm from the injury epicenter. $^{**}P = 7.86E\text{-}05$, control versus TBI (0 μm), $^{**}P = 1.01E\text{-}03$, control versus TBI (300 μm); two-way repeated-measures ANOVA with Tukey's post hoc test; N = 4–6 mice per group. **d** Quantification of GAD67-GFP+ cell density in uninjured control, sham, and brain-injured mice 0.5 months after CCI. $^{**}P = 1.07E\text{-}06$, ipsilateral control versus ipsilateral TBI, $^{**}P = 1.58E\text{-}03$, ipsilateral sham versus ipsilateral TBI, $^{**}P = 9.25E\text{-}05$, ipsilateral TBI versus contralateral TBI, two-way ANOVA with Tukey's post hoc test, $n = 4$–6 per group. **e** GAD67-GFP+ cell density at 0–600 μm from the injury epicenter. $^{**}P = 3.53E\text{-}03$, control versus TBI (0 μm), $^{**}P = 1.66E\text{-}03$, control versus TBI (300 μm), $^{**}P = 1.74E\text{-}04$, control versus TBI (600 μm); two-way repeated-measures ANOVA with Tukey's post hoc test; $N = 4$–6 mice per group. Scale bars, 500 μm; error bars, SEM. See Supplementary Data 1 for statistical analyses.

3 months, respectively, compared to the high average spike rate in control mice (Fig. 6b). After TBI, less than half of the isolated neurons were visually responsive (32% at 0.5 months; 49% at 3 months), compared to 90% of control V1 cells (Chi-square = 56.3, df = 2, $P = 5.94E\text{-}13$; Fig. 6c). Similarly, average peak firing rates were significantly lower in brain-injured V1 (control: 42.7 ± 5.7 spikes/s, compared to 5.2 ± 0.4 spikes/s 0.5 months after TBI and 9.9 ± 1.7 spikes/s 3 months after TBI; $P = 3.6E\text{-}20$, Kruskal–Wallis H test; Fig. 6d). Prior to stimulation, background activity was highest for the uninjured control group (7.6 ± 2.8 spikes/s) and included one outlier with a baseline firing rate over 150 spikes/second; whereas background activity for cells

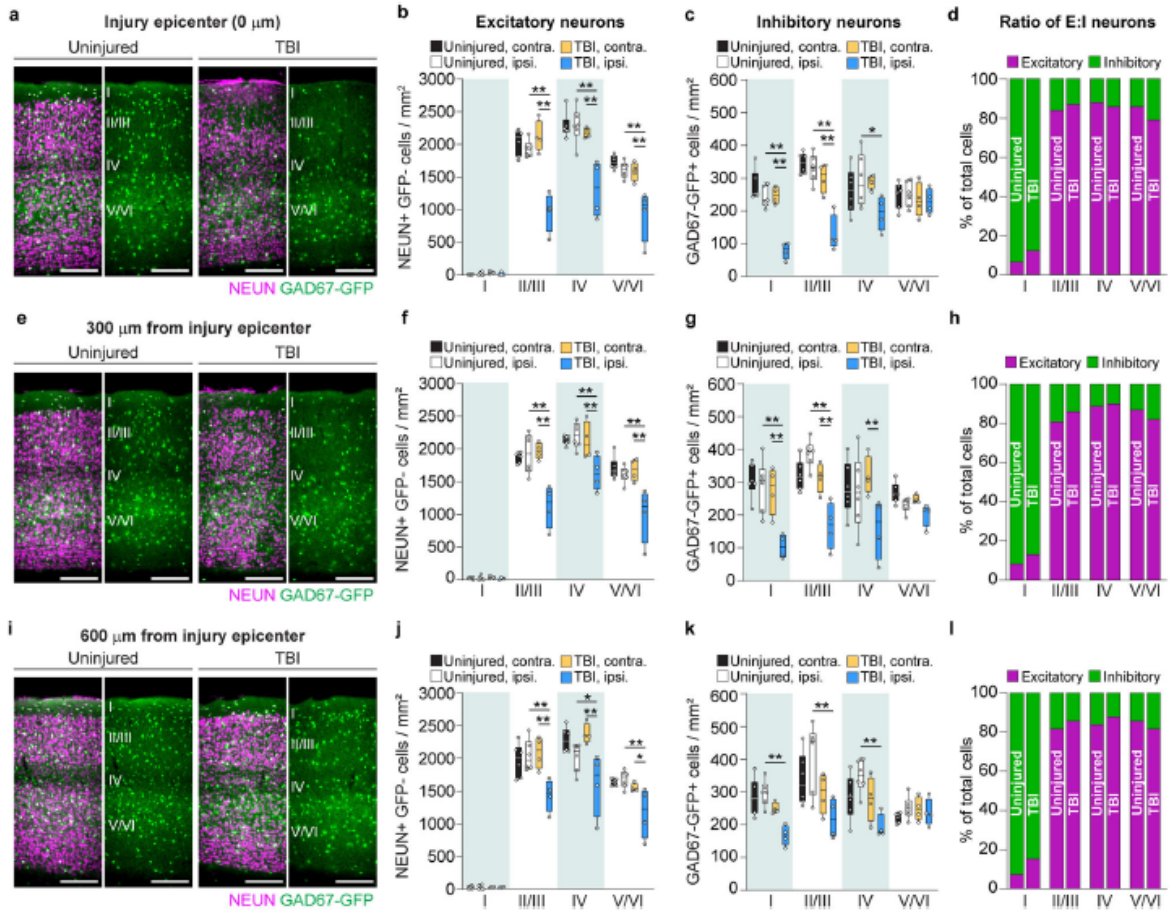

Fig. 3 V1 injury produces subtype- and layer-specific loss of neurons. a, e, i Coronal images of control and CCI-injured V1 labeled for NEUN (magenta) and GAD67-GFP (green) at 0 (**a**), 300 (**e**), and 600 μm (**i**) from the injury. **b, f, j** Quantification of NEUN+/GFP- cell density in layers I, II/III, IV, and V/VI. $N = 4$–6 mice per group. **c, g, k** Quantification of GAD67-GFP cell density in layers I, II/III, IV, and V/VI. $N = 4$–6 mice per group. **d, h, l** Analysis of the proportion of excitatory to inhibitory neuron density at the injury site (Chi-square = 2.17, df = 3, P = 0.54; **d**), 300 μm (Chi-square = 1.32, df = 3, $P = 0.72$; **h**), or 600 μm caudal to the epicenter (Chi-square = 2.68, df = 3, $P = 0.44$; **l**). Scale bars, 500 μm. Box and whisker plots show median, 25th and 75th percentiles, and the whisker bars represent maximum and minimum values. $^{**}P < 0.01$, $^{*}P < 0.05$; random intercept mixed model with Tukey-Kramer post hoc test. See Supplementary Data 2 for statistical analyses.

0.5 months (2.7 ± 2.5 spikes/s) and 3 months (1.6 ± 2.2 spikes/s) after TBI was significantly lower than in uninjured controls ($P = 3.59E\text{-}20$, Kruskal–Wallis H test; Fig. 6e). Together, these findings suggest there is damage to the local V1 neuron population that lasts for several months after TBI.

To evaluate the functional profile of injured V1 in more detail, we next measured single-neuron responses to a range of fundamental visual stimuli, including orientation, size, spatial frequency, and temporal frequency in vivo (Fig. 7; Supplementary Fig. 6). For these analyses, only visually responsive cells were included (see criteria in Methods). Brain-injured mice showed weaker tuning and selectivity to all four types of stimulus parameters compared to uninjured controls (Fig. 7) and had a substantial percentage of cells that were nonresponsive to one or more stimulus conditions (Supplementary Fig. 6). For the cell population, these differences were significant for orientation (Fig. 7b), size (Fig. 7d), and spatial frequency (Fig. 7f), but not temporal frequency (Fig. 7h). The difference was quite striking for orientation and size tuning, both of which are strongly mediated through local cortical inhibition[47–49]. For orientation, the tuning width, measured as the half-width at half-height (HWHH) of the preferred direction (90° in the example cells) was nearly twice as sharp in the control example (23.7°) compared to 0.5 months after injury (45.0°), and more than 50% broader 3 months after injury (36.3°; Fig. 7a). These differences were also seen for the population (control: 30.9° ± 1.9°, 0.5 months after TBI: 43.1° ± 4.0°; 3 months after TBI: 42.6° ± 2.8°; $P = 0.0013$, Kruskal–Wallis H test; Fig. 7b). Broader tuning after TBI is consistent with orientation tuning mediated more through intact thalamocortical feed-forward mechanisms and impairments in cortical inhibition[48,49]. Similarly, the larger size preference in TBI compared to control neuron examples (73° and 60° at 0.5 and 3 months post-TBI vs. 35° in uninjured controls; Fig. 7c) and populations (control: 41.5 ± 2.1°, compared to 79.3 ± 3.4° at 0.5 months and 52.1 ± 3.1° at 3 months postinjury; $P = 1.16E\text{-}13$, Kruskal–Wallis H test; Fig. 7d) is also consistent with a loss of cortical inhibition[47]. This is because stimulus size is normally kept small through a process of lateral suppression mediated by long-range intrinsic excitatory V1 neurons synapsing onto local inhibitory neurons[47,50]. We note that the 3 months postinjury group had a statistically smaller preferred size than the 0.5-month group ($P = 2.04E\text{-}05$, Kruskal–Wallis H test; Fig. 7d). This could be a sign of recovery, however, a larger

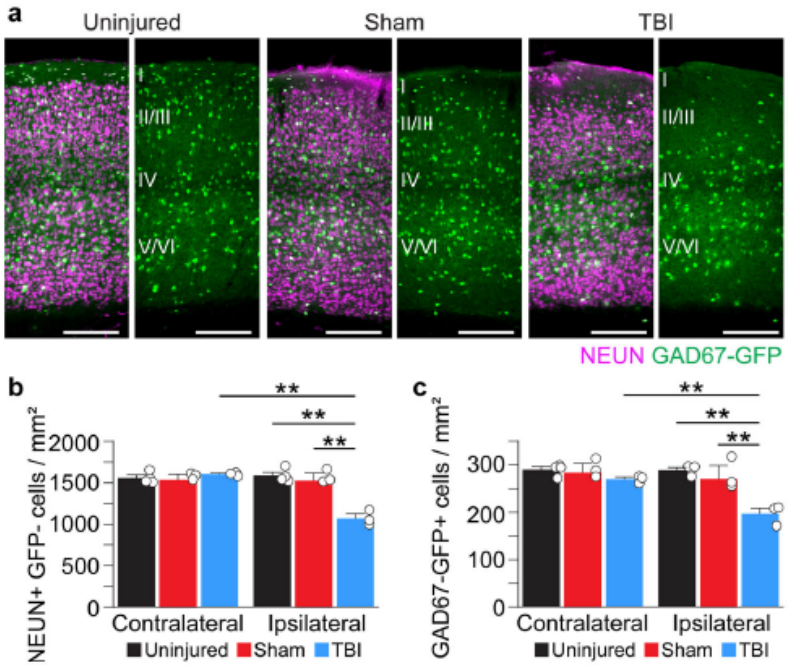

Fig. 4 Chronic neuron loss in V1 after TBI. a Coronal images of control, sham and CCI-injured V1 labeled for NEUN (magenta) and GAD67-GFP (green) 3 months after TBI. **b** Quantification of NEUN+/GFP- cell density in control, sham, and brain-injured mice 3 months after CCI. $^{**}P = 1.33E-06$, ipsilateral control versus ipsilateral TBI, $^{**}P = 4.23E-06$, ipsilateral sham versus ipsilateral TBI, $^{**}P = 1.99E-06$, ipsilateral TBI versus contralateral TBI; two-way ANOVA with Tukey's post hoc test, $N = 3$–4 mice per group. **c** Quantification of GAD67-GFP+ cell density in control, sham, and brain-injured mice 90 d after CCI. $^{**}P = 1.60E-04$, ipsilateral control versus ipsilateral TBI, $^{**}P = 1.02E-03$, ipsilateral sham versus ipsilateral TBI, $^{**}P = 2.96E-03$, ipsilateral TBI versus contralateral TBI; two-way ANOVA with Tukey's post hoc test, $N = 3$–4 mice per group. Scale bar, 500 µm; error bars, SEM. See Supplementary Data 3 for statistical analyses.

percentage of 3-month animal cells did not even respond to the size stimuli (Supplementary Fig. 6c).

Discussion

Patients with TBI can show long-lasting deficits in visual system function, such as visual acuity and field loss, binocular dysfunction, and spatial perceptual deficits[1]. Here, we delivered a mild focal contusion injury directly to V1 to model occipital contusion injuries, which occur almost exclusively after a direct blow to the back of the head[2,51]. Although V1 was relatively well-preserved, compared to traditional approaches that produce substantial tissue damage[13,16,18], we found neuron loss at the injury site that extended into deep cortical layers. Interestingly, the degree of neuron loss was different in excitatory versus inhibitory systems. Excitatory neurons were lost throughout all layers of brain-injured V1, but the greatest degree of cell loss was contained at the injury site. In contrast, inhibitory neurons were uniformly lost by ~35% across all sections examined, but cell loss was restricted to superficial layers I-IV of V1. These observations are different from TBI to the hippocampus, where hilar interneurons are widely considered to be the most vulnerable to injury despite being the deepest layer from the site of impact[13,17,20]. The cellular mechanism for these cell-type-specific responses to injury is unknown. In vivo recordings revealed a massive reduction in VEP amplitudes, consistent with damage to the local V1 neuron population, and dramatically altered single-neuron tuning to visual stimuli, including changes in orientation and size, which have been shown to be modulated by cortical interneurons[52]. These findings are consistent with human studies showing visual field dysfunction can occur in individuals with no measurable lesion[53].

Structural and functional damage following V1 injury appear to be permanent. This is different from damage to other sensory areas. For example, in the whisker barrel cortex, previous in vivo electrophysiology studies have shown there is an initial hypoactivity of neuronal responses 24 h after TBI that recovers within 12 weeks after injury, despite persistent structural changes[28,29]. In the current study, we evaluated the effect of V1 TBI on all GABAergic neurons, but specific subtypes may be more or less vulnerable to injury, as has been seen in other brain areas[13]. Further studies evaluating synaptic plasticity and neuronal connectivity in brain-injured V1 will ultimately be required to determine potential candidate mechanisms underlying the permanent disruption of V1 neuron tuning after TBI.

Individuals with TBI can develop visual impairments independent from other injury-induced motor or cognitive deficits[54–57]. Increases in light intensity evoke inhibitory synaptic activity to prevent changes in luminance intensity from disrupting cortical circuit function[58] and inability to modulate cortical gain has been proposed as a potential mechanism of injury-related photosensitivity[54,57]. Here we show that basic visual processes in V1 are altered to reflect a loss of cortically mediated inhibition. We found significantly broader orientation tuning widths consistent with reduced local inhibitory neuron activity[52]. Instead, in brain-injured animals, V1 orientation tuning resembles the broader widths mediated through feed-forward mechanisms from the thalamus[48,49], which are likely more intact. Similarly, increased spatial summation indicated by larger stimulus size preference in TBI is consistent with the loss of local inhibitory neurons mediating surround suppression[59] and likely reflects preservation of feed-forward mediated mechanisms[47].

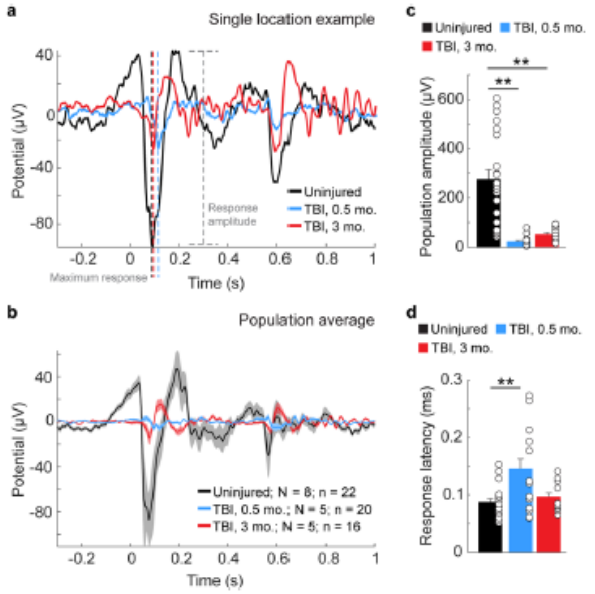

Fig. 5 TBI disrupts V1 responses to visual stimuli. a Representative example of VEPs in layer 5 of an uninjured control animal (black trace) and animals 0.5 months (blue trace) and 3 months after CCI (red trace). The maximum response for each trace is indicated by dotted black, blue and red lines. Response amplitude for the control condition is indicated by the dotted gray line. **b** Average evoked potentials from recording sites in uninjured control (black) and 0.5 months (blue) and 3 months (red) after TBI. *N* = number of animals; *n* = number of recording locations. Shading indicates S.E.M. **c** Quantification of average evoked amplitude. $^{**}P = 6.23E\text{-}09$, control versus 0.5 months after TBI; $^{**}P = 1.88E\text{-}03$, control versus 3 months after TBI; Kruskal–Wallis H with Dunn's post hoc. **d** Quantification of average response latency. $^{**}P = 0.02$, control versus 0.5 months after TBI, Kruskal–Wallis H with Dunn's post hoc. Individual data points represent the value for each of the recording locations. N, animals; n, recording location; error bars, SEM.

In V1, GABAergic inhibition is essential for a wide range of basic V1 functions, such as tuning a neuron's preference for stimulus contrast, size, and orientation[52,60,61], as well as higher-order processing, such as contrast perception[62]. During development, cortical inhibition modulates critical periods, a transient time of enhanced sensitivity to sensory experience. This has been most extensively studied in juvenile V1, in which obstructing vision through one eye results in cortical blindness to this eye, even after normal vision is restored[63]. Cortical inhibition is required for opening the developmental critical period in the visual cortex[64] and inactivating interneurons can prolong the critical period[65] or impair cortical plasticity[66]. Even in adulthood, after binocular vision is well established, manipulating inhibition through pharmacology[61,67] or interneuron transplantation[68,69] can have dramatic effects on cortical plasticity in response to monocular visual deprivation. Given our recent success using interneuron transplantation to treat post-traumatic memory problems and epilepsy[70,71], future studies evaluating the effect of manipulating excitatory versus inhibitory activity in brain-injured V1 may reveal new avenues for circuit-based therapy.

Methods

Animals. Mice were maintained in standard housing conditions on a 12 h light/dark cycle with food and water provided *ad libitum*. All protocols and procedures were approved by and followed the guidelines of the University Laboratory Animal Resources at the University of California, Irvine and adhered to National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. For electrophysiology experiments, we used C57Bl/6J mice (Jackson Laboratories, cat no. 000664), and for anatomy experiments, we used a hemizygous glutamic acid decarboxylase—enhanced green fluorescence protein (GAD67-GFP) knock-in line[46] maintained on a CD-1 background for > 10 generations.

Experimental design. Male and female mice were randomly allocated to experimental groups prior to TBI. Brain injury was performed at P60, and experiments were performed 0.5 or 3 months after TBI. Brain injuries were only considered to be successful if the lesion was found to be centered over the rostral end of V1. Three animals were excluded from the immunostaining analysis, because upon histological inspection the lesion was not found to be centered over the rostral end of V1. No additional animals were generated to replace these mice. All other brain-injured mice survived and remained otherwise healthy until the day of experimentation.

Controlled cortical impact (CCI). Unilateral controlled cortical impact was performed as previously described[13,71], with modifications to the location and depth of injury. Mice were anesthetized with 2% isoflurane until unresponsive to toe-pinch, then placed into a stereotactic frame and maintained on 1% isoflurane. The fur overlying the skull was trimmed and the scalp was scrubbed with betadine before exposing the skull with a midline incision. The skull was rotated 20 degrees counterclockwise along the rostral-caudal axis and the rostral end of the skull was lowered 20 degrees relative to skull-flat. This orientation centered the impactor tip at the rostral end of V1. A ~4-5 mm craniotomy was centered 3 mm lateral to the midline and 3 mm rostral to the lambdoid suture in the right hemisphere. The skull cap was removed leaving the dura intact. A computer-controlled pneumatically driven impactor (TBI-0310, Precision Systems and Instrumentation) with a 3 mm beveled stainless-steel tip was used to deliver a 0.2 mm depth contusive injury perpendicular to the dura at 3.5 m/s velocity and 500 ms of impactor dwell time. The skull cap was not replaced, and the incision was closed with silk sutures. Animals undergoing surgical procedures received buprenorphine hydrochloride (Buprenex, 0.05 mg/kg, delivered i.p.) preoperatively and once daily for 3d. A postoperative health assessment was performed for 5d following surgical procedures.

Immunostaining. At 0.5 or 3 months after injury, mice were transcardially perfused with 4% paraformaldehyde (PFA) and free-floating vibratome sections (50 μm) were processed using standard immunostaining procedures[71]. Sections were stained with the following primary antibodies: GFP (1:1000; cat. no. GFP-1020, Aves Labs), NEUN (1:1000; cat. no. MAB377, Millipore), GFAP (1:500, cat. no. MAB3402, Millipore) and IBA1 (1:1000, cat. no. 019-19740, Fujifilm). Secondary antibodies were Alexa 488, 546, 594, and 647 (1:1000; cat. nos. A-11039, A-11005, A-11030 and A-21244, Fisher Scientific). Sections were then mounted on charged slides (Superfrost plus; Fisher Scientific) with Fluoromount-G containing DAPI (Southern Biotech). Images were obtained with a Leica DM6 epifluorescence microscope. Brightness and contrast were adjusted manually using Adobe Photoshop; z-stacks were generated using Leica software.

Volumetric analysis. Quantification of cortical lesion volume was performed by measuring the area of cortical tissue remaining in both hemispheres in eight DAPI-labeled coronal sections along ~2400 μm of the rostral-caudal axis spaced 300 μm apart as previously described[13,71]. Borders of the cortical plate were drawn between the dorsal aspect of the corpus callosum and the pial surface using ImageJ. Regions of the cortical subplate (e.g., amygdala) were excluded from analysis. The % of the ipsilateral cortex remaining for each animal was calculated using the following formula:

$$\%\,\text{Cortex Remaining} = \left(\frac{\sum i_n}{\sum c_n}\right) \times 100$$

where i = the area of the ipsilateral cortex and c = the area of the contralateral cortex and n = the section number.

Cortical thickness measurement. Average cortical thickness was measured from a series of three DAPI-labeled x10 images of the entire cortical column centered at the injury epicenter and two 300 μm serial sections caudal to the epicenter. The area of tissue between the pial surface and the ventral aspect of layer V/VI was divided by the width of the frame (958.29 μm) to obtain an average cortical thickness value along the width of the frame. For uninjured controls, images were taken in corresponding brain sections at the most central portion of V1 as defined in the 2017 Allen Reference Atlas.

Cell quantification. Fluorescently labeled coronal brain sections (50 μm) were imaged using a Leica DM6 fluorescence microscope with an x20 objective and quantification was performed in ImageJ, as previously described[13,71]. For quantification of cell density, three brain sections spaced 300 μm apart were counted, with the rostral-most section at the injury epicenter and the next two additional sections

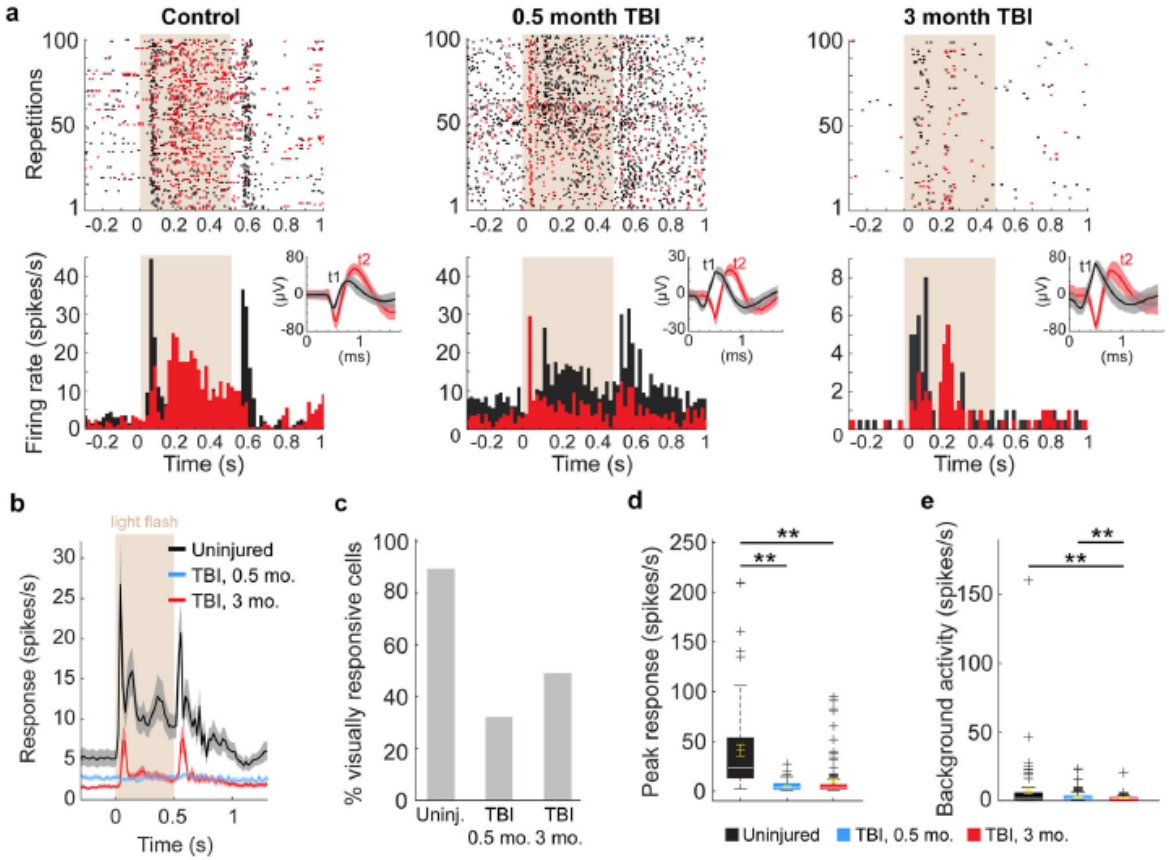

Fig. 6 Reduced V1 neuron firing following TBI. a Light-evoked responses of action potential firing for two example neurons (black and red) for each animal group: control (left), 0.5 months (middle) and 3 months after injury (right). The top row shows raster plots to 100 repetitions of the flash stimulus. The bottom row shows the spikes/s averaged over 20 ms bins. In both rows, the 500 ms light stimulus is indicated by beige background shading. Insets in the upper right of the bottom row show raw wave forms isolated by two templates (t1, black and t2, red) based on differences in spike amplitude (uV) and timing (ms). Shading indicates spike variability. **b** Population averages of light-evoked single-unit responses of action potential firing in uninjured controls (black) and CCI-injured mice 0.5 months (blue) and 3 months after injury (red). $n = 67$ cells from 8 controls, 110 cells from 5 mice 0.5 months after TBI, and 115 cells from 5 mice 3 months after TBI. Shading indicates S.E.M. The 500 ms light stimulus is indicated by beige background shading. **c**. Percentage of visually responsive cells. **d** Quantification of peak single-neuron firing rates from each group in response to light stimulus. $^{**}P = 9.56E\text{-}10$, control versus 0.5 months after TBI, $^{**}P = 9.56E\text{-}10$, control versus 3 months after TBI; Kruskal–Wallis H with Dunn's post hoc. **e** Quantification of single-neuron firing rates from each group in the 500 ms prior to the light stimulus. $^{**}P = 2.00E\text{-}03$, control versus 0.5 months after TBI, $^{**}P = 3.02E\text{-}04$, control versus 3 months after TBI; Kruskal–Wallis H with Dunn's post hoc. For box plots, dashed error bars represent the maximum and minimum observations within 1.5 inter-quartile range of the 25th and 75th percentile; values greater than 1.5 inter-quartile range of the 75th percentile are indicated by +.

caudal to the epicenter. For layer analysis, the border of each layer (layers I, II/III, IV, and V/VI) were defined manually by visual inspection of neuron densities in NEUN epifluorescence images, as previously described[72,73]. For quantification of GFAP and IBA1 immunostaining, measurements were analyzed at three different locations and the percentage of the area above fluorescence threshold was applied using ImageJ according to a previous protocol[71]. The same settings were used for all sections.

Neurophysiology. Animals were initially anesthetized with 2% isoflurane in a mixture of N_2O/O_2 (70%/30%) then placed into a stereotaxic apparatus. A small, custom-made plastic chamber was secured to the exposed skull using dental acrylic. After one day of recovery, re-anesthetized animals were placed in a custom-made hammock, maintained under isoflurane anesthesia (1–2% in N_2O/O_2) and multiple single tungsten electrodes were inserted into V1 layers II–V1 using the same craniotomy produced during the injury phase. All recording locations were within the CCI damaged region of V1 (defined as being within the craniotomy). Following electrode placement, the chamber was filled with sterile agar and sealed with sterile bone wax. Animals were then sedated with chlorprothixene hydrochloride (1 mg/kg; IM;[74]) and kept under light isoflurane anesthesia (0.2–0.4% in 30% O_2) throughout the recording procedure. EEG and EKG were monitored throughout and body temperature was maintained with a heating pad (Harvard Apparatus, Holliston, MA).

Data was acquired using a multi-channel Scout recording system (Ripple, UT, USA). Local field potentials (LFP) from multiple locations at matching cortical depths were band-pass filtered from 0.1 Hz to 250 Hz and stored along with spiking data at 1 kHz sampling rate. LFP signal was aligned to stimulus time stamps and averaged across trials for each recording depth in order to calculate visually evoked potentials (VEP)[75–77]. Single-neuron spike signals were band-pass filtered from 500 Hz to 7 kHz and stored at a 30 kHz sampling frequency. Spikes were sorted online in Trellis (Ripple, UT, USA) while performing visual stimulation. Action potentials were detected based on negative and positive thresholds that were at least twice as large (S/N > 2:1) as the background noise. For each recording location, thresholds were adjusted to maintain a high signal-to-noise ratio. Waveforms were sorted by marking templates based on the clear amplitude difference, positive or negative peak detection, and the slope between negative and positive component (see insets in Fig. 6a), which can be defined as the spike width. Visual stimuli were generated in Matlab (Mathworks, USA) using Psychophysics Toolbox[78–80] and displayed on a gamma-corrected LCD monitor (55 inches, 60 Hz; 1920 ×1080 pixels; 52 cd/m^2 mean luminance). Stimulus onset times were corrected for monitor delay using an in-house designed photodiode system[81].

Visual responses were assessed according to previously published methods[76,81,82]. For recordings of visually evoked responses, cells were first tested with 100 repetitions of a 500 ms bright flash stimulus (105 cd/m^2). Receptive fields for visually responsive cells were then located using square-wave drifting gratings,

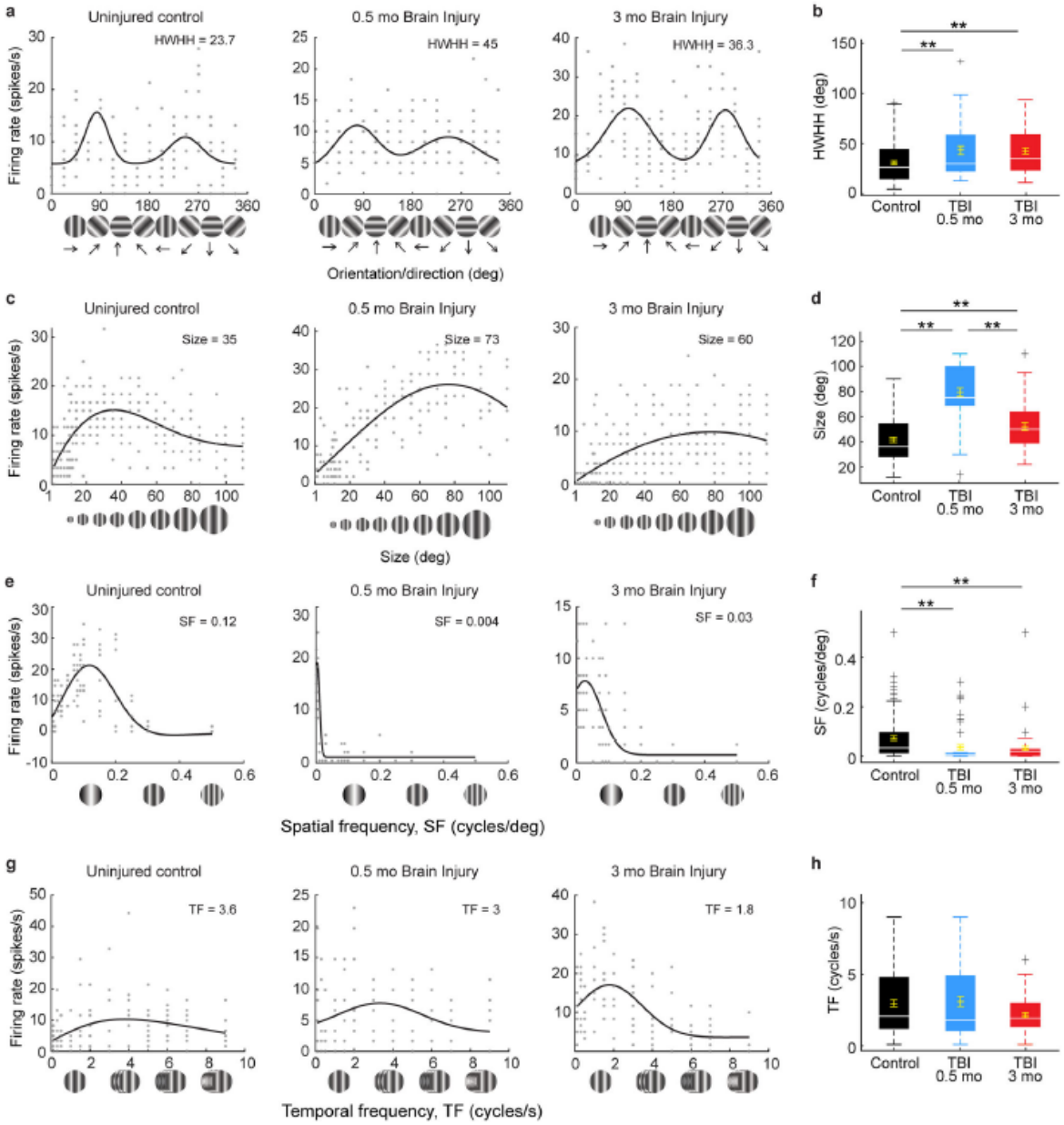

Fig. 7 TBI disrupts V1 neuron tuning curves in response to drifting gratings. a, b Orientation tuning curves for single neurons in an uninjured control (black) and CCI-injured mice 0.5 months (blue) and 3 months (red) after injury. To facilitate comparisons across examples orientation preferences have been aligned to 90° and 270°, with 90° representing the preferred direction. Tuning values are given as the half-width at half-height (HWHH) in degrees in each panel (**a**) and the population averages are quantified in (**b**). $^{**}P = 0.03$, control versus 0.5 months after TBI, $^{**}P = 1.99E\text{-}03$, control versus 3 months after TBI; Kruskal–Wallis H with Dunn's post hoc. $n = 109$ cells from 7 uninjured controls, 54 cells from 5 mice 0.5 months after TBI, and 75 cells from 5 mice 3 months after TBI. **c**, **d** Single neuron examples and population average quantification of aperture size (in degrees). $^{**}P = 9.56E\text{-}10$, control versus 0.5 months after TBI, $^{*}P = 0.042$, control versus 3 months after TBI, $^{**}P = 2.04E\text{-}05$, 0.5 versus 3 months after TBI; Kruskal–Wallis H with Dunn's post hoc. $n = 81$ cells for control, 45 cells 0.5 months after TBI and 41 cells 3 months after TBI. **e**, **f** Single neuron examples and quantification of spatial frequency (SF). $^{**}P = 3.80E\text{-}06$, control versus 0.5 months after TBI, $^{**}P = 6.51E\text{-}03$, control versus 3 months after TBI, Kruskal–Wallis H with Dunn's post hoc. $n = 105$ cells for control, 55 cells 0.5 months after TBI, 71 cells 3 months after TBI. **g**, **h** Single neuron examples and quantification of temporal frequency (TF). $P = 0.26$; Kruskal–Wallis H test. Optimal values for each parameter are given in each panel. $n = 95$ cells for control, 59 cells 0.5 months after TBI, and 70 cells 3 months after TBI. Background activity for each cell is indicated by gray dashed lines. For box plots, dashed error bars represent the maximum and minimum observations within 1.5 inter-quartile range of the 25th and 75th percentile; values greater than 1.5 inter-quartile range of the 75th percentile are indicated by +.

after which optimal orientation, direction, and spatial and temporal frequencies were determined using sine-wave gratings. Shown at optimal orientation, spatial frequencies used ranged from 0.001 to 0.5 cycles/°; Temporal frequencies used were from 0.1 to 10 cycles/s. Using optimal parameters, size tuning was assessed with apertures ranging from 1 to 110° at 100% contrast. With optimal size, orientation tuning of the cell was re-assessed using 8 orientations × 2 directions each, stepped by 22.5° increments. Background activity was calculated as average activity from 500 ms before stimulus onset for each repetition. A cell was determined to be visually responsive if the average firing rate was more than 2 standard deviations above background activity and at least 3 spikes/s. Any cell that was nonresponsive to the flash stimulus was not probed using sine-wave gratings. A percentage of flash-responsive cells in the 0.5- and 3-month conditions did not respond to every sine-wave stimulus condition used (Supplemental Fig. 6). Non-responses to sine-wave stimuli were excluded from population analyses because they could not be fit to a curve.

Local field potential (LFP) analysis. Amplitude of response was calculated as a difference between the peak of the positive and negative components of the VEP. Response latency was defined as the time from stimulus onset to maximum response. Maximum of the response was defined at the larger of the negative or positive peak. For uninjured control animals, depths corresponding to layer 5 were always used (~500 µm). This is because layer 5 amplitude responses were the highest in control animals (see example in Supplemental Fig. 3a). For TBI animals, the depth with the highest amplitude was used. This is because VEPs were more erratic in TBI animals and not always the most responsive at layer 5 (see examples in Supplemental Fig. 3b, c).

Single-unit analysis. Tuning curves were calculated based on the average spike rate centered around the preferred direction (peak response). Optimal visual parameters were chosen as the maximum response value. Orientation tuning was measured in degrees at the half-width at half-height (HWHH; 1.18 × σ) based on fits to Gaussian distributions[47,48,81–84] using:

$$R_{O_i} = baseline + R_p e^{-\frac{(O_i - O_p)^2}{2\sigma^2}} + R_n e^{\frac{(O_i - O_p + 180)^2}{2\sigma^2}}, \quad (1)$$

where O_i is the stimulus orientation, R_{O_i} is the response to different orientations, O_p is the preferred orientation, R_p and R_n are the responses at the preferred and nonpreferred direction, σ is the tuning width, and 'baseline' is the offset of the Gaussian distribution. Gaussian fits were estimated without subtracting spontaneous activity, similar to the procedures of Alitto and Usrey[83].

Size tuning curves were fitted by a difference of Gaussian (DoG) function:

$$R_s = K_e \int_{-s}^{s} e^{\left(\frac{-x}{re}\right)^2} dx - K_i \int_{-s}^{s} e^{(-x/r_i)^2} dx + R_0, \quad (2)$$

in which R_s is the response evoked by different aperture sizes. The free parameters, K_e and re, describe the strength and the size of the excitatory space, respectively; Ki and ri represent the strength and the size of the inhibitory space, respectively; and R_0 is the spontaneous activity of the cell.

The optimal spatial and temporal frequency was extracted from the data fitted to Gaussian distributions using the following equation:[81,82,85,86]

$$R_{SF/TF} = baseline + R_{pref} e^{-\frac{\left(\frac{SF}{SF_{pref}} - \frac{SF}{SF_{pref}}\right)^2}{2\sigma^2}}, \quad (3)$$

Where $R_{SF/TF}$ is the estimated response, R_{pref} indicates response at preferred spatial or temporal frequency, SF/TF indicates spatial or temporal frequency, σ is the standard deviation of the Gaussian, and baseline is Gaussian offset.

Statistics and reproducibility. Anatomical data analysis was performed in Graphpad Prism 9, Microsoft Excel, and SAS 9.4 software. Experimental groups were averaged across groups (i.e., N = animals) compared by two-way ANOVA with Tukey's post hoc test, or repeated-measures two-way ANOVA followed by Sidak's post hoc test. For layer analysis, data were fitted to a random intercept mixed model followed by Tukey-Kramer post hoc. Cell density was defined as the response variable and distance from the injury, cell layer, group, the interaction of layer by group, and the interaction of distance by layer by the group as explanatory variables. Neurophysiology data analysis was performed in Matlab (Mathworks, USA). Neural responses were averaged across recording locations (i.e., N = animals; n = recording locations) or cells (in single-unit recordings) and groups were compared by Kruskal–Wallis H test followed by multiple comparisons using Dunn's post hoc. All data are expressed as mean ± SEM. Significance was set at $P < 0.05$.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data that support the findings of this study are available as source data in Supplementary Data 4. All other data are available from the corresponding author upon reasonable request.

Code availability

Data were collected with previously published custom MatLab script[76] and is available from the corresponding author upon reasonable request.

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Author contributions
J.C.F contributed to the execution and analysis of experiments, funding and wrote the first draft of the manuscript. A.T.F designed and performed neurophysiology experiments, analyzed data, contributed funding, and edited the manuscript. A.T. performed immunostaining and analysis and edited the manuscript. J.R.M. performed preliminary cell quantifications. D.C.L designed neurophysiology experiments, analyzed data, contributed funding, and edited the manuscript. R.F.H contributed to the concept, design, analysis of experiments, funding, and wrote the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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