

Tools and methods for cell ablation and cell inhibition in *Caenorhabditis elegans*

Dennis Rentsch, Amelie Bergs, Jiajie Shao, Nora Elvers, Christiane Ruse, Marius Seidenthal, Ichiro Aoki, and Alexander Gottschalk

NOTE: The reviews and decision letters are unedited and appear as submitted by the reviewers.

In extremely rare instances and as determined by a Senior Editor or the EIC, portions of a review may be redacted. If a review is signed, the reviewer has agreed to no longer remain anonymous.

The review history appears in chronological order.

Review Timeline:

Submission Date:	2024-05-12
Editorial Decision:	2024-06-24
Revision Received:	2024-07-16
Accepted:	2024-07-16

June 24, 2024

RE: GENETICS-2022-305010

Dear Dr. Gottschalk:

I am pleased to accept your manuscript entitled "Tools and methods for cell ablation and cell inhibition in *Caenorhabditis elegans*" for publication in GENETICS, pending minor revision.

Please submit your revision along with a response to the reviewers' concerns and suggestions, which can be viewed at the bottom of this email. I expect this can be done within 30 days.

Upon resubmission, please include:

1. A clean version of your manuscript;
2. A marked version of your manuscript in which you highlight significant revisions carried out in response to the major points raised by the editor/reviewers (track changes is acceptable if preferred);
3. A detailed response to the editor's/reviewers' comments and to the concerns listed above. Please reference line numbers in this response to aid the editors.

Additionally, please ensure that your revision is formatted for GENETICS: <https://academic.oup.com/genetics/pages/general-instructions>.

Follow this link to submit the revised manuscript: Link Not Available

Thank you for submitting your research to Genetics.

Sincerely,

Roger Pocock
Associate Editor
GENETICS

Approved by:
Oliver Hobert
Senior Editor
GENETICS

Reviewer comments:

Reviewer #1 (Comments for the Authors (Required)):

The review by Rentsch et al., provides an extensive overview of tools and methods for cell ablation and inhibition in *C. elegans*. It discusses various approaches including pharmacological treatments, laser ablation, genetically encoded cell death, and optogenetic tools, emphasizing their applications and effectiveness in understanding the role of specific cells and neurons. The latter is an important addition, as the specific examples of neurons tested using the different tools, and, when possible, the shortcomings of the mentioned system, provide an added immediate advantage to the reader. Rentsch et al take an extra step and highlight the importance of these methods in providing insights into neuronal function, behavior regulation, and the overall cellular architecture of *C. elegans*. Each method's temporal and spatial precision, reversibility, and potential applications in broader biological contexts are thoroughly examined. I highly enjoyed reading this review, I think it will be a very useful resource for *C. elegans* researchers. I only have a few minor comments

Section 1- perhaps its worth mentioning that the paralysis induced by most of the pharmacological drugs is reversible?

Section 2- Cell ablation requires a special nitrogen pulsed UV laser which produces microjoule-level pulses with nanosecond durations. Thus, this method required a specialized laser, which doesn't fit all microscopes and is usually rather expensive. This should be mentioned as a limitation. I would also mention that femtolasers (two-photon or more) could also be used for ablation,

with higher precision and less tissue damage (see Chung et al <https://link.springer.com/article/10.1007/s00339-009-5201-7>).

Section 6- A very recently published tool could also be integrated into the review in chapter 6.5- Wietek et al characterized a bistable inhibitory optoGPCR for multiplexed optogenetic control of neural circuits (<https://www.nature.com/articles/s41592-024-02285-8#Sec30>). PdCO is an opsin isolated from *Platynereis dumerilii*. It seems to nicely complement OPN3 and LcPPO. It was codon optimized for *C. elegans* and tested for its effects on locomotion.

Reviewer #2 (Comments for the Authors (Required)):

Rentsch and co-authors provide a detailed overview of various techniques for silencing or killing neurons in *C. elegans*. The work is a valuable contribution because it describes in one place many approaches even those that are mechanistically quite distinct. In general I thought there was a good balance between practical description of what a given method is useful for, vs minor technical details, and of descriptions of how the methods was developed.

The classes of method described cover many decades of intense innovation, and so the review necessarily omits some papers, but on the whole I thought the work was sufficiently comprehensive to be useful to the community.

It also did a good job directing the reader to still relevant prior worm-specific reviews on optogenetics and laser ablation. I believe the work will be of benefit to the community and urge publication.

Below are comments to improve readability and style:

Figure 1: The box describing pharmacology tools is hard to see and oddly lacks pictures and is out of order with respect to the caption. Suggest reordering to be consistent.

Figure 3: Light treatment bar is hard to see on the bottom axis. It might be more visible at the top.

-Introduction lacks citations. Is this intentional?

-Please confirm nomenclature for "Chapters". I'm more used to seeing these described as "sections."

-It would be useful to define "dark activity" when first mentioned as this is less commonly known.

-In section 5.1, 6th line: typo "maker" should be "selection marker"

Reviewer #3 (Comments for the Authors (Required)):

The authors present a review of methods for ablating and inhibiting cells in *C. elegans*. It provides a broad overview of an important topic and will be potentially useful to researchers in the field.

My primary concern with the manuscript is that the quality of writing could use some improvement. Much of the text is unnecessarily convoluted. Many examples are given below.

Detailed comments:

Abstract: "To understand the function of cells within an organism, and of neurons within a nervous system..."

This sentence strikes me as odd. Are neurons not also cells within an organism? I suggest removing "and of neurons within a nervous system" or perhaps rephrasing as "such as neurons within a nervous system"

"A range of approaches and tools were developed and used over the past few decades, that act either constitutively, or acutely and reversibly, in systemic or local fashion, using either drugs or genetically encoded tools, and exogenous triggers like light."

I find this sentence confusing to read. I'm not sure what "and exogenous triggers like light" is being added to. I suggest revising to emphasize clarity of expression rather than trying to cover every possible parameter over which the tools may vary.

In the same sentence, "drugs or genetically encoded tools" seems like a false dichotomy, as there are a number of methods that

employ both drugs and genetically encoded tools.

Introduction: "Eliminating (the function of) neurons, and/or the inhibition of their function..." is phrased in an unnecessarily complicated manner.

"In the following chapters, we briefly describe the tools and some of their applications"

"Chapters" usually refers to large sections of a book-sized work. In an article the authors should instead refer to "Sections".

"Thus, we would like to apologize to those colleagues whose work we may not have covered in the depth that they deserve, or where we may have overlooked important contributions."

In my opinion this type of statement does not belong in a review article, especially in the introduction. It is understood by all that a review article does not need to cite every possible paper in a given field.

Fig. 1A. "photosensitive" is misspelled

Fig. 1C: Instead of listing irreversible tools as having off kinetics time scales of 10,000 seconds, it would be more clear to write "irreversible" as the Y label there.

p. 3: "Last, also muscle can be inhibited by direct drug action, likely on the actin-myosin contractile apparatus, namely BDM"

This sentence is confusing. It seems to imply that the actin-myosin contractile apparatus is also known as BDM. "Last, also" does not make sense.

Sec. 1. The Worm Breeder's Gazette should not be cited in bibliographies. The relevant information should be cited as personal communication only, and only with permission of the authors. See <http://dev.wormbook.org/wbg/citing-the-gazette/>

Sec. 2. "The first approaches used for cell ablation were using (UV) laser irradiation of the nucleus of the respective cell..."

Ablation can be done using a range of wavelengths. The most common ablation methods do not use UV but rather visible light with wavelength around 440 nm.

End of Sec. 2.: "Animals" is misspelled.

Sec. 2. It would be helpful to say a few words about the mechanisms by which laser ablation kills cells.

Sec. 3: "Therefore, other approaches were chosen and / or developed" is not a good opening sentence for a section.

Sec. 4.2: "While KillerRed requires dimerization and is relatively large, miniSOG only consists of 106 amino acids..."

What does relatively large mean? If the idea is to compare with miniSOG, why not provide the number of amino acids?

Sec. 5.1: The authors should describe the logic by which a constitutively open potassium channel causes excitable cell inactivation.

5.2: "However, a photoactivated version of BoNT (PA- BoNT) has been generated and used to silence motor neurons, see chapter 5.5."

"Chapter" should be replaced by "Section" throughout the manuscript.

Also, it would be helpful to organize the discussions of BoNT and PA-BoNT together. Perhaps PA-BoNT could be described in 5.3 instead of 5.5.

6.1. "...channelrhodopsin-2 (ChR2), a depolarizing non-selective cation channel, is complemented and counteracted by a growing repertoire of optogenetic silencers."

In what sense is ChR2 "counteracted" by silencers? In this context it is enough to say that it is "complemented".

Rebuttal letter

Comments by the reviewers are in black, [our responses in blue](#). Please also find a version of the manuscript with our changes / additions marked in red.

Reviewer #1 (Comments for the Authors (Required)):

The review by Rentsch et al., provides an extensive overview of tools and methods for cell ablation and inhibition in *C. elegans*. It discusses various approaches including pharmacological treatments, laser ablation, genetically encoded cell death, and optogenetic tools, emphasizing their applications and effectiveness in understanding the role of specific cells and neurons. The latter is an important addition, as the specific examples of neurons tested using the different tools, and, when possible, the shortcomings of the mentioned system, provide an added immediate advantage to the reader. Rentsch et al take an extra step and highlight the importance of these methods in providing insights into neuronal function, behavior regulation, and the overall cellular architecture of *C. elegans*. Each method's temporal and spatial precision, reversibility, and potential applications in broader biological contexts are thoroughly examined. I highly enjoyed reading this review, I think it will be a very useful resource for *C. elegans* researchers. I only have a few minor comments

[Thank you for the encouraging feedback !](#)

Section 1- perhaps its worth mentioning that the paralysis induced by most of the pharmacological drugs is reversible?

[Thank you, yes this is important to add and we did this.](#)

Section 2- Cell ablation requires a special nitrogen pulsed UV laser which produces microjoule-level pulses with nanosecond durations. Thus, this method required a specialized laser, which doesn't fit all microscopes and is usually rather expensive. This should be mentioned as a limitation. I would also mention that femtolasers (two-photon or more) could also be used for ablation, with higher precision and less tissue damage (see Chung et al <https://link.springer.com/article/10.1007/s00339-009-5201-7>).

[Thank you, we added a few sentences about this, though wanted to keep this brief, as there are extensive and excellent reviews on this matter, which we have referenced, including the suggested review article.](#)

Section 6- A very recently published tool could also be integrated into the review in chapter 6.5- Wietek et al characterized a bistable inhibitory optoGPCR for multiplexed optogenetic control of neural circuits (<https://www.nature.com/articles/s41592-024-02285-8#Sec30>). PdCO is an opsin isolated from *Platynereis dumerilii*. It seems to nicely complement OPN3 and LcPPO. It was codon optimized for *C. elegans* and tested for its effects on locomotion.

[Thank you for bringing this to our attention. The work demonstrating this tool in *C. elegans* was kind-of hidden in the supplements of this paper, which is why we overlooked it. We now mention this tool and give a brief description of its properties.](#)

Reviewer #2 (Comments for the Authors (Required)):

Rentsch and co-authors provide a detailed overview of various techniques for silencing or killing neurons in *C. elegans*. The work is a valuable contribution because it describes in one place many approaches even those that are mechanistically quite distinct. In general I thought there was a good balance between practical description of what a given method is useful for, vs minor technical details, and of descriptions of how the methods was developed.

The classes of method described cover many decades of intense innovation, and so the review necessarily omits some papers, but on the whole I thought the work was sufficiently comprehensive to be useful to

the community.

It also did a good job directing the reader to still relevant prior worm-specific reviews on optogenetics and laser ablation. I believe the work will be of benefit to the community and urge publication.

Thank you for your helpful and constructive feedback!

Below are comments to improve readability and style:

Figure 1: The box describing pharmacology tools is hard to see and oddly lacks pictures and is out of order with respect to the caption. Suggest reordering to be consistent.

Thank you, we now extended the figure by pictograms briefly describing pharmacology approaches.

Figure 3: Light treatment bar is hard to see on the bottom axis. It might be more visible at the top.

We changed this to having transparent shading for the duration of the light stimulus, so it is also visible right at the x-axis.

-Introduction lacks citations. Is this intentional?

Not really, but as the introduction is very general and could easily include dozens of citations that, however, are being cited throughout the sections of the review, we initially did not add references here. There are now some references, mainly for reviews or tool benchmarking papers, but we did not want to extend this too much.

-Please confirm nomenclature for "Chapters". I'm more used to seeing these described as "sections."

Absolutely, "chapters" makes no sense. We changed this to "sections".

-It would be useful to define "dark activity" when first mentioned as this is less commonly known.

Thank you, yes, done.

-In section 5.1, 6th line: typo "maker" should be "selection marker"

Thank you for spotting this.

Reviewer #3 (Comments for the Authors (Required)):

The authors present a review of methods for ablating and inhibiting cells in *C. elegans*. It provides a broad overview of an important topic and will be potentially useful to researchers in the field.

We are thankful for your positive feedback.

My primary concern with the manuscript is that the quality of writing could use some improvement. Much of the text is unnecessarily convoluted. Many examples are given below.

Thank you for suggesting to improve the text at these places, which we have done.

Detailed comments:

Abstract: "To understand the function of cells within an organism, and of neurons within a nervous system..."

This sentence strikes me as odd. Are neurons not also cells within an organism? I suggest removing "and of neurons within a nervous system" or perhaps rephrasing as "such as neurons within a nervous system"

We have done this.

"A range of approaches and tools were developed and used over the past few decades, that act either constitutively, or acutely and reversibly, in systemic or local fashion, using either drugs or genetically encoded tools, and exogenous triggers like light."

I find this sentence confusing to read. I'm not sure what "and exogenous triggers like light" is being added to. I suggest revising to emphasize clarity of expression rather than trying to cover every possible parameter over which the tools may vary.

We altered this sentence and split it in two, to make it clearer.

In the same sentence, "drugs or genetically encoded tools" seems like a false dichotomy, as there are a number of methods that employ both drugs and genetically encoded tools.

We have clarified this, thank you.

Introduction: "Eliminating (the function of) neurons, and/or the inhibition of their function..." is phrased in an unnecessarily complicated manner.

We have simplified this sentence.

" In the following chapters, we briefly describe the tools and some of their applications"

"Chapters" usually refers to large sections of a book-sized work. in an article the authors should instead refer to "Sections".

We have altered this.

"Thus, we would like to apologize to those colleagues whose work we may not have covered in the depth that they deserve, or where we may have overlooked important contributions."

In my opinion this type of statement does not belong in a review article, especially in the introduction. It is understood by all that a review article does not need to cite every possible paper in a given field.

We removed this sentence.

Fig. 1A. "photosensitive" is misspelled

Thank you for spotting this.

Fig. 1C: Instead of listing irreversible tools as having off kinetics time scales of 10,000 seconds, it would be more clear to write "irreversible" as the Y label there.

We altered the figure accordingly.

p. 3: "Last, also muscle can be inhibited by direct drug action, likely on the actin-myosin contractile apparatus, namely BDM"

This sentence is confusing. It seems to imply that the actin-myosin contractile apparatus is also known as BDM. "Last, also" does not make sense.

Thank you for spotting this, it was changed.

Sec. 1. The Worm Breeder's Gazette should not be cited in bibliographies. The relevant information should be cited as personal communication only, and only with permission of the authors. See <http://dev.wormbook.org/wbg/citing-the-gazette/>

Thank you for this remark. We altered this, but did not do so initially, since many journals no longer permit authors to cite personal communication. However, we asked the two authors, Miriam Goodman and Martin Chalfie, for permission to cite them as personal communication (e-mails will be provided upon request, and to the editor), and we also now cite two references by Dr. Chalfie that describe the use of BDM.

Sec. 2. "The first approaches used for cell ablation were using (UV) laser irradiation of the nucleus of the respective cell..."

Ablation can be done using a range of wavelengths. The most common ablation methods do not use UV but rather visible light with wavelength around 440 nm.

Thank you for this. We added a few sentences to the section on laser ablation, to emphasize the one- and two- / multi-photon regimes that can be used to ablate cells.

End of Sec. 2.: "Animals" is misspelled.

Thank you for spotting this.

Sec. 2. It would be helpful to say a few words about the mechanisms by which laser ablation kills cells.

We added a few sentences on this.

Sec. 3: "Therefore, other approaches were chosen and / or developed" is not a good opening sentence for a section.

We now have an additional sentence to start this section.

Sec. 4.2: "While KillerRed requires dimerization and is relatively large, miniSOG only consists of 106 amino acids..."

What does relatively large mean? If the idea is to compare with miniSOG, why not provide the number of amino acids?

We added this information.

Sec. 5.1: The authors should describe the logic by which a constitutively open potassium channel causes excitable cell inactivation.

We added this information.

5.2: "However, a photoactivated version of BoNT (PA- BoNT) has been generated and used to silence motor neurons, see chapter 5.5."

"Chapter" should be replaced by "Section" throughout the manuscript.

We did this now.

Also, it would be helpful to organize the discussions of BoNT and PA-BoNT together. Perhaps PA-BoNT could be described in 5.3 instead of 5.5.

1st Accepted Version - Authors' Response to Reviewers: July 16, 2024

We would like to keep it like it is, for the following reason: We first wanted to cover non-light activated tools for protein disruption (5.2), and then switched to light-induced tools (5.3. and beyond). In principle, one could show PA-BoNT first; yet, we wanted to first cover the 'unspecific' light-activated tools that generate ROS, and then move to the protein-specific, light-activated proteolysis tools. We refer to PA-BoNT in the last sentence of section 5.2 ("However, a photoactivated version of BoNT (PA-BoNT) has been generated and used to silence motor neurons, see section 5.5"), to refer the readers to it.

6.1. "...channelrhodopsin-2 (ChR2), a depolarizing non-selective cation channel, is complemented and counteracted by a growing repertoire of optogenetic silencers."

In what sense is ChR2 "counteracted" by silencers? In this context it is enough to say that it is "complemented".

Thank you, we removed this.

July 16, 2024

RE: GENETICS-2022-305010R1

Prof. Alexander Gottschalk
Goethe-Universität Frankfurt am Main
Buchmann Institute and Institute of Biophysical Chemistry
Max von Laue Strasse 15
Frankfurt D-60438
Germany

Dear Dr. Gottschalk:

Congratulations, your Review entitled "Tools and methods for cell ablation and cell inhibition in *Caenorhabditis elegans*" is accepted for publication in GENETICS! Many thanks for contributing to GENETICS.

To Proceed to Publication:

1. Format your article according to GENETICS style: <https://academic.oup.com/genetics/pages/general-instructions>
2. Ensure that you comply with data and community resource citation guidelines:
<https://academic.oup.com/genetics/pages/general-instructions#Data-Policy>
3. Upload your final files at <https://genetics.msubmit.net>
4. Add oupsupport@scipris.com and genetics.oup@novatechset.com (or the domains @scipris.com and @novatechset.com) to your email program's "safe senders" list. You will be contacted by both at various points during the production process.

Notes:

- We invite you to submit an original color figure related to your paper for consideration as cover art. Please email your submission to the editorial office or upload it with your final files. You can submit a small-sized image for evaluation, and if selected, the final image must be a TIFF file 2513px wide by 3263px high (8.375 by 10.875 inches; resolution of 600ppi). Please avoid graphs and small type.

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If you have any questions or encounter any problems while uploading your accepted manuscript files, please email the editorial office at sourcefiles@thegsajournals.org.

Sincerely,

Roger Pocock
Associate Editor
GENETICS

Approved by:
Oliver Hobert
Senior Editor
GENETICS