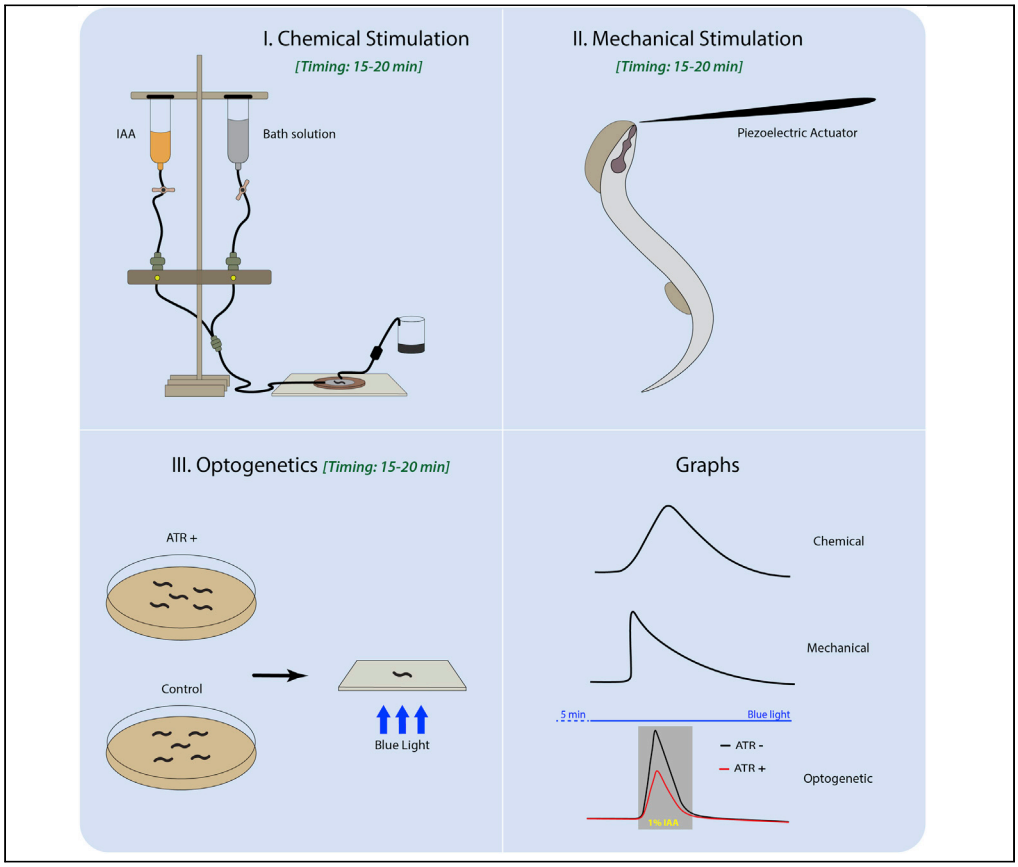
STAR Protocols

CellPress
OPEN ACCESS

Protocol

Protocol for glial Ca^{2+} imaging in *C. elegans* following chemical, mechanical, or optogenetic stimulation



Caenorhabditis elegans is an exceptionally transparent model to analyze calcium (Ca^{2+}) signals, but available protocols for neuronal $Ca2^{+}$ imaging may not be suitable for studying glial cells. Here, we present a detailed protocol for glial Ca^{2+} imaging in *C. elegans* following three different approaches including chemical, mechanical, and optogenetic stimulation. We also provide the details for imaging analysis using Image-J.

Hankui Cheng, Umar Al-Sheikh, Du Chen, Duo Duan, Lijun Kang

11818106@zju.edu.cn (H.C.)
kanglijun@zju.edu.cn (L.K.)

Highlights

Comprehensive approaches for glial Ca^{2+} imaging in *C. elegans*

Measuring glial Ca^{2+} levels following chemical, mechanical, or optogenetic stimulation

Detailed steps for image analysis with Image J

Cheng et al., STAR Protocols 3, 101169
March 18, 2022
https://doi.org/10.1016/j.xpro.2022.101169

STAR Protocols

CellPress
OPEN ACCESS

Protocol

Protocol for glial Ca^{2+} imaging in *C. elegans* following chemical, mechanical, or optogenetic stimulation

Hankui Cheng,[1,2,3,4,*] Umar Al-Sheikh,[1,2,3] Du Chen,[1,2] Duo Duan,[1,2] and Lijun Kang[1,2,4,5,*]

[1]Department of Neurobiology and Department of Neurology of the Fourth Affiliated Hospital, Zhejiang University, School of Medicine, Hangzhou, Zhejiang 310053, China

[2]NHC and CAMS Key Laboratory of Medical Neurobiology, MOE Frontier Science Center for Brain Research and Brain-Machine Integration, School of Brain Science and Brain Medicine, Zhejiang University, Hangzhou, Zhejiang 310053, China

[3]These authors contributed equally

[4]Technical contact

[5]Lead contact

*Correspondence: 11818106@zju.edu.cn (H.C.), kanglijun@zju.edu.cn (L.K.)
https://doi.org/10.1016/j.xpro.2022.101169

SUMMARY

***Caenorhabditis elegans* is an exceptionally transparent model to analyze calcium (Ca^{2+}) signals, but available protocols for neuronal $Ca2^{+}$ imaging may not be suitable for studying glial cells. Here, we present a detailed protocol for glial Ca^{2+} imaging in *C. elegans* following three different approaches including chemical, mechanical, and optogenetic stimulation. We also provide the details for imaging analysis using Image-J.**

For complete details on the use and execution of this protocol, please refer to Duan et al. (2020).

BEFORE YOU BEGIN

This protocol describes the reagents, equipment, and experimental steps required in *C. elegans* glial Ca^{2+} imaging under chemical, mechanical and optogenetic stimulation. Please refer to www.wormbook.org for information related to the basic biology of *Caenorhabditis elegans*.

Preparation of sylgard round coverslip

Timing: 3 days

1. Prepare 1:10 sylgard (Dow Corning) A and B silicon elastomer to act as protective padding for the touch needle in the following experiments.
2. Drop 10 μL of silicon on one side of a coverslip then cover it with another one to spread the silicon appropriately.
3. Separate the coverslips and one side should be covered with silicon.
4. Incubate the prepared coverslips at 75°C for 3 days then store at 4°C.

Note: The sylgard coverslips can be stored at 4°C for a year.

Preparation of all-trans-retinal (ATR) plates for optogenetics

Timing: 3 days approximately

5. Prepare 100 mM ATR stock by dissolving ATR powder in pure ethyl alcohol or absolute ethanol.
6. Dilute the 100 mM ATR stock to 0.1% with OP50 bacteria suspension.

7. Seed the mixed OP50 bacteria with 0.1% (v/v) 100 mM ATR on clean NGM plates.
8. Leave the plates to dry for 2–3 days, store at 4°C (up to 3 weeks), and use the plates away from light.
9. Prepare ethanol control plates (without ATR) by adding ethanol to OP50 bacteria suspension at 0.1% (v/v). Seed the mixed OP50 bacteria with 0.1% (v/v) ethanol on clean NGM plates. Dry and store the plates as described above.

Note: ATR is an essential co-factor required for channelrhodopsin activity in *C. elegans* (Schild and Glauser, 2015; Zou et al., 2018).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Sodium Chloride	Sigma-Aldrich	Cat#S3014
Potassium Chloride	Sigma-Aldrich	Cat#P5405
Magnesium Chloride	Sigma-Aldrich	Cat#M2393
Calcium Chloride	Sigma-Aldrich	Cat#C1016
HEPES	Sigma-Aldrich	Cat#7365-45-9
Glucose	Sigma-Aldrich	Cat#G7021
Sodium Hydroxide	Sigma-Aldrich	Cat#S5881
Isoamyl alcohol (IAA)	Sigma-Aldrich	Cat#W205702
All-trans-Retinal (ATR) powder	Sigma-Aldrich	Cat#R2500
Ethyl alcohol, Pure	Sigma-Aldrich	Cat#459836
Experimental models: Organisms/strains		
C. elegans: Bristol N2	Caenorhabditis Genetics Center (CGC)	N2
C. elegans: kanIs3[*Pvap-1::mCherry+ Pvap-1::GCaMP5.0*]	(Duan et al., 2020)	ST1121
C. elegans: lite-1(ce314); kanEx195 [*Pvap1::frt2::CoChR::sl2::TagRFP+ PT02B11.3::Flp+ Pvap-1::GCaMP5.0*]	(Duan et al., 2020)	ST1761
C. elegans: lite-1(ce314); kanEx631 [*PR11D1.3::CoChR::sl2e::TagRFP+ Psra-6::GCaMp5.0+ Punc122::GFP*]	(Duan et al., 2020)	ST2162
Software and algorithms		
Adobe Illustrator/Lightroom/ Premiere/Photoshop CC	Adobe Inc.	https://www.adobe.com/
ImageJ	National Institutes of Health	https://imagej.nih.gov/ij/
Micro-Manager	Vale Lab, UCSF	https://micro-manager.org/
Other		
3D printed resin Circular chamber	SHINING 3D Tech. Co., Ltd.	https://www.shining3d.com/
3D printed resin Square chamber	SHINING 3D Tech. Co., Ltd.	https://www.shining3d.com/
Borosilicate Glass capillaries	WPI	1B100F-4
Borosilicate Glass capillaries	Vital Sense	B15024F
Corning® cover glasses (square)	Sigma-Aldrich	CLS285522
Greiner round coverslip	Sigma-Aldrich	GN501870
Sylgard™ Silicone Elastomer Kit (A & B)	Dow Corning	184
GLUture® topical tissue adhesive	Zoetis	ZE-10013
Vaseline®	Sigma-Aldrich	Cat#16415
Soft Silicone tubing	AliExpress	N/A
Flaming/Brown micropipette puller	Sutter	P-1000
Inverted microscope	Olympus	IX71
EMCCD camera	Andor	DL-604M-OEM

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Blue light (460–480 nm) LED	Thorlabs	M470L3
Upright microscope	Olympus	BX51WI
T-Cube LED Driver	Thorlabs	LEDD1B
Genuino Uno Rev3	Arduino	A000066
optiMOS™ Scientific CMOS	QImaging	N/A
Multichannel Perfusion system	WPI	MPS-2
Peristaltic Pump	Kamoer	NKCP
Piezoelectric actuator	Physik Instrumente	P-840.30
Micromanipulator	Sutter	MPC-325
HEKA patch clamp amplifier	Warner	EPC10 USB
Worm picker	Beijing Xuesi Chuang Biological Technology	XSCWP08

MATERIALS AND EQUIPMENT

Bath solution

Reagent	Final concentration	Amount
NaCl	145 mM	8.47 g
KCl	2.5 mM	0.186 g
$MgCl_2$	1 mM	0.20 g
$CaCl_2$	5 mM	0.555 g
HEPES	10 mM	2.36 g
glucose	20 mM	3.6 g
ddH_2O	N/A	Fill up to 1000 mL
Total	**N/A**	**1,000 mL**

Note: pH adjusted to 7.3 with NaOH. Can be stored at 4°C for 3 weeks.

Equipment note

3D printed perfusion chambers: In-figure measurements of 3D printed resin circular and square chambers (Figures 3Aa and 4Ab).

Alternatives: Series 20 Perfusion Imaging and Recording Chambers (Warner Instruments, LLC; cat #RC-21BRW) can be used.

STEP-BY-STEP METHOD DETAILS

Preparation before stimulation

Timing: 5–10 min

1. Acquire glass needles from borosilicate glass capillaries using Flaming/Brown micropipette puller.
2. Procure a 30 cm long soft silicone tubing about 1 mm inner diameter.
3. Insert a 200 μL yellow pipette tip at one end of the tube, and a glass needle at the other end to build a glue pipette (Figure 2).
4. Carefully break the needle tip at the edge of the square glass to a worm body size opening. The diameter of the needle tip in our experiment was 10 μm.
5. Place the sterilized yellow pipette tip on the lip to carefully pump the glue (GLUture®).
6. Insert needle tip in the glue.
7. Pump the glue slowly to $1/8^{th}$ of the tip of the needle.

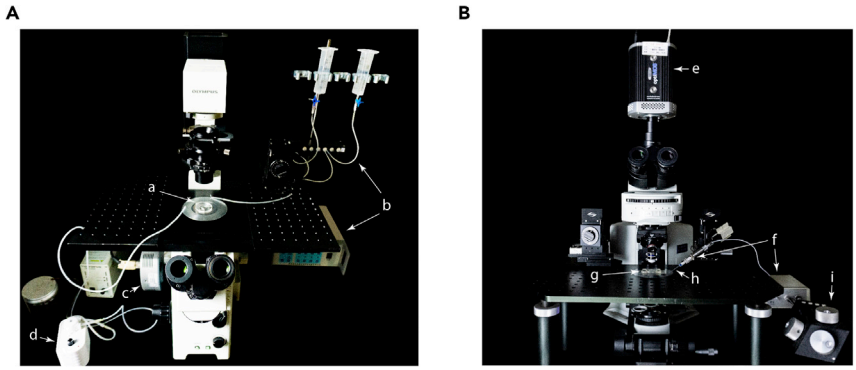


Figure 1. Platforms for calcium imaging

(A) Inverted microscope with perfusion system. a. chamber; b. perfusion system; c. EMCCD camera; d. pump.

(B) Upright microscope with physical stimulus system. e. OptiMOS camera; f. piezoelectric actuator; g. chamber; h. touch needle; i. Micromanipulator.

⚠ CRITICAL: When sucking the glue, one should constantly watch the needle tip under the microscope to avoid overflow of glue in the needle.

Approach I: Odorant/chemical stimuli for glial Ca^{2+} imaging

Timing: 15–20 min

8. Drop 20–40 μL bath solution in the middle of a 22*22 mm square cover glass (Figure 3Ab).
9. Transfer one worm gently using a worm picker from OP50 plate to the bath solution.

Note: If the worm to be glued is dirty (before transferring to bath solution), relocate to a clean NGM plate without OP50 bacteria and drop 5–10 μL bath solution to wash. Then the worm can be moved back to the square glass to be glued.

10. Stabilize the worm on the square glass with the glue using the glue pipette (Figure 3B).

⚠ CRITICAL: Worms should be treated gently and smoothly. The nose of the worm should not be immersed in the glue at any cost, otherwise steps 8–10 should be repeated (practice is required to master these steps). The stabilized part of the worm is decided by the location of the target glia. In the case of AMsh glia, the amphid of worm should be fixed to the square glass, and a small amount of glue is used to cement the tail to avoid movement interference (Figure 3B). Appropriate amount of glue is required; too much would result in death of the worm and too little would allow the worm to move and interfere with the experiment.

11. Wait for 10 min (more or less).

⚠ CRITICAL: There might be some interference when the worms are glued to the square glass. The worm especially its nervous system need time to be at rest before any experiment.

12. Mount the square glass to the bottom of the circular chamber with Vaseline.

Note: Vaseline should be used appropriately not to stick the circular chamber. Vaseline is used as a thin protective layer to avoid friction between the circular chamber and the square glass.

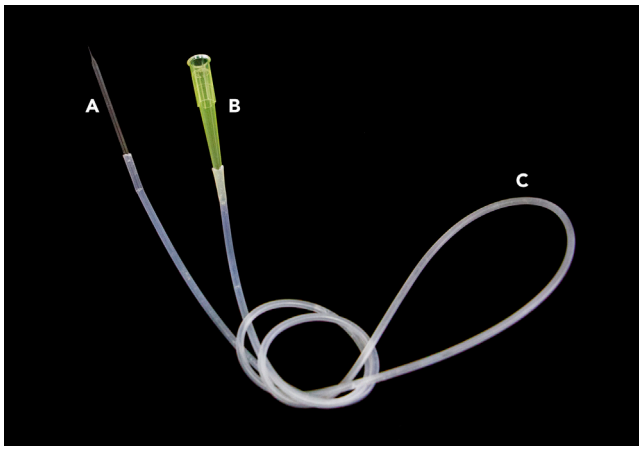


Figure 2. Schematic of glue pipette
(A–C) Glass needle; (B) yellow pipette tip; (C) hollow tube.

13. Place the prepared chamber on the platform of an inverted microscope and focus on the target glia labeled with fluorescence protein and genetically encoded calcium indicators like *GCaMP5.0* in this study.
14. Use the fluid system (MPS-2 Multichannel Perfusion system) to add odorants and chemicals which are mixed with bath solution at appropriate concentration at 0.2 mL/s flow rate (Calculated using the *Hagen-Poiseuille equation* from the instruction manual of MPS-2 Multichannel perfusion system).
15. Keep the pump active all the time to remove bath solution or Isoamyl alcohol (IAA) from the chamber (Figure 1A). IAA is used in this study as an odorant to activate the nematode's glia.

⚠ CRITICAL: The fluid system allows easy switch from bath solution to chemicals. The fluid switch time is crucial in the experiment and the pump should be active before the recording starts.

16. Acquire images using CCD camera.
17. Record the glial calcium responses every second using the Micro-Manager software (Vale Lab, UCSF) at 0–30 s of bath solution application then 60 s of IAA and finally wash with bath solution for 110 s.
18. After the recording is completed, remove the square glass, transfer a new worm and repeat.
19. Use image-J to analyze the calcium imaging data.

Approach II: Mechanical stimuli

Timing: 15–20 min

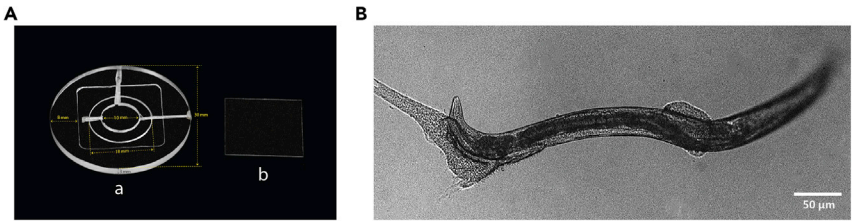


Figure 3. Chemical stimuli set up
(A and B) A a. 3D printed circular chamber; b. square glass; (B) a worm adhered for chemical stimuli experiment.

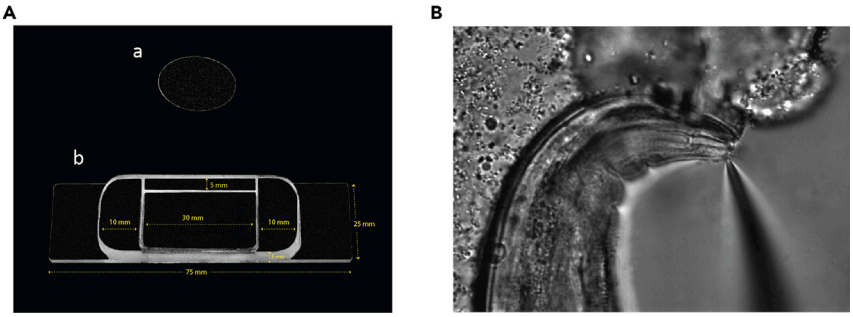


Figure 4. Physical stimuli set up
(A and B) A a. coverslip; b. 3D printed square chamber; (B) a worm adhered for physical stimuli experiment.

Set the amplifier to give a pulse at a specific time point. The piezoelectric actuator can transform 1 volt pulse to 4.5 μm mechanical stimuli (Fan et al., 2021).

20. Drop 20–40 μL bath solution in the middle of the prepared sylgard round coverslip.
21. Transfer one worm from the NGM plate to bath solution.
22. Stabilize one side of the worm (Ventral or Dorsal) with the glue (Figure 4).

⚠ CRITICAL: Delicate manipulations are recommended to stabilize worms under the objective lens. The worm's nose should not be submerged in glue, as it would prevent the worm from mechanical stimuli. Stick one side of the worm to expose specific area to provide stimuli (Figure 4B). In this study, we exposed the nose where the dendrites of AMsh glia can be stimulated by mechanical touch (Methods video S1).

23. Wait for 10 min (more or less).
24. Insert the coverslip into the square chamber filled with bath solution (Figure 4).

Note: Vaseline is not required in this part as square chamber is filled with bath solution so that the coverslip is fully immersed, leaving no bubbles.

25. Position the head of the worm (target area) toward the touch needle by moving either the coverslip or square chamber (Adjust the worm's head adjacent to the piezo-driven needle).
26. Place the prepared chamber on the platform of the microscope and focus on the target glia. (The chamber can be taped on both sides to the platform to avoid movement).
27. Operate the objective lens and touch needle side by side carefully; the touch needle should always be observed under the microscope. The touch needle tip should be very close to the head of the worm (Figure 4B), but should not touch or press it before the experiment starts.

⚠ CRITICAL: The touch needle should be operated very carefully since the piezoelectric actuator is fragile and the needle can easily be broken when in contact to the bottom of the chamber. The touch needle should not be sharp as it will pierce the worm's body instead of providing touch stimuli.

28. Acquire images using a CCD camera. Start recording using the Micro-Manager software, and provide mechanical stimuli though the HEKA EPC-10 amplifier and piezoelectric actuator at a time point of 20 s or 30 s.

Note: Activation of the glia by mechanical stimuli can be observed (Arrowhead denote touch stimuli at 30 s; Methods video S1; Figure 6).

29. Move the microscope and the touch needle out of the bath solution.
30. Repeat with a new coverslip.
31. Use image-J to analyze the calcium imaging data.

Approach III: Optogenetics

Timing: 15–20 min

Note: For optogenetic experiments, worms are cultured on OP50- All-trans retinal (ATR) plates at 20°C for two generations prior to assays. The chromophore all-trans retinal is light-sensitive, so ATR-supplemented NGM plates need to be wrapped in aluminum foil.

32. Adhere the CoChR-transgenic worm which is fed with or without ATR (control) on the square glass using the glue pipette to stabilize the worm.
33. Wait for 10 min (more or less).
34. Place the prepared chamber on the platform of the microscope and focus on the target glia.
35. Reset the exposure time and the interval time to try different stimulation frequency, which is very important to activate glial responses. The interval time was set at 100 ms and the frequency adjusted to 5 Hz (460–480 nm). The exposure time should not be longer than the interval time.

Note: Glia may not be easy to activate if the blue light is kept always on. Under 5Hz blue light, activated glial cells have Ca^{2+} responses (Figure 8). Some glial cells have different activation frequency so a range of frequencies can be tested.

36. After 5 min activation, then start the recording of ASH neurons fluorescence intensity under 1 % IAA stimuli (Similar to Step 13-16 odorant/chemical stimulation).

Note: We used the *lite-1* mutant background strain with CoChR expression in glia like in the *Pvap-1::frt2::CoChR::sl2::TagRFP* strain. Without ATR, the glial cells cannot be activated. We activated the AMsh glia and recorded the ASH neurons' activities under chemical stimuli as an example (Figure 7). Previously, we showed that when the AMsh glia are activated, the ASH neuronal response to 1% IAA will be inhibited (Duan et al., 2020). This model is used to study the functions of glial cells. Step 36 is not required to activate and record AMsh glial calcium signals.

37. Acquire the image.
38. Use image-J to analyze the calcium imaging data.

Note: The Figure 8 is to show that AMsh glia with CoChR expression can be activated by 5 Hz blue light, but it is not suitable for statistical analysis of calcium increases in AMsh glia due to similar baselines of GCaMP and CoChR. To activate and record AMsh glia calcium signals at the same time; mCherry, RCaMP, Cameleon, Chrimson or others are recommended instead of GCaMP and CoChR together (Kerr, 2006; Schild and Glauser, 2015).

Image acquisition

Timing: 5–10 min

Raw images were acquired by a QImaging optiMOS™ Scientific CMOS (sCMOS) or CCD camera under the control of the Micro-Manager software.

⚠ CRITICAL: The exposure time should not be longer than the interval time. In this study, the exposure time was 100 ms and the interval time was 1 s. The interval time should be

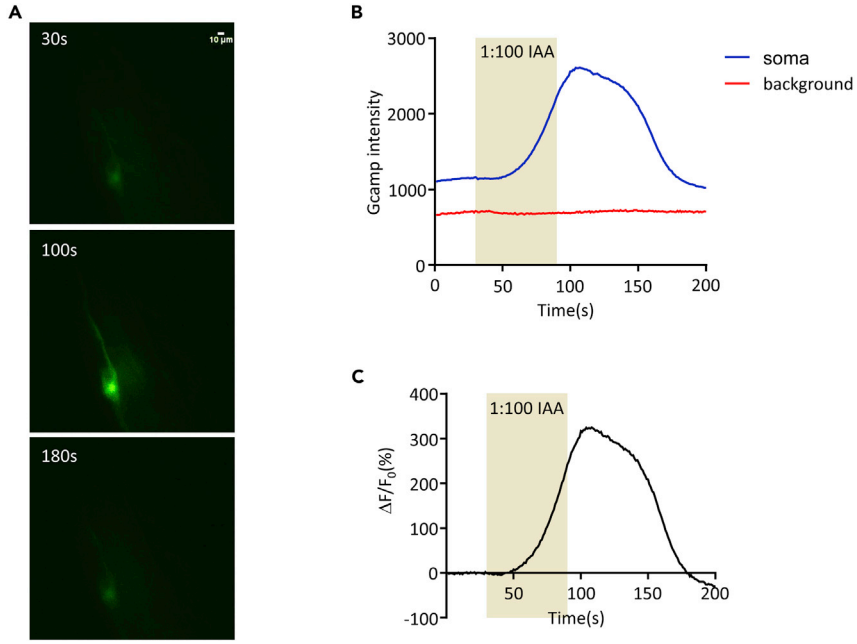


Figure 5. IAA induces calcium elevation in AMsh glia

(A) Calcium transients in AMsh glia visualized with fluorescence and GCaMP5.0. Individual frames taken before, during and after application of 1:100 IAA are shown. Bar: 10 μm.

(B) Fluorescence intensities of calcium increase in AMsh glia with background.

(C) The ratio reflects an increase in intracellular calcium level in AMsh glia under 1:100 IAA stimulation.

between 0.2-1 s. If the time is too long then some small and quick Ca^{2+} signals can be missed; too short will require enough space to store the data.

EXPECTED OUTCOMES

In study, we recorded and analyzed calcium responses of AMsh glia in *C. elegans* under chemical and mechanical stimulation as well as ASH neurons after optogenetical activation of AMsh glia. We used 1/100 IAA odorant to stimulate AMsh glia and measured the calcium levels (Figure 5). For mechanical stimulation of AMsh glia, we applied a 15 μm touch stimuli (Methods video S1; Figure 6B-Touch y-axis) to a properly glued worm (Figure 4B) and the calcium transients were recorded (Figure 6). Lastly, we used a 5 Hz blue light (460–480 nm) to optogenetically activate AMsh glia and record the activities of ASH neurons in response to IAA (Figure 7). We also showed that AMsh glia can be activated with 5 Hz blue light but the calcium recordings are statistically erroneous due to similar baselines of CoChR and GCaMP (Figure 8).

QUANTIFICATION AND STATISTICAL ANALYSIS

Image analysis

Timing: 5–10 min

All image stacks were analyzed using Image-J software.

1. Open the Image-J software and choose "polygon selection" to select around the AMsh glial soma.
2. Choose "intensity v Time Monitor" under "stacks-T-function". The list of intensity data at each time point is named A. The background is the dark area (Figure 9).

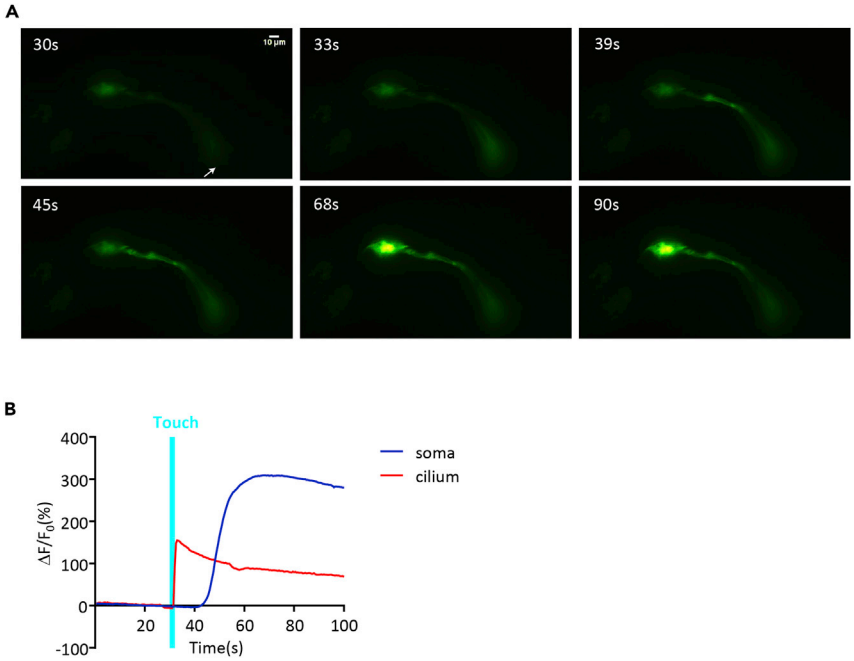


Figure 6. Touch induced-calcium transients in AMsh glia

(A) Calcium transients in AMsh glia visualized with fluorescence and GCaMP5.0. Individual frames taken before, during and after application of 15 µm physical touch (arrowhead) are shown. Bar: 10 µm.

(B) Calcium increase of the cilium and soma in AMsh glia activated by 15 µm physical touch (Touch y-axis).

3. Select the area beside the glia as a background as the ROI (Region of Interest) is not stable since it is more like an average data of that part. It is recommended to keep the same background area compared to glial soma.
4. Rename the background list also as B.
5. F = A-B. The average GCaMP5.0 signal in the first 10 s before stimulation is set as F0 and ΔF/F0 is calculated for each data point.

Note: Similar steps for neuronal Image-J analysis after optogenetical glial activation. When selecting the area around the glia, it should be less than 1.5 times the soma size. The data will vary if the selection is too large or small. In the odorant stimulation, the head of the worm is not fully stabilized, so the nose will move leading to the glia to be out of the selected area for analysis – a larger area around the glia can be selected. Please see Figure 10 for a summary of the protocol steps.

LIMITATIONS

This protocol is suitable for sensory glial cells such as the OL socket glia, AMsh glia (Ding et al., 2015), CEPsh glia and so on. It can be used to test the calcium levels of some sensory neurons (Ge et al., 2020), muscles (Yue et al., 2018) and intestine but the waiting time for the glue to dry varies accordingly.

Note: For optogenetics, specific glial promoters are required; otherwise nearby neurons, glial cells or neuronal circuits can interfere with the calcium recording and analysis (Refer to Fung et al. (2020) for specific glial promoters).

TROUBLESHOOTING

Problem 1

The prepared sylgard coverslip is sticky (step 3 in the preparation of sylgard coverslip).

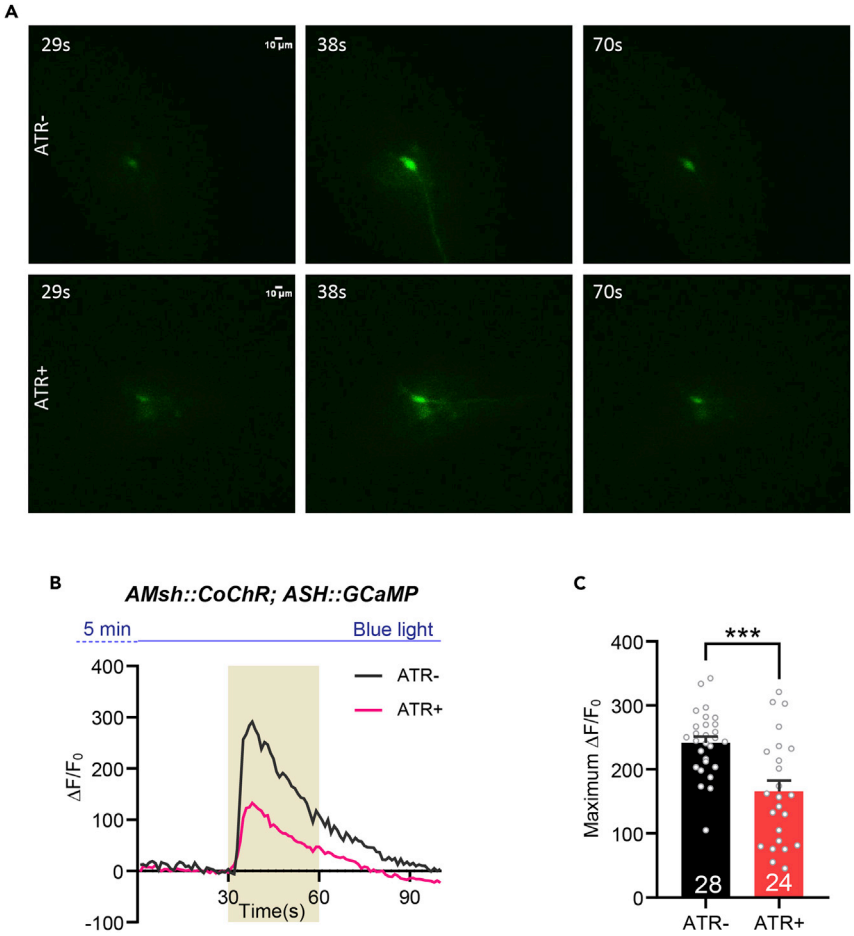


Figure 7. Optogenetic activation of AMsh glia using 5Hz blue light and recording of IAA-induced ASH neuronal calcium transients

(A) IAA-induced calcium transients in ASH neurons visualized with fluorescence and GCaMP5.0. Individual frames taken before, during and after application of 1:100 IAA and optogenetic activation of AMsh glia under blue light are shown. Bar: 20 μm.

(B) Fluorescence intensities of calcium increase in ASH neurons with or without ATR under optogenetic activation of AMsh glia.

(C) Summary bar graph of (B).

Potential solution

If the prepared coverslip is sticky, change the ratio of the Sylgard Dow Corning A and B elastomer from 1/10 to 1/7 or 1/6 (step 1 in the preparation of sylgard round coverslip).

Problem 2

The efficiency of the glue to stick the worm varies and bath solution results are fickle (step 10 in odorant, step 22 in mechanical stimuli, and step 32 in optogenetics).

Potential solution

The glue will dry fast when exposed to the air. After each use, store the glue at 4°C or −20°C, and the same applies to the bath solution. Since the pH of the bath solution has an effect on the sticking power of the glue, the bath solution should not be stored for long. It is recommended to prepare new bath solution every two weeks.

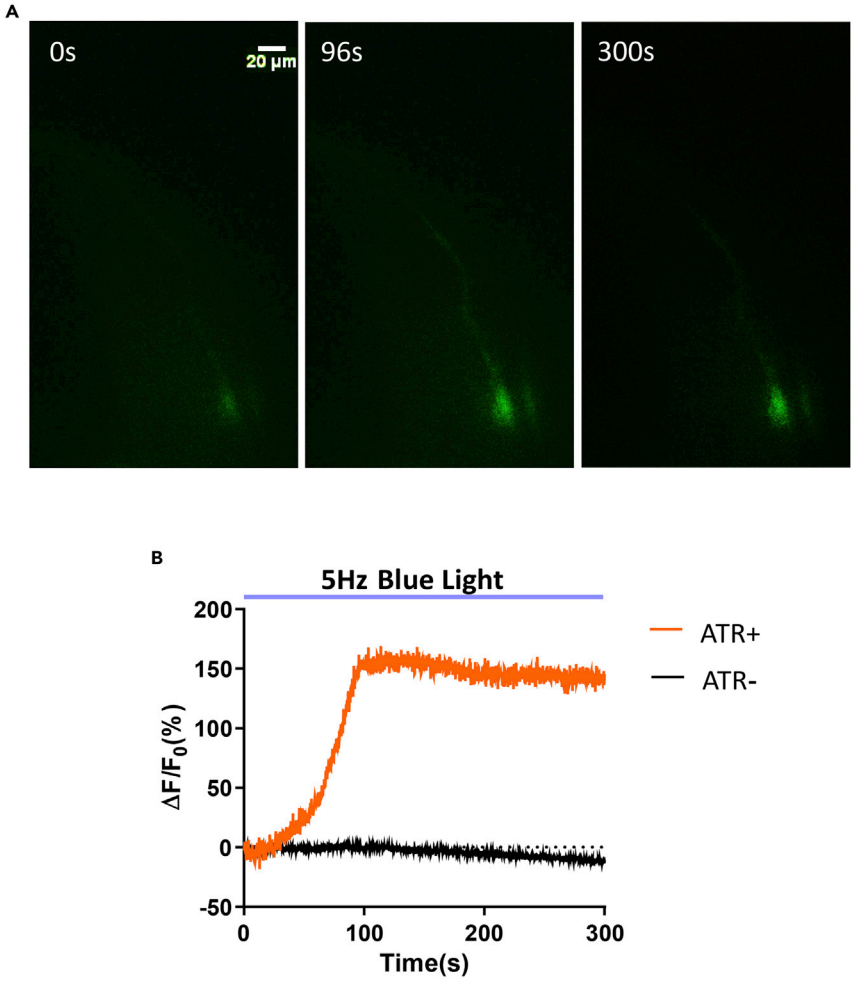


Figure 8. AMsh glia can be activated by 5Hz blue light

(A) Calcium transients in AMsh glia visualized with fluorescence and GCaMP5.0. Individual frames taken before, during and after application of 5Hz blue light are shown. Bar: 20 μm.

(B) Typical fluorescence intensity increase of the AMsh glia activated by 5Hz blue light of cultivated worms with or without ATR.

Problem 3

The mouth of the worm is easily glued or the glue is spread across (step 10 in odorant, step 22 in mechanical stimuli, and step 32 in optogenetics).

Potential solution

The nematode's cilia which extend to environment should not be glued. The worm's body should be glued to the square glass or pad first then the head. Although this procedure can be tricky at the beginning, it will be easier with practice.

Problem 4

While recording the target glia, the worm moves or jiggles, disturbing the focus and recording (step 16 in odorant stimuli).

Potential solution

This may be due to the short rest time after gluing the worm. If the rest time is short, wait some more minutes until the worm is stabilized. If the worm is not stabilized properly, the head of worm can be glued completely avoiding the nose (step 10 in odorant stimuli and Step 32 in optogenetics).

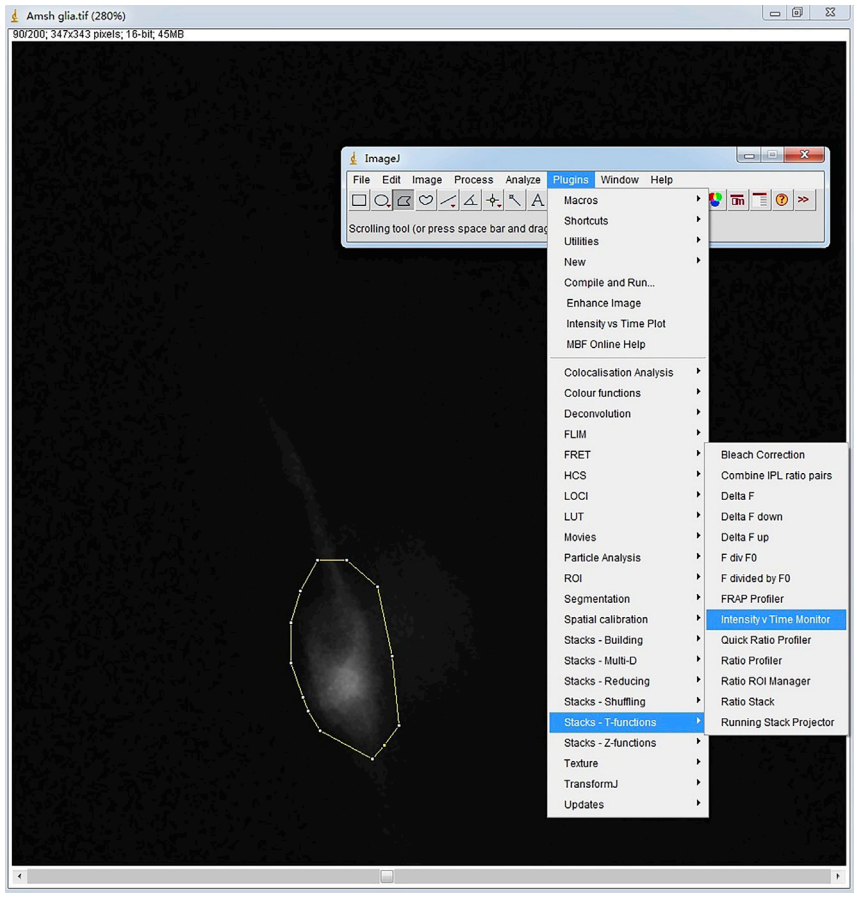


Figure 9. Image-J analysis

Problem 5

When stimulating the worm by touch, there was no response (step 28 in mechanical stimuli).

Potential solution

To find the touch area, several trials are needed as the touch needle might not be at the right position. The coverslip, on which the worm is glued, can be moved to adjust the head of the worm near the touch needle (step 25 in mechanical stimuli). Once the touch area is located then the worm should not be moved.

Problem 6

If the touch area is located but there was low or no response upon touch stimulation (step 28 in mechanical stimuli).

Potential solution

This may occur if the angle of the touch needle is not appropriate. The mechanical arm of the piezoelectric actuator can be adjusted to a steep angle (30°–55°) and slow manipulation of touch needle to the touch area. If the problem is not the touch angle (step 25 in mechanical stimuli) and still there was low or no response upon touch stimulation then it might be due to the width of the touch needle. Therefore, the needle tip can be broken carefully at the edge of the coverslip to make a wider needle tip.

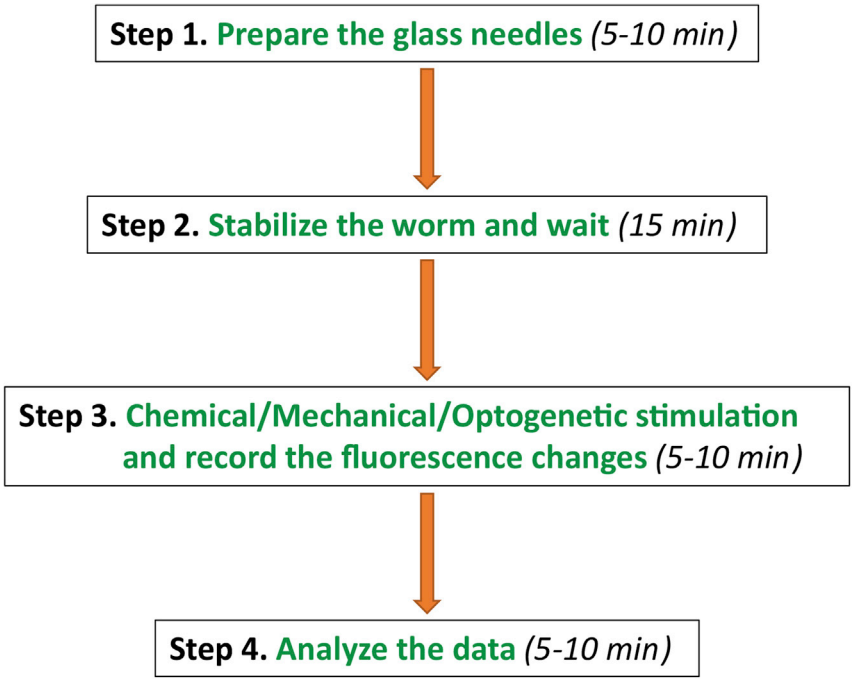


Figure 10. Step summary

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the lead contact - Kang Lijun, kanglijun@zju.edu.cn or Technical Contact - Cheng Hankui, 11818106@zju.edu.cn.

Materials availability

The strains generated in this study are available upon request.

Data and code availability

This study did not generate unique datasets or codes.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2022.101169.

ACKNOWLEDGMENTS

This work was supported by grants from the National Foundation of Natural Science of China (31771113, 31471023, 31800878, and 31900736), Zhejiang Provincial Natural Science Foundation (LZ22C090001), and The Fellowship of China National Postdoctoral Program for Innovative Talents (BX20200297). We thank the *Caenorhabditis Genetic Center* (CGC), which is supported by the National Institutes of Health - Office of Research Infrastructure Programs (P40 OD010440), for strains.

AUTHOR CONTRIBUTIONS

L.K., H.C., and U.A. conceived and optimized the protocol. H.C., U.A., D.C., and D.D. performed the experiments and analyzed the data. H.C. and U.A. wrote the manuscript. L.K., H.C., and U.A. revised and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Ding, G., Zou, W., Zhang, H., Xue, Y., Cai, Y., Huang, G., Chen, L., Duan, S., and Kang, L. (2015). In vivo tactile stimulation-evoked responses in Caenorhabditis elegans amphid sheath glia. PLoS ONE *10*, e0117114.

Duan, D., Zhang, H., Yue, X., Fan, Y., Xue, Y., Shao, J., Ding, G., Chen, D., Li, S., Cheng, H., et al. (2020). Sensory glia detect repulsive odorants and drive olfactory adaptation. Neuron *108*, 707–721.e708.

Fan, Y., Zou, W., Liu, J., Al-Sheikh, U., Cheng, H., Duan, D., Du, C., Liu, S., Chen, L., Xu, J., et al. (2021). Polymodal functionality of *C. elegans* OLL neurons in mechanosensation and thermosensation. Neurosci. Bull *37*, 611–622.

Fung, W., Wexler, L., and Heiman, M.G. (2020). Cell-type-specific promoters for *C. elegans* glia. J. Neurogenet. *34*, 335–346.

Ge, M.H., Wang, W., Wu, T.H., Wen, X., Al-Sheikh, U., Chen, L.L., Yin, S.W., Wu, J.J., Huang, J.H., He, Q.Q., et al. (2020). Dual recombining-out system for spatiotemporal gene expression in C. elegans. iScience *23*, 101567.

Kerr, R.A. (2006). Imaging the Activity of Neurons and Muscles (WormBook), pp. 1–13.

Schild, L.C., and Glauser, D.A. (2015). Dual color neural activation and behavior control with chrimson and CoChR in *Caenorhabditis elegans*. Genetics *200*, 1029–1034.

Yue, X., Zhao, J., Li, X., Fan, Y., Duan, D., Zhang, X., Zou, W., Sheng, Y., Zhang, T., Yang, Q., et al. (2018). TMC proteins modulate egg laying and membrane excitability through a background leak conductance in *C. elegans*. Neuron *97*, 571–585.e575.

Zou, W., Fu, J., Zhang, H., Du, K., Huang, W., Yu, J., Li, S., Fan, Y., Baylis, H.A., Gao, S., et al. (2018). Decoding the intensity of sensory input by two glutamate receptors in one *C. elegans* interneuron. Nat. Commun. *9*, 4311.