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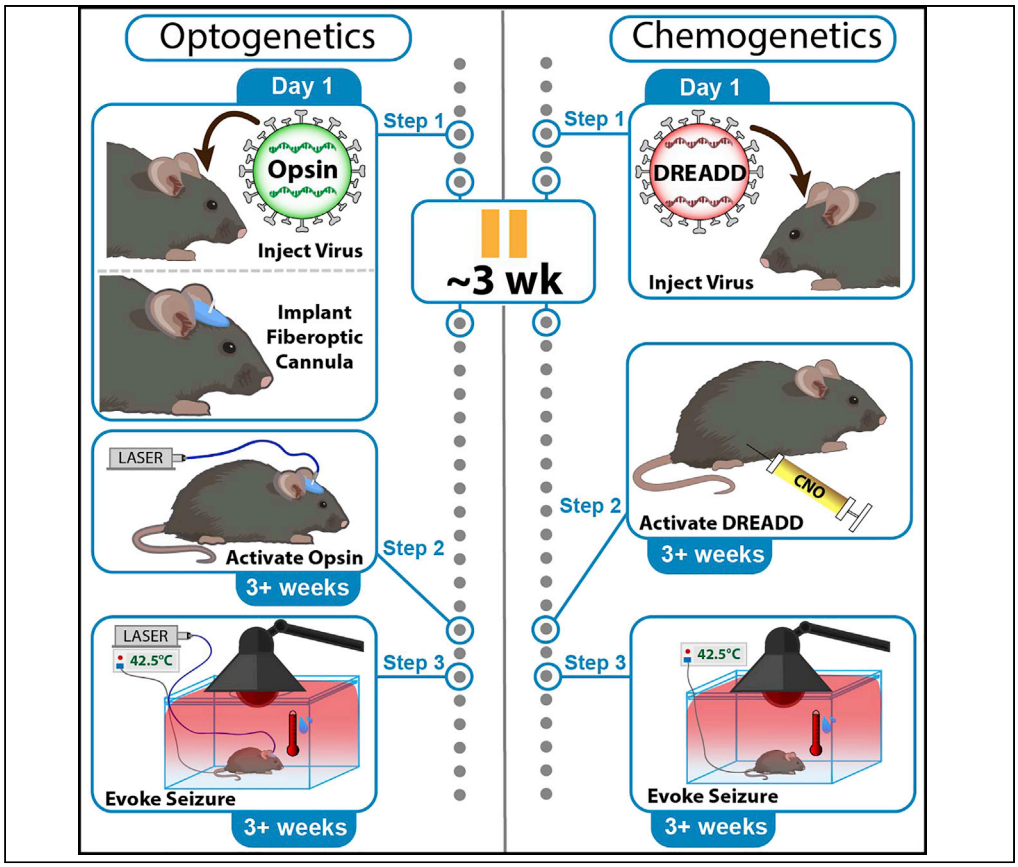
Optogenetic and chemogenetic manipulation of seizure threshold in mice



Here, we present a protocol using optogenetics or chemogenetics to assess the neuronal circuits contributing to seizure initiation. Both approaches allow for targeted control of neuronal populations *in vivo* and can be combined with experimental manipulations to acutely induce seizures in rodent models. We describe how to (1) introduce and (2) activate optogenetic or chemogenetic actuators while (3) inducing seizures via hyperthermia in a mouse model of epilepsy. This protocol can be adapted for use in other induced seizure models.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Julia A. Kravchenko, Ethan M. Goldberg, Joanna Mattis

jkravche@umich.edu (J.A.K.)
jomattis@med.umich.edu (J.M.)

Highlights

Stereotactic surgery for targeted infusion of virus into the rodent brain

Manipulation of neuronal activity *in vivo* via optogenetics or chemogenetics

Acute seizure induction via hyperthermia

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Optogenetic and chemogenetic manipulation of seizure threshold in mice

Julia A. Kravchenko,[1,6,*] Ethan M. Goldberg,[2,3,4] and Joanna Mattis[1,5,7,*]

[1]Department of Neurology, University of Michigan Medical Center, Ann Arbor, MI 48109, USA

[2]Division of Neurology, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA

[3]Department of Neurology, The University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA

[4]Department of Neuroscience, The University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA

[5]Neuroscience Graduate Program, University of Michigan Medical Center, Ann Arbor, MI 48109, USA

[6]Technical contact

[7]Lead contact

*Correspondence: jkravche@umich.edu (J.A.K.), jomattis@med.umich.edu (J.M.)
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SUMMARY

Here, we present a protocol using optogenetics or chemogenetics to assess the neuronal circuits contributing to seizure initiation. Both approaches allow for targeted control of neuronal populations *in vivo* and can be combined with experimental manipulations to acutely induce seizures in rodent models. We describe how to (1) introduce and (2) activate optogenetic or chemogenetic actuators while (3) inducing seizures via hyperthermia in a mouse model of epilepsy. This protocol can be adapted for use in other induced seizure models.

For complete details on the use and execution of this protocol, please refer to Mattis et al. (2022).[1]

BEFORE YOU BEGIN

Epilepsy is a neurologic condition defined by the propensity for recurrent seizures, or paroxysmal bursts of uncontrolled electrical activity in the brain. To progress our understanding of epilepsy, and to identify future therapeutic targets, it is essential to identify circuit mechanisms of seizure initiation.

Classical neuroscience techniques, including targeted lesions and electrical stimulation, have identified key brain regions involved in seizure initiation.[2–5] However, these strategies lack the cell-type resolution necessary to causally implicate defined neuronal populations (which may be co-mingled within a brain region) in ictogenesis. This limitation may be overcome by the development of optogenetics and chemogenetics. Optogenetics introduces light-sensitive opsins into neural tissue which, when exposed to light, can activate or inhibit opsin-expressing neurons with millisecond-timescale precision.[6,7] Chemogenetics involves the targeted expression of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) which, when activated by a synthetic ligand, can activate neurons on a timescale of minutes to hours without impacting endogenous signaling.[8,9]

When applied in pre-clinical models of epilepsy, optogenetic and chemogenetic tools can enable causal investigation of the contributions of specific cell types involved in seizure onset. One experimental approach is to study spontaneous seizures in animals with epilepsy, either by measuring a change in seizure frequency following stimulation,[10–13] or by detecting seizures online and stimulating upon seizure onset.[14–18] However, it may be difficult to achieve adequate statistical power to detect an effect if spontaneous seizure frequency is low at baseline, and an

automated closed-loop paradigm is challenging to implement. An alternative approach is to test the impact of circuit manipulation on acutely induced seizures, for example via electrical kindling or chemoconvulsant administration.[19–21] However, the circuit mechanisms of exogenously triggered seizures may differ from those underlying spontaneous seizures, limiting the predictive validity of these induced seizure models.

Acutely engaging endogenous seizure predisposition in animals that have epilepsy offers a third approach to circuit dissection. Fevers are a common cause of breakthrough seizures in patients with epilepsy,[22,23] and fever-induced seizures are a defining feature of Dravet Syndrome and other genetic epilepsies.[24–26] Consistent with this, multiple animal models of epilepsy have been developed to exhibit hyperthermia-evoked seizures, triggered by elevation of environmental temperature.[27–31] Combining hyperthermia-evoked seizures with optogenetic or chemogenetic techniques offers a naturalistic, acute seizure method to dissect circuit mechanisms of ictogenesis.[1] In this protocol, we describe how to manipulate hyperthermia-evoked seizure temperature threshold using optogenetics or chemogenetics *in vivo*.

Institutional permissions

All experimental procedures were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) at the University of Michigan.

Obtain virus for optogenetic or chemogenetic manipulation of neurons

Timing: 3 weeks

Note: Timing is based on shipping estimates.

1. Order Virus.
   a. Viruses can be ordered through a plasmid repository or Vector Core (such as Addgene or UNC Vector Core).
      i. Be sure to select the appropriate serotype, promoter, recombinase dependence (if desired), gene, and fluorophore.
      ii. A control virus (if necessary) should encode the fluorophore alone, with matched serotype, promoter, and recombinase dependence.

Note: In Mattis et al.,[1] we used AAV9-CaMKIIα-hChR2(H134R)-EYFP to optogenetically target excitatory projection neurons in the entorhinal cortex. We used AAV8-hSyn-DIO-hM4D(G_i)-mCherry to chemogenetically target parvalbumin-expressing inhibitory interneurons in the dentate gyrus (in transgenic mice expressing Cre recombinase in parvalbumin neurons).

2. Aliquot and Store.
   a. Virus must be stored at −80°C for long-term stability and protected from repeated freeze thaw cycles.
      i. Upon receiving the virus, thaw and make aliquots, storing them at −80°C.
      ii. Thaw individual aliquots at time of use.

Set up for surgery

Timing: 30 min

Prior to completing surgery, ensure proper compliance with the regulatory guidelines from your institution for rodent survival surgery. Note the materials from step 3c (Figure 1C) are unnecessary for the chemogenetic approach.

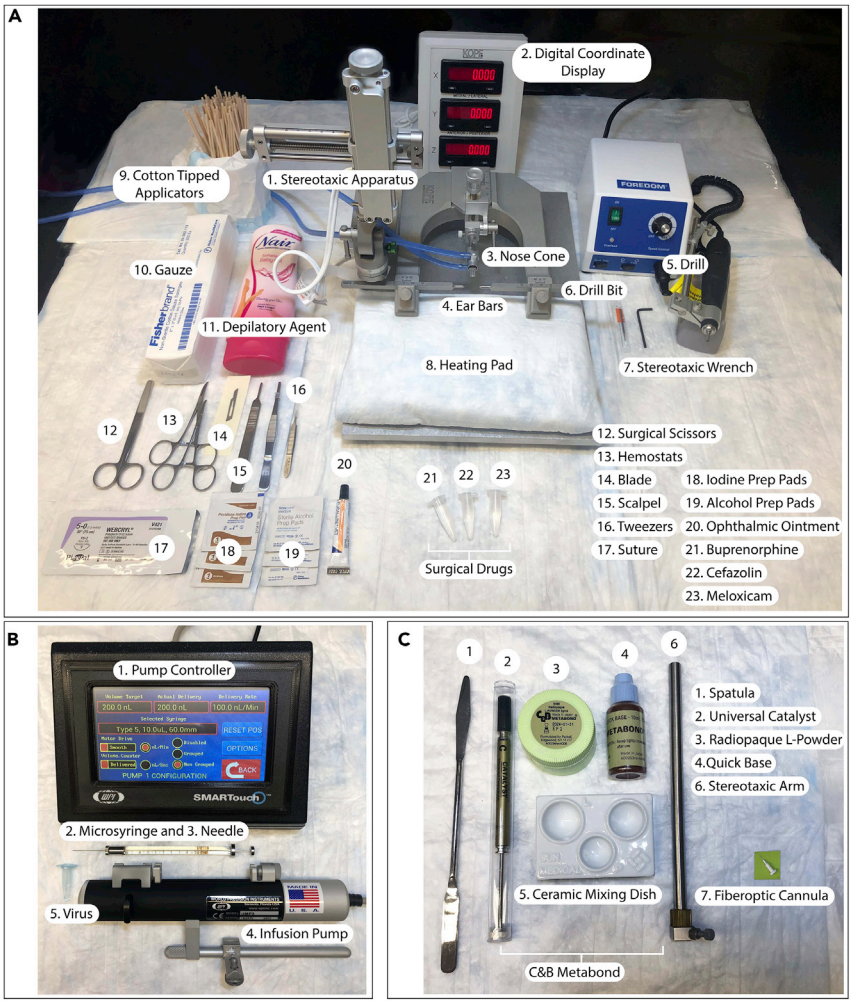

Figure 1. Surgical supplies

(A) Basic surgical supplies needed for this protocol.

(B) Additional supplies required for virus infusion surgery.

(C) Additional supplies required for fiberoptic implantation surgery.

3. Gather supplies for surgery (Figure 1).
   a. Basic surgical supplies, including hardware, surgical materials, and drugs (Figure 1A).
      i. Stereotaxic Apparatus.
      ii. Digital Coordinate Display.
      iii. Nose Cone.
      iv. Ear Bars.
      v. Drill.
      vi. Drill Bit.
      vii. Stereotax Wrench.
      viii. Heating Pad.
      ix. Cotton Tipped Applicators.
      x. Gauze.
      xi. Depilatory Agent.
      xii. Surgical Scissors.
      xiii. Hemostats.

xiv. Blade.
xv. Scalpel.
xvi. Tweezers.
xvii. Suture.
xviii. Iodine Prep Pads.
xix. Sterile Alcohol Prep Pads.
xx. Ophthalmic Ointment.
xxi. Buprenorphine.
xxii. Cefazolin.
xxiii. Meloxicam.

Note: A stereoscope (not pictured) may be helpful for visualization and troubleshooting purposes.

b. Supplies for virus infusion (Figure 1B).
   i. Pump Controller.
   ii. 10 μL Micro-Syringe.
   iii. 33G Beveled Needle.
   iv. Infusion Pump.
   v. Individual Virus Aliquot.

Note: Dilute the virus aliquot as necessary in pre-chilled saline to the final working concentration. Individual aliquots are typically stable at 4°C for several days, which allows some flexibility for surgeries. Discard any residual virus rather than re-freezing.

c. Supplies for cannula implantation, including application tools and adhesive cement (C&B Metabond) (Figure 1C).
   i. Spatula.
   ii. Universal Catalyst.
   iii. Radiopaque L-Powder.
   iv. Quick Base.
   v. Ceramic Mixing Dish.
   vi. Stereotaxic Arm.
   vii. Fiberoptic Cannula.

4. Prepare experimental animals.
   a. Ensure that your mouse is at the appropriate age and weight for survival surgery with anesthetic and that the procedure has been approved by your regulatory institution.

Note: In Mattis et al.,[1] we used haploinsufficient *Scn1a*$^{+/-}$ mice,[31–34] available commercially via The Jackson Laboratory. We maintained the *Scn1a*$^{+/-}$ mouse line on a 129S6.SvEvTac background, on which they do not express an overt phenotype. To generate experimental mice, we crossed male *Scn1a*$^{+/-}$ mice with female wild type or Cre-driver mice on a C57BL/6J background. The F1 mice (on a 50:50 mixed 129S6:BL6/J background) were used for experiments. Further details regarding this breeding scheme, as well as specific Cre-driver lines, can be found in Favero et al.,[35] and Mattis et al.[1]

Configure laser hardware

Timing: 10 min

The laser should be selected for wavelength based on the activation spectrum of the expressed opsin. Note that this preparation is only necessary for the optogenetic approach.

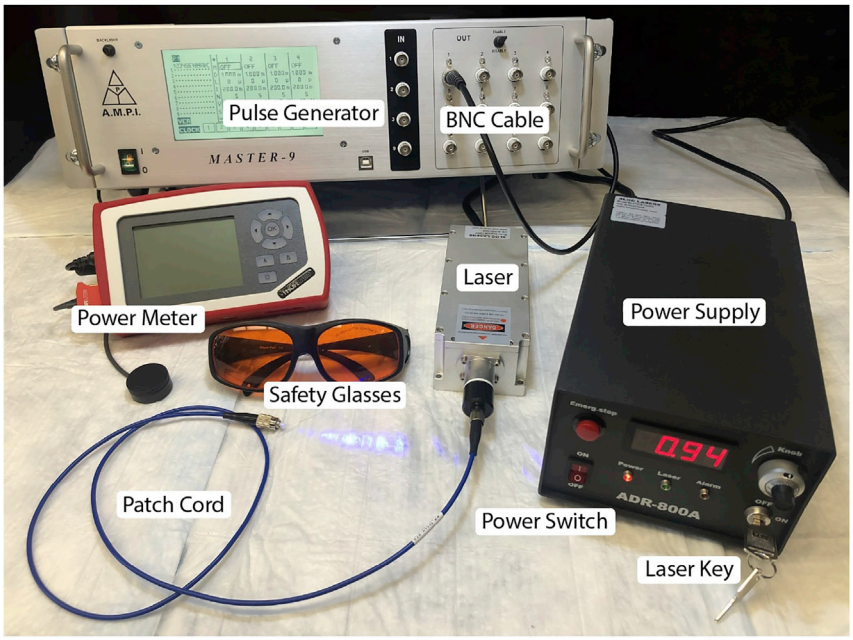

Figure 2. Laser hardware
The equipment necessary for activation of opsins *in vivo*.

5. Gather hardware (Figure 2).
   a. Pulse Generator (e.g., Master-9).
   b. Laser.
   c. Power Supply.
   d. Patch Cord.
   e. Male-Male BNC Cable.
   f. Power Meter.
   g. Safety Glasses.
6. Connect the components.
   a. Use the cords provided by the manufacturer to connect the laser to the power supply.
   b. Connect the pulse generator to the power supply using a male-male BNC cable.
   c. Connect the patch cord to the laser.
   d. For global power control, use the power switch on the power supply. To control light emission from the laser, use the laser key.

⚠ CRITICAL: Always use safety glasses while operating the laser.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
pAAV-CaMKIIα-hChR2(H134R)-EYFP	Lee et al.[36]	RRID:Addgene_26969
AAV9-CamKIIα-eYFP-WPRE-hGH	Lee et al.[36]; UNC Vector Core	Available upon request.
pAAV-hSyn-DIO-hM4D(G_i)-mCherry	Krashes et al.[9]	RRID:Addgene_44362
Chemicals, peptides, and recombinant proteins		
Buprenorphine	Millipore Sigma	Cat#B-044

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Cefazolin	Fisher Scientific	Cat#C22425G
Meloxicam	Fisher Scientific	Cat#AC459550050
Isoflurane	Unit for Laboratory Animal Medicine – University of Michigan	N/A
Clozapine N-oxide	Fisher Scientific	Cat#NC1044836
DMSO	Fisher Scientific	Cat#D12345
Saline	Henry Schein	Cat#1048688
Experimental models: Organisms/strains		
Mouse: B6;129P2-Pvalb$^{tm1(cre)Arbr/J}$ (Male and female mice; 4–10 weeks)	The Jackson Laboratory	RRID:IMSR_JAX: 017320
Mouse: Scn1a$^{+/-}$: 129S-Scn1a^{tm1Kea}/Mmjax (Male and female mice; 4–10 weeks)	The Jackson Laboratory	RRID:MMRRC_037107-JAX
Mouse: C57BL/6J (Male and female mice; 4–10 weeks)	The Jackson Laboratory	RRID: IMSR_JAX:000664
Other		
Stereotaxic apparatus with accessories	David Kopf Instruments	Model 940
Leica Stereoscope S9i	Leica	S9i
Anesthesia equipment	Vetamac Inc.	N/A
K.1070 Micromotor Kit	Foredom	K.1070
Burrs for micro drill	Fine Science Tools	Cat#19007-14
Heating pad	Scrip Hessco	Cat#2320086
Cotton-tipped applicators	Fisher Scientific	Cat#10-000-692
Gauze	Fisher Scientific	Cat#22-362178
Nair hair removal lotion	Amazon	N/A
Surgical scissors	Fine Science Tools	Cat#14007-14
Hartman hemostats	Fine Science Tools	Cat#13003-10
Blade	Fine Science Tools	Cat#10015-00
Scalpel	Fine Science Tools	Cat#10003-12
Tweezers	Fine Science Tools	Cat#11064-07
Suture	AllMedTech	Cat#21275168
Iodine prep pads	Fisher Scientific	Cat#19-027048
Alcohol prep pads	Fisher Scientific	Cat#22-363-750
Ophthalmic ointment	Fisher Scientific	Cat#NC1886507
Microinjection syringe pump	World Precision Instruments	Cat#UMP3T-2
Nanofil syringe (10 μL)	World Precision Instruments	Cat#0204C
Nanofil 33G Beveled Needle	World Precision Instruments	Cat#NF33BV-2
Spatula	Fisher Scientific	Cat#14-357Q
Fiberoptic cannula	Doric	Cat#ZF1.25
C&B Metabond	Patterson Dental	Cat#S380
Master-9	A.M.P. Instruments	Cat#318814
Laser	Shanghai Laser & Optics Century Co., Ltd.	BL473T8-100FC
BNC cable	ThorLabs	Cat#22-49-C12
Power meter	ThorLabs	Cat#PM121D
Ceramic sleeve	Precision Fiber Products	Cat# 176331
Heat lamp with table clamp	Physitemp	HL-1
Microprobe thermometer	Physitemp	BAT-12
Rectal probe for small animals	Physitemp	RET4
Heating chamber	This Paper	N/A
Scale	Fisher Scientific	Cat#S72422
Disposable syringes	Fisher Scientific	Cat#14-955-464
Fluorescent microscope	Nikon	Eclipse Ni-E

MATERIALS AND EQUIPMENT

Clozapine N-oxide (CNO) is the agonist for DREADD human M4 muscarinic (hM4) receptor. The CNO solution is made as a stock that is then thawed and diluted immediately prior to experimentation. If using the optogenetic approach, this preparation is unnecessary.

CNO stock solution		
Reagent	Final concentration	Amount
Clozapine N-oxide	N/A	10 mg
DMSO, anhydrous	N/A	117 μL
Sterile Saline	N/A	2.803 mL
Total	**10 mM**	**2.92 mL**

Make six 450 μL aliquots and one 200 μL aliquot of the CNO stock solution. The 450 μL aliquot will be enough for 5 mice weighing no more than 30 g while the 200 μL aliquot will be enough for 2 mice weighing no more than 30 g. Freeze aliquots −20°C and store for up to 3 months.

STEP-BY-STEP METHOD DETAILS

Virus injection surgery

Timing: ~60 min / mouse

Virus injection surgery is completed using a stereotaxic apparatus to enable precise targeting of neuronal structures. The methods in Mattis et al.[1] targeted both the entorhinal cortex and the dentate gyrus. However, for illustration, coordinates used in this protocol will only target the entorhinal cortex. Note that this same procedure can be used to introduce virus into any brain region using coordinates identified via a mouse brain atlas.[37]

1. Anesthetize the mouse.
   a. Weigh the mouse to calculate appropriate dosages for drugs for post-operative pain and infection control. We used the following:
      i. Buprenorphine (0.5–1.0 mg/kg in sterile saline; sub-cutaneous).
      ii. Cefazolin (500 mg/kg in sterile saline; sub-cutaneous).
      iii. Meloxicam (5 mg/kg in sterile saline; sub-cutaneous).

   Alternatives: Check with your institution for up-to-date guidelines and official recommendations.

   b. Anesthetize with inhaled isoflurane (4%) in 100% oxygen in an induction chamber.
      i. This takes approximately 1–3 min depending on the age and size of the mouse.
      ii. Monitor respiratory rate (1/s) and loss of toe/tail-pinch reflex for successful induction. Troubleshooting 1.
   c. Reduce anesthesia to 0.5%–2% isoflurane in 100% oxygen and redirect the flow to the stereotax. Isoflurane levels should be continually titrated for the remainder of the surgery to maintain appropriate depth of anesthesia.
2. Position and prepare the mouse in the stereotaxic frame (Figure 3A).
   a. Transfer the mouse from the induction box to the stereotax. Place the nose in the nose cone and secure it in place by hooking the upper incisors over a bar within the nose cone.
   b. Place a heating pad underneath the mouse to prevent hypothermia.
   c. Gently insert the ear bars and tighten to secure the mouse.

Figure 3. Virus injection

(A) The mouse is secured in the stereotax via ear bars, with its nose in the nose cone for maintenance of inhaled anesthesia. The fur is removed from the scalp in preparation for an incision.

(B) The skull is exposed. The drill is posed to bore a hole at the desired coordinates.

(C) The needle tip is positioned at the desired coordinates to enable viral injection into the mouse brain.

⚠ CRITICAL: Ensure that when securing the ear bars in the frame, the position markings on the ear bars relative to the stereotax are aligned on both sides – i.e., the skull is centered within the stereotax. This will ensure minimal repositioning during subsequent steps.

Note: Check positioning by gently attempting to adjust the head side to side as well as up and down. Proper positioning of the ear bars will prevent this movement.

d. Cover the eyes in ophthalmic eye ointment to prevent drying.
e. Apply a depilatory agent onto the scalp. Remove it with a sterile cotton tipped applicator after 2–3 min.

Alternatives: Shaving the surgical area is also acceptable, though it may increase the risk of potential infection if not thoroughly disinfected.

f. Sterilize the scalp with three rounds of iodine and alcohol wipes.

3. Make an incision.
   a. Use a blade to make a single midline incision.
   b. Use the same blade to gently scrape away the tissue from the top of the skull.
   c. Use hemostats secured to the skin to keep the surgical field exposed.
   d. Clear the surgical field with gauze and cotton tipped applicators.
4. Center and level the skull.
   a. Attach a drill to the stereotaxic arm, navigate to Bregma, and set this location as A/P: 0, M/L: 0, D/V: 0.

b. Navigate to Lambda, typically ~4.5 mm posterior to Bregma in an adult mouse. Ensure that the D/V coordinates are within 0.05 mm relative to Bregma.
   i. If not, change the angle of skull by either raising or lowering the nose cone; repeat steps 4a and 4b.

5. Drill (Figure 3B).
   a. Navigate to the virus injection coordinate(s) and drill a small hole at each site. For instance, for the (right) entorhinal cortex, the injection coordinate (relative to Bregma in mm) is:
      i. A/P: -4.5, M/L: 3.0.
   b. If completing *in vivo* optogenetics, drill an additional hole (if needed) for the fiberoptic cannula placement.

⚠ CRITICAL: Drill slowly by applying only gentle pressure to the skull to avoid causing trauma to the brain. The sound of the drill will increase in pitch as the drill advances through the skull. You may also see a small amount of fluid leak. Stop immediately.

6. Prepare the injection system.
   a. Attach a 33 G beveled needle on a 10 μL micro-syringe to the infusion pump secured to the stereotax.
   b. Withdraw an appropriate volume of your chosen virus. This can be done manually or automatically with the infusion pump.
   c. Ensure that the pump is set to injection mode.
   d. Navigate to Bregma, ensuring that the tip of the needle just touches the surface of the skull and set this location as A/P: 0, M/L: 0, D/V: 0.
   e. Navigate to the injection site: A/P: -4.5, M/L: 3.0, D/V: -4.2.
      i. Move slowly in the D/V axis to prevent excessive tissue damage.
7. Inject virus (Figure 3C). Troubleshooting 2.
   a. Slowly inject the desired volume of virus.
      i. We injected 200 nL at 100 nL/min at each site.
   b. Leave the needle inserted for 5–10 min for proper virus diffusion and to prevent leak of virus from the injection site upon removal of the needle.
   c. Slowly withdraw the needle.
   d. If completing additional injections, repeat steps 6b–7c.

⚠ CRITICAL: If utilizing *in vivo* chemogenetics, continue with step 8. If using optogenetic virus proceed directly to step 10.

8. Complete the Surgery.
   a. Remove the hemostats.
   b. Close the incision site using sutures.

   Alternatives: Depending on preference and existing institutional guidelines, glue may also be used.

   c. Remove the mouse from the stereotax.
   d. Place the mouse in a recovery chamber with a heating pad.
      i. Monitor every 15 min until regular mobility and behavior is observed.
   e. Return the animal to its home cage.
9. Provide post-operative care.
   a. Administer a second dose of Meloxicam 24 h post-surgery.
      i. If the mouse continues to show signs of pain (i.e., decreased activity, hunched posture, scruffy or greasy coat) after the first day, provide additional injections spaced 24 h apart.
   b. Monitor pain and activity levels twice a day on the first day of post-op.
      i. Continue monitoring daily for the duration of the experiment.

Alternatives: Check with your institution for up-to-date post-operative care guidelines.

Pause point: Wait for optimal viral expression before performing experiments. Typical waiting time is at least 3 weeks, but the precise time must be empirically determined based on the specific experimental parameters, including viral serotype, promoter, titer, and volume.

Fiberoptic cannula implantation

Timing: ~30 min/mouse

For *in vivo* optogenetics, fiberoptic cannulas are implanted to allow laser light to target the desired brain structure. The implantation surgery can be completed in the same surgical session as the viral injections.

10. Implant the fiberoptic cannula.
    a. Attach the fiberoptic cannula to the stereotaxic arm (Figure 4B).
    b. Navigate to Bregma using the cannula and set this location as A/P: 0, M/L: 0, D/V: 0.
    c. Find the implant coordinates and insert slowly.
        i. For the right entorhinal cortex, insert at A/P: -4.5, M/L: 3.0, D/V: -3.9.

Note: The desired activation volume of the targeted brain region, wavelength, power density, and opsin sensitivity play a role in determining the optimal distance between the virus

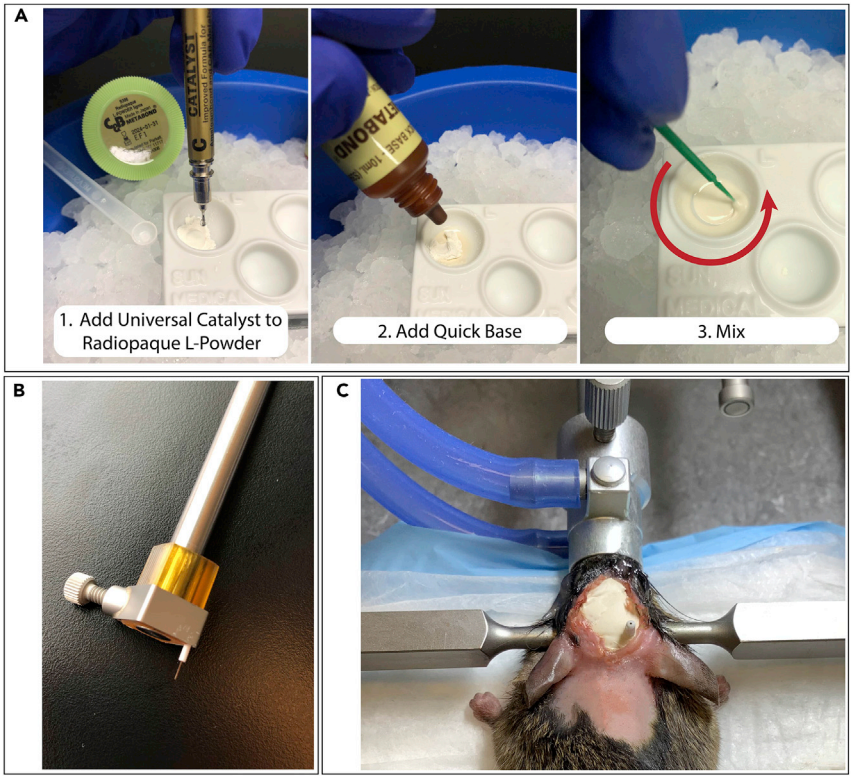

Figure 4. Fiberoptic cannula implantation

(A) Mixing of C&B Metabond adhesive cement, which is used to affix the fiberoptic cannula to the skull.

(B) A fiberoptic cannula is secured to a detachable arm of the stereotax.

(C) The mouse with an implanted fiberoptic cannula secured with C&B Metabond. Note that this image is prior to suturing the skin, in order to visualize that the dental cement is in direct contact with a large surface area of the skull.

injection site and fiberoptic cannula.[6] In practice, we placed the fiberoptic cannula 0.1–0.4 mm dorsal to the virus injection site.

⚠ CRITICAL: The fiberoptic tip is fragile; perform 10b-c with care.

⚠ CRITICAL: Do not remove the cannula or the arm until the cannula has been cemented.

11. Make the dental cement (Figure 4A).
    a. Pre-chill the ceramic mixing dish on ice.

    Note: This slows down the reaction allow adequate working time.

    b. Add 4 drops of the liquid QuickBase into a well in the dish.
    c. Add 1 drop of Universal Catalyst into the same well.
    d. Add 1–3 leveled scoops of L-Powder into the same well.
        i. The amount can be varied to change the viscosity per user preference.
    e. Rapidly mix.

    Note: The Radiopaque (versus clear) L-powder may be preferable for optogenetic experiments since it results in less visible light around the fiber – thus avoiding an inadvertent visual cue to the animal.

12. Secure the cannula to the skull. Troubleshooting 3.
    a. Apply the solution to the skull.
        i. The solution for the first layer should be thick enough to harden quickly, yet loose enough that it can spread over the skull to maximize adhesion.

    ⚠ CRITICAL: Take care that the cement does not adhere to the skin or eyes.

        ii. The solution for the second layer should be medium viscosity so it can be built up around the cannula.
        iii. The solution for the final layer should be thin to smooth any bumps or sharp edges that can harbor bacteria or injure the animal.

    Pause point: Wait for 10–20 min for the dental cement to fully dry before removing the holder from the cannula. The solidity of the headcap can be tested with gentle tapping.

13. Remove the arm holding the fiberoptic cannula (Figure 4C). Place the cap on top of the cannula to protect it.
14. Return to step 8 to complete the surgery.

In vivo optogenetics

Timing: 10 min

In the optogenetic approach, stimulation of neurons is achieved by photoactivation of opsins expressed in the brain (Figure 5). Here we calibrate the lasers to stimulate the opsins expressed in the entorhinal cortex. Ensure that you are using the correct wavelength to stimulate your chosen virus. In this protocol we utilize a blue light at a wavelength of 473 nm, appropriate for activation of Channelrhodopsin.[38]

⚠ CRITICAL: Ensure that you are always wearing the proper eye protection when using the laser. Do not look directly into the laser or the laser scatter as this can cause eye damage.

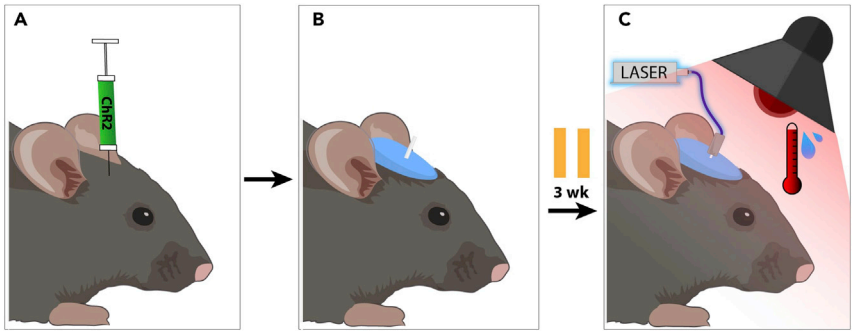

Figure 5. Workflow for *in vivo* optogenetics

(A) Infusion of an opsin (ChR2)-encoding virus into the brain of a mouse.

(B) Implanted fiberoptic cannula secured with opaque dental acrylic.

(C) Opsin activation (via laser stimulation through the fiberoptic cannula) coupled with hyperthermia. These experiments can typically be performed 3+ weeks after surgery.

15. Turn the laser on to calibrate the output (Figure 2).
    a. Use a power meter to measure the output at the tip of a fiberoptic cannula connected to the patch cord.
        i. Adjust the output to the desired power (e.g., 3 mW).
    b. Using a pulse generator (e.g., Master-9), set the laser parameters to generate the desired stimulation sequence.
        i. We used 5 ms pulse at a rate of 20 Hz, with alternating epochs of 5 s ON / 5 s OFF.[39]

Note: The parameters of laser stimulation (i.e., frequency, pulse width, power, and activation pattern) may differ depending on experimental requirements. It is important to choose a pulse width that is sufficient to activate the opsin (>2 ms), and a frequency that is low enough to allow the desired cell type to fire at physiological rates.

16. Turn the laser key to the "OFF" position prior to connecting the animal.
17. Attach the mouse via a fiberoptic patch cord with a plastic sleeve.
18. Turn the laser key to the "ON" position immediately prior to initiating the hyperthermia experiment.

Note: To control for the possible heat-inducing effects of light, use a fluorophore-only virus paired with light stimulation. Alternatively, to control for opsin expression, use an opsin-expressing virus without light stimulation.

In vivo chemogenetics

Timing: 15 min

In the chemogenetic approach, stimulation of neurons is achieved with an injection of 10 mg/kg CNO 30 min prior to hyperthermia experiments (Figure 6).

19. Make CNO working solution.
    a. Thaw a 450 μL aliquot of CNO stock solution.

⚠ CRITICAL: Thaw the CNO stock solution aliquot immediately prior to use.

    b. Add 450 μL of sterile saline to dilute the 450 μL CNO stock solution aliquot 1:1.

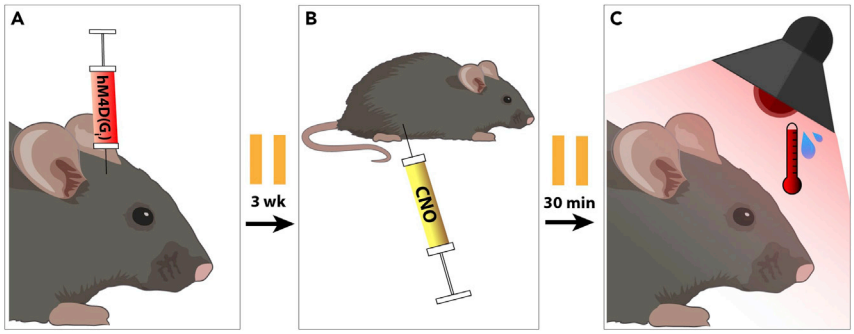

Figure 6. Workflow for *in vivo* chemogenetics

(A) Infusion of a DREADD (hM4D(G_i))-encoding virus into the brain of a mouse.

(B) Intraperitoneal injection of designer drug CNO, to activate the DREADD.

(C) Hyperthermia experiments performed 30 min after CNO injection.

20. Prepare individual doses of CNO in disposable syringes.
    a. Weigh each mouse.
    b. Calculate the appropriate dose of CNO working solution using this formula:
        i. 5.8 (μL) *X (weight of mouse in grams) = Y (μL of CNO working solution).
21. Inject the mouse intraperitoneally.

Note: We suggest using 2% DMSO in saline as a control.

Pause point: To ensure that the CNO has enough time to exert its effect, wait 30 min prior to seizure induction experiments.

Hyperthermia

Timing: 20–30 min

To determine the effect of optogenetic or chemogenetic stimulation on acute seizure temperature threshold, we used a passive heating procedure to induce an acute hyperthermia-induced seizure.

Note: To account for circadian confounds we triggered any repeated seizures at the same time of day each time – i.e., approximately 24 h apart.

22. Assemble Equipment.
    a. Set up the control chamber, the heating chamber, and the cooling chamber.
        i. The control chamber should be completely empty and located in the same room as the heating chamber (Figure 7A).

Note: The chamber should remain at room temperature (20°C–22°C). To avoid any passive heat transfers, keep the chamber at least 2 feet away from the heating and cooling chambers. We used an empty cage.

        ii. The heating chamber should contain a lid and heating lamp (Figure 7B).

Note: Mice with generalized tonic-clonic seizures can easily jump out of the box. A lid is strongly recommended.

        iii. The cooling chamber should contain a thick layer of crushed ice covered by a damp paper towel (Figure 7C).

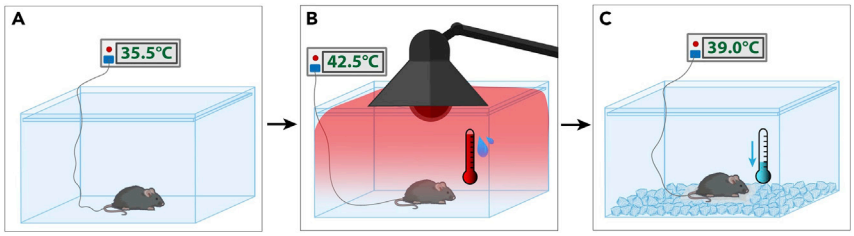

Figure 7. Experimental set up for hyperthermia testing

(A) The mouse is connected to a temperature probe and placed in an ambient temperature control chamber.

(B) The mouse is moved to the heating chamber and exposed to a heating lamp, with continuous monitoring of internal body temperature.

(C) The mouse is moved to the cooling chamber at the conclusion of the experiment.

Note: A lid here is similarly suggested to encourage contact between the ice and the mouse and to prevent escape.

b. Set up a camera to record the procedure to score seizures off-line.
c. If using the optogenetic approach, ensure that the lasers have been set to the appropriate settings and calibrated.
d. Using an alcohol swab, disinfect the rectal probe and let it air dry.

23. Prepare the mouse.
    a. If using the chemogenetic approach, ensure that at least 30 min have elapsed since the CNO injection.
    b. If using the optogenetic approach, connect the cable to the implanted cannula(s).

Note: While we did not include EEG recordings in this study, they are easily compatible with hyperthermia experiments. EEG cables can be connected at this time.

c. Lubricate the now disinfected and dry rectal probe and insert into the rectum of the mouse. Secure the probe with tape around the tail.

Optional: Tape can be labeled with an animal number for easier off-line ID.

24. Place the mouse into the control chamber for ~5 min, or at least until the recorded body temperature is stable.
    a. If using the optogenetic approach, laser stimulation can be initiated while the mouse is in the control chamber, or upon transfer to the heating chamber, as desired.
25. Transfer the mouse into the heating chamber and turn on the heating lamp. troubleshooting 4 and 5.
    a. Record the temperature of the mouse at least every minute to ensure heating is occurring.
    b. If/when a seizure occurs, record the body temperature, and immediately remove the animal from the heating chamber.

Note: Animals were removed upon observation of a Stage 4–5 behavioral seizure on the Modified Racine Scale. Seizures usually began with a vocalization that was quickly followed by jumping, falling over, and generalized tonic-clonic activity.

c. If the animal reaches 42.5°C without having a seizure, stop the experiment.

26. Transfer the mouse to the cooling chamber.
    a. Once the body temperature is below 40°C, remove the rectal probe and continue to monitor for any adverse effects.
27. Once the mouse has regained regular mobility and is exhibiting normal behavior, return it to its home cage. Troubleshooting 6.

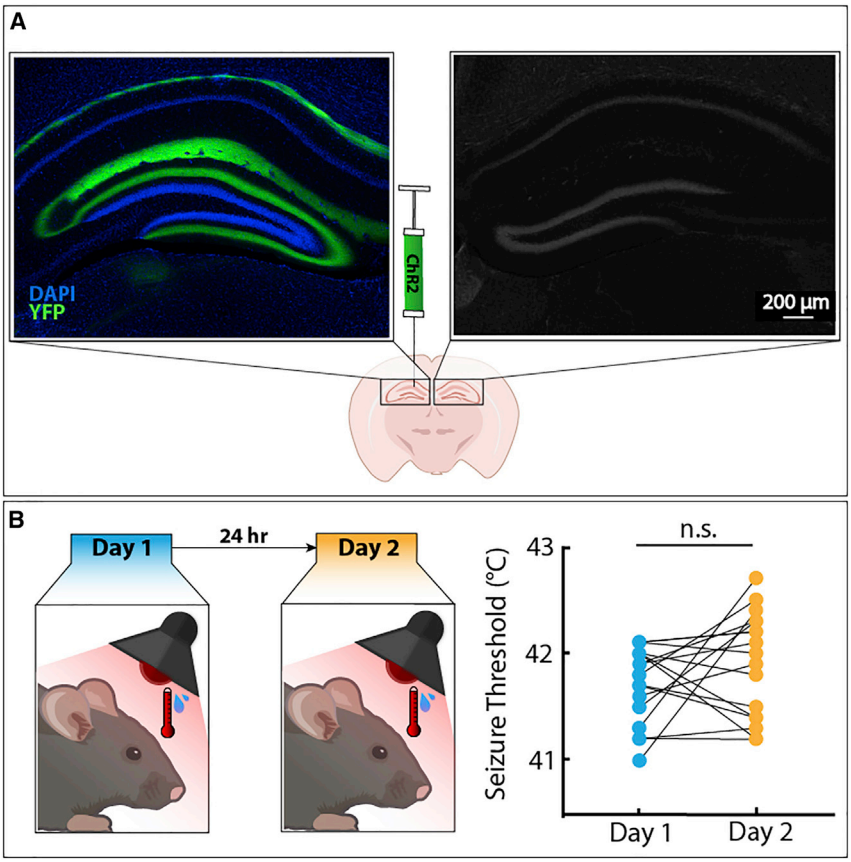

Figure 8. Expected outcomes

(A) Present *(left)* and absent *(right)* virus expression in the hippocampus as visualized under a fluorescent microscope.

(B) Hyperthermia testing on Day 1 does not significantly affect the acute seizure temperature threshold on Day 2, supporting the use of a repeated measures design. Not significant (n.s.) is defined here as $p > 0.05$ by paired t-test.

EXPECTED OUTCOMES

This protocol describes methods to introduce and activate optogenetic and chemogenetic tools in the brain. Successful viral expression can be verified post-hoc via visualization of fluorophore in the targeted brain region (Figure 8A).

This protocol also describes methods for induction of seizures in the *Scn1a*$^{+/-}$ mouse model via hyperthermia. If the cell population being activated or inhibited is relevant to ictogenesis then the observed temperature threshold for seizure initiation may increase or decrease.

QUANTIFICATION AND STATISTICAL ANALYSIS

We observed that hyperthermia-evoked seizure threshold was stable within mice across sequential days (Figure 8B). Therefore, testing may be completed with a cross-over, repeated measures design, wherein each mouse experiences both stimulation and control conditions on sequential days of hyperthermia. This data can be analyzed using a paired t-test.

However, it is critical to consider whether the optogenetic or chemogenetic stimulation itself may kindle seizures (i.e., whether stimulation on day 1 will lower seizure threshold on day 2). If upon control testing a kindling effect is observed, a single round of hyperthermic testing – in which half of the mice receive optogenetic or chemogenetic stimulation, and the other half receive a control – is preferred. An unpaired t-test should be used in this case.

LIMITATIONS

This protocol is specifically designed to test the effect of optogenetic or chemogenetic stimulation on the onset of behaviorally observed induced hyperthermic seizures. To measure the impact of stimulation on (1) severity, propagation, or duration of seizures, (2) subclinical seizures, (3) spontaneous seizures, or (4) other types of acute seizures, the protocol must be adapted accordingly.

Further, this protocol assumes that neither optogenetic stimulation nor hyperthermia results in seizures in wild-type mice. Optogenetic stimulation at certain parameters can be used to kindle and induce seizures in mice,[40,41] so thorough control testing should be completed to avoid confounding results.

Finally, this protocol is limited by the capabilities of optogenetic and chemogenetic stimulation. Both techniques provide a degree of spatiotemporal control over neuronal tissue.[42] Chemogenetics can modify excitability over a large brain volume and offers a broad (~30–60 min) manipulation window but cannot achieve precise control over spike rate and timing. Optogenetics, in contrast, provides precise temporal control, yet is spatially constrained to the volume of brain that is exposed to light. Further, the optogenetic strategy requires additional hardware and increased surgery time for implantation of fiberoptic fibers, which may increase potential post-operative complications. Specific experimental requirements will determine which strategy is optimal.

TROUBLESHOOTING

Problem 1

Irregular breathing pattern under anesthesia (step 1b).

Potential solution

If the mouse is exhibiting slow, irregular, or shallow breathing, it may indicate excessive anesthesia or airway occlusion. Reduce the isoflurane levels and loosen the nose cone, monitoring the mouse until vital signs are in an acceptable range. It may be necessary to remove the mouse from the stereotax entirely to ensure that regular vitals return. Resume isoflurane at a lower level to continue the procedure. It is important to note that the amount of isoflurane needed to properly anesthetize the animal gradually decreases over the course of surgery, so active monitoring is necessary to maintain proper depth throughout.

On the other hand, if the mouse is exhibiting irregular, rapid breathing and/or is exhibiting the toe/tail-pinch reflex, it may indicate inadequate anesthesia. Ensure that there is enough isoflurane and oxygen in each tank, and that all the tubing is securely connected. Verify that the nose cone is correctly placed before increasing isoflurane levels. Note that younger mice (<P21) may have more volatile reactions to inhaled anesthesia.

Problem 2

Absent or inadequate virus expression (step 7).

Potential solution

If upon examining your tissue there is no fluorescent virus expression (Figure 8A), several possibilities should be considered:

First, the quality of the virus may have decreased after too many freeze/thaw cycles, resulting in diminished or absent expression.

Second, an insufficient amount of virus may have been injected. This may be due to clogging of the injection needle, preventing virus from entering the brain. To monitor for this during the surgery, we confirm flow of virus before and after every injection by visualizing the needle through a stereoscope

and slowly injecting until a small amount of virus can be seen exiting the needle. If there is no outflow, the needle is likely clogged. It may be possible to remove the clog by (1) increasing the injection rate on the pump or (2) manually working the plunger in saline to remove the clog. If these methods fail, switch to a new needle. An alternative technical issue is that the virus may leak out from the brain as the needle was withdrawn; we recommend visualizing the injection site while withdrawing the needle to monitor for this. To prevent leaking, leave the needle inserted for a longer time and withdraw it at a slower rate. If the viral amount is inadequate even in absence of these technical issues, a higher titer and/or volume of virus can be used.

Third, non-expression may be a result of inadequate waiting time post-injection. We typically wait at least 3 weeks for sufficient expression, although the minimum waiting time depends on multiple parameters including viral titer, volume, serotype, and promoter.

Finally, verify that the correct virus-genotype pairing has been achieved. For example, injecting a Cre-dependent virus into an animal without Cre recombinase will not result in viral expression.

Problem 3

High rate of headcap and implant loss (step 12).

Potential solution

Headcap loss indicates that there is inadequate adhesion between the cement and the skull. Ensure that the bone is totally dry prior to applying the initial layer of dental cement. Lightly score the surface of the skull with a scalpel or surgical scissors to increase surface area to which the cement can bind. While usually unnecessary in mice (but required in larger animals such as rats), consider adding a partially inserted surgical screw (1.5–2 turns) into the skull as another anchor point.

Mice typically do not require single housing after the implant of a fiberoptic cannula, although if cage mates chew on the implants to the point of loss it may be necessary.

Problem 4

The body temperature of the mouse is not increasing (step 25).

Potential solution

If during the hyperthermia procedure the mouse exhibits behavior suggestive of hyperthermia yet the recorded temperature does not reflect this, there may be an issue with the temperature probe accurately measuring body temperature. To ensure that the temperature probe is inserted at an adequate and consistent depth, use a piece of tape to mark ~1 inch on the probe (for adult mice) and insert to that depth. Use tape to tightly secure the probe to the tail, paying particular attention to the proximal tail. Any sliding movement of the probe indicates that it is inadequately secured.

If the mouse exhibits normal behavior and the core body temperature does not adequately rise, the mouse may be exposed to inadequate heat. Move the lamp closer to the mouse (<8 inches) and make sure that the bulb is at least 250 Watts. To reduce heat leakage from the chamber, switch to a smaller, heat proof box.

Problem 5

There are no changes to acute seizure temperature threshold (step 25).

Potential solution

In the case of both *in vivo* optogenetics and chemogenetics, there may be inadequate virus expression (see problem 2). The function of both types of viruses can also be verified via post-hoc c-Fos immunohistochemistry (for activating opsins or DREADDs) and/or via slice physiology.

If using *in vivo* chemogenetics, check the quality and age of the CNO solution. Ensure that appropriate time passed between the CNO injection and hyperthermia testing, such that the DREADD should have been activated.

If using *in vivo* optogenetics, check the power of the laser and ensure tight coupling between the patch cord and the fiberoptic implant. Consider whether the stimulation parameters (frequency, pulse-width, etc.) need to be optimized. Confirm that the implanted fiber is correctly located just above the target region such that the opsin is exposed to light: on coronal slices, the location of the implanted fiber will result in a tear in the tissue that is accompanied by gliosis.

Problem 6

Death following hyperthermic seizure (step 27).

Potential solution

Ensure that the temperature probe is working properly (see problem 4), and that mouse body temperature does not exceed 42.5°C. Move the lamp further away (>8 inches) from the chamber to heat the mouse more gradually. Empirically, we have found that actively cooling mice – transferring the mouse to the cooling chamber as soon as seizure is reached and maintaining close contact with the ice during immediate recovery – improves survival rates (< 1 death per 20–25 mice).

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Joanna Mattis (jomattis@umich.edu).

Materials availability

This study generated a design for a heating chamber for hyperthermia experiments that was manufactured by the LSA Scientific Instrument Shop at the University of Michigan. To request blueprints for the hyperthermia box, reach out to the lead contact, Joanna Mattis (jomattis@umich.edu).

Data and code availability

This study did not create nor analyze data with any novel code/software.

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AUTHOR CONTRIBUTIONS

J.A.K. wrote the first draft of the manuscript. J.M. contributed toward writing the manuscript. J.A.K., E.M.G., and J.M. were involved in editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Mattis, J., Somarowthu, A., Goff, K.M., Jiang, E., Yom, J., Sotuyo, N., Mcgarry, L.M., Feng, H., Kaneko, K., and Goldberg, E.M. (2022). Corticohippocampal circuit dysfunction in a mouse model of Dravet syndrome. Elife *11*, e69293. https://doi.org/10.7554/eLife.69293.

2. Mirski, M.A., and Ferrendelli, J.A. (1984). Interruption of the mammillothalamic tract prevents seizures in Guinea pigs. Science *226*, 72–74. https://doi.org/10.1126/science.6433485.

3. Garant, D.S., and Gale, K. (1983). Lesions of substantia nigra protect against experimentally induced seizures. Brain Res. *273*, 156–161. https://doi.org/10.1016/0006-8993(83)91105-8.

4. Browning, R.A. (1986). VII. Neuroanatomical localization of structures responsible for seizures in the GEPR: lesion studies. Life Sci. *39*, 857–867. https://doi.org/10.1016/0024-3205(86)90367-X.

5. Takebayashi, S., Hashizume, K., Tanaka, T., and Hodozuka, A. (2007). The effect of electrical stimulation and lesioning of the anterior thalamic nucleus on kainic acid–induced focal cortical seizure status in rats. Epilepsia *48*, 348–358. https://doi.org/10.1111/j.1528-1167.2006.00948.x.

6. Yizhar, O., Fenno, L.E., Davidson, T.J., Mogri, M., and Deisseroth, K. (2011). Optogenetics in neural systems. Neuron *71*, 9–34. https://doi.org/10.1016/j.neuron.2011.06.004.

7. Zhang, F., Wang, L.-P., Brauner, M., Liewald, J.F., Kay, K., Watzke, N., Wood, P.G., Bamberg, E., Nagel, G., Gottschalk, A., and Deisseroth, K. (2007). Multimodal fast optical interrogation of neural circuitry. Nature *446*, 633–639. https://doi.org/10.1038/nature05744.

8. Campbell, E.J., and Marchant, N.J. (2018). The use of chemogenetics in behavioural neuroscience: receptor variants, targeting approaches and caveats. Br. J. Pharmacol. *175*, 994–1003. https://doi.org/10.1111/bph.14146.

9. Krashes, M.J., Koda, S., Ye, C., Rogan, S.C., Adams, A.C., Cusher, D.S., Maratos-Flier, E., Roth, B.L., and Lowell, B.B. (2011). Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. J. Clin. Invest. *121*, 1424–1428. https://doi.org/10.1172/JCI46229.

10. Soper, C., Wicker, E., Kulick, C.V., N'Gouemo, P., and Forcelli, P.A. (2016). Optogenetic activation of superior colliculus neurons suppresses seizures originating in diverse brain networks. Neurobiol. Dis. *87*, 102–115. https://doi.org/10.1016/j.nbd.2015.12.012.

11. Paschen, E., Elgueta, C., Heining, K., Vieira, D.M., Kleis, P., Orcinha, C., Häussler, U., Bartos, M., Egert, U., Janz, P., and Haas, C.A. (2020). Hippocampal low-frequency stimulation prevents seizure generation in a mouse model of mesial temporal lobe epilepsy. Elife *9*, e54518. https://doi.org/10.7554/eLife.54518.

12. Zhou, Q.-G., Nemes, A.D., Lee, D., Ro, E.J., Zhang, J., Nowacki, A.S., Dymecki, S.M., Najm, I.M., and Suh, H. (2019). Chemogenetic silencing of hippocampal neurons suppresses epileptic neural circuits. J. Clin. Invest. *129*, 310–323. https://doi.org/10.1172/JCI95731.

13. Lybrand, Z.R., Goswami, S., Zhu, J., Jarzabek, V., Merlock, N., Aktar, M., Smith, C., Zhang, L., Varma, P., Cho, K.-O., et al. (2021). A critical period of neuronal activity results in aberrant neurogenesis rewiring hippocampal circuitry in a mouse model of epilepsy. Nat. Commun. *12*, 1423. https://doi.org/10.1038/s41467-021-21649-8.

14. Bui, A.D., Nguyen, T.M., Limouse, C., Kim, H.K., Szabo, G.G., Felong, S., Maroso, M., and Soltesz, I. (2018). Dentate gyrus mossy cells control spontaneous convulsive seizures and spatial memory. Science *359*, 787–790. https://doi.org/10.1126/science.aan4074.

15. Kim, H.K., Gschwind, T., Nguyen, T.M., Bui, A.D., Felong, S., Ampig, K., Suh, D., Ciernia, A.V., Wood, M.A., and Soltesz, I. (2020). Optogenetic intervention of seizures improves spatial memory in a mouse model of chronic temporal lobe epilepsy. Epilepsia *61*, 561–571. https://doi.org/10.1111/epi.16445.

16. Krook-Magnuson, E., Armstrong, C., Bui, A., Lew, S., Oijala, M., and Soltesz, I. (2015). Evaluation of the dentate gate theory in epilepsy: dentate gate in temporal lobe epilepsy. J. Physiol. *593*, 2379–2388. https://doi.org/10.1113/JP270056.

17. Krook-Magnuson, E., Armstrong, C., Oijala, M., and Soltesz, I. (2013). On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. Nat. Commun. *4*, 1376. https://doi.org/10.1038/ncomms2376.

18. Paz, J.T., Davidson, T.J., Frechette, E.S., Delord, B., Parada, I., Peng, K., Deisseroth, K., and Huguenard, J.R. (2013). Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury. Nat. Neurosci. *16*, 64–70. https://doi.org/10.1038/nn.3269.

19. Nakajima, T., Daval, J.-L., Gleiter, C.H., Deckert, J., Post, R.M., and Marangos, P.J. (1989). C-fos mRNA expression following electrical-induced seizure and acute nociceptive stress in mouse brain. Epilepsy Res. *4*, 156–159. https://doi.org/10.1016/0920-1211(89)90020-X.

20. Giardina, W.J., and Gasior, M. (2009). Acute seizure tests in epilepsy research: electroshock- and chemical-induced convulsions in the mouse. Curr. Protoc. Pharmacol. *45*. 5-22.1-5. 22.37. https://doi.org/10.1002/0471141755.ph0522s45.

21. Morales-Villagrán, A., Ureña-Guerrero, M.E., and Tapia, R. (1996). Protection by NMDA receptor antagonists against seizures induced by intracerebral administration of 4-aminopyridine. Eur. J. Pharmacol. *305*, 87–93. https://doi.org/10.1016/0014-2999(96)00157-4.

22. Bauer, J., Saher, M.S., Burr, W., and Elger, C.E. (2000). Precipitating factors and therapeutic outcome in epilepsy with generalizedtonic–clonic seizures. Acta Neurol. Scand. *102*, 205–208. https://doi.org/10.1034/j.1600-0404.2000.102004205.x.

23. Kaddumukasa, M., Kaddumukasa, M., Matovu, S., and Katabira, E. (2013). The frequency and precipitating factors for breakthrough seizures among patients with epilepsy in Uganda. BMC Neurol. *13*, 182. https://doi.org/10.1186/1471-2377-13-182.

24. Camfield, P., and Camfield, C. (2015). Febrile seizures and genetic epilepsy with febrile seizures plus (GEFS+). Epileptic Disord. *17*, 124–133. https://doi.org/10.1684/epd.2015.0737.

25. Villas, N., Meskis, M.A., and Goodliffe, S. (2017). Dravet syndrome: characteristics, comorbidities, and caregiver concerns. Epilepsy Behav. *74*, 81–86. https://doi.org/10.1016/j.yebeh.2017.06.031.

26. Wallace, R.H., Wang, D.W., Singh, R., Scheffer, I.E., George, A.L., Phillips, H.A., Saar, K., Reis, A., Johnson, E.W., Sutherland, G.R., et al. (1998). Febrile seizures and generalized epilepsy associated with a mutation in the Na+-channel ß1 subunit gene SCN1B. Nat. Genet. *19*, 366–370. https://doi.org/10.1038/1252.

27. Cwetsch, A.W., Ziogas, I., Narducci, R., Savardi, A., Bolla, M., Pinto, B., Perlini, L.E., Bassani, S., Passafaro, M., and Cancedda, L. (2022). A rat model of a focal mosaic expression of PCDH19 replicates human brain developmental abnormalities and behaviours. Brain Commun. *4*, fcac091. https://doi.org/10.1093/braincomms/fcac091.

28. Escayg, A., and Goldin, A.L. (2010). Sodium channel SCN1A and epilepsy: mutations and mechanisms. Epilepsia *51*, 1650–1658. https://doi.org/10.1111/j.1528-1167.2010.02640.x.

29. Gu, B., Zhu, M., Glass, M.R., Rougié, M., Nikolova, V.D., Moy, S.S., Carney, P.R., and Philpot, B.D. (2019). Cannabidiol attenuates seizures and EEG abnormalities in Angelman syndrome model mice. J. Clin. Invest. *129*, 5462–5467. https://doi.org/10.1172/JCI130419.

30. Kruger, L.C., O'Malley, H.A., Hull, J.M., Kleeman, A., Patino, G.A., and Isom, L.L. (2016). β1-C121W is down but not out: epilepsy-associated Scn1b-C121W results in a deleterious gain-of-function. J. Neurosci. *36*, 6213–6224. https://doi.org/10.1523/JNEUROSCI.0405-16.2016.

31. Mistry, A.M., Thompson, C.H., Miller, A.R., Vanoye, C.G., George, A.L., and Kearney, J.A. (2014). Strain- and age-dependent hippocampal neuron sodium currents correlate with epilepsy severity in Dravet syndrome mice. Neurobiol. Dis. *65*, 1–11. https://doi.org/10.1016/j.nbd.2014.01.006.

32. Han, Z., Chen, C., Christiansen, A., Ji, S., Lin, Q., Anumonwo, C., Liu, C., Leiser, S.C., Meena, Aznarez, I., et al. (2020). Antisense oligonucleotides increase Scn1a expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome. Sci. Transl. Med. *12*, eaaz6100. https://doi.org/10.1126/scitranslmed.aaz6100.

33. Hawkins, N.A., Anderson, L.L., Gertler, T.S., Laux, L., George, A.L., and Kearney, J.A. (2017). Screening of conventional anticonvulsants in a genetic mouse model of epilepsy. Ann. Clin. Transl. Neurol. *4*, 326–339. https://doi.org/10.1002/acn3.413.

34. Miller, A.R., Hawkins, N.A., McCollom, C.E., and Kearney, J.A. (2014). Mapping genetic modifiers of survival in a mouse model of Dravet syndrome. Genes Brain Behav. *13*, 163–172. https://doi.org/10.1111/gbb.12099.

35. Favero, M., Sotuyo, N.P., Lopez, E., Kearney, J.A., and Goldberg, E.M. (2018). A transient developmental window of fast-spiking interneuron dysfunction in a mouse model of Dravet syndrome. J. Neurosci. *38*, 7912–7927. https://doi.org/10.1523/JNEUROSCI.0193-18.2018.

36. Lee, J.H., Durand, R., Gradinaru, V., Zhang, F., Goshen, I., Kim, D.-S., Fenno, L.E., Ramakrishnan, C., and Deisseroth, K. (2010). Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. Nature *465*, 788–792. https://doi.org/10.1038/nature09108.

37. Paxinos, G., and Franklin, K.B.J. (2019). Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates (Academic Press).

38. Mattis, J., Tye, K.M., Ferenczi, E.A., Ramakrishnan, C., O'Shea, D.J., Prakash, R., Gunaydin, L.A., Hyun, M., Fenno, L.E., Gradinaru, V., et al. (2011). Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. Nat.

Methods *9*, 159–172. https://doi.org/10.1038/nmeth.1808.

39. Leung, C., Cao, F., Nguyen, R., Joshi, K., Aqrabawi, A.J., Xia, S., Cortez, M.A., Snead, O.C., Kim, J.C., and Jia, Z. (2018). Activation of entorhinal cortical projections to the dentate gyrus underlies social memory retrieval. Cell Rep. *23*, 2379–2391. https://doi.org/10.1016/j.celrep.2018.04.073.

40. Cela, E., McFarlan, A.R., Chung, A.J., Wang, T., Chierzi, S., Murai, K.K., and Sjöström, P.J. (2019). An optogenetic kindling model of neocortical epilepsy. Sci. Rep. *9*, 5236. https://doi.org/10.1038/s41598-019-41533-2.

41. Khoshkhoo, S., Vogt, D., and Sohal, V.S. (2017). Dynamic, cell-type-specific roles for GABAergic interneurons in a mouse model of optogenetically inducible seizures. Neuron *93*, 291–298. https://doi.org/10.1016/j.neuron.2016.11.043.

42. Vlasov, K., Van Dort, C.J., and Solt, K. (2018). Chapter eleven - optogenetics and chemogenetics. In Methods in Enzymology Chemical and Biochemical Approaches for the Study of Anesthetic Function Part B., R.G. Eckenhoff and I.J. Dmochowski, eds. (Academic Press), pp. 181–196. https://doi.org/10.1016/bs.mie.2018.01.022.