

Submission ID #: 71594

Scriptwriter Name: Sri Lakshmi Priya

Project Page Link: <https://review.jove.com/account/file-uploader?src=21836218>

Title: Inducing Daytime Circadian Phase Shifts Using Chemogenetic and Spectral Approaches

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **yes**

If **Yes**, can you record movies/images using your own microscope camera?

No

Zeiss STEMI 508, Nikon Eclipse Ti2

Scope shots: 2.5.2, 2.6.1, 2.6.2, 2.7.1, 2.7.2. 2.8.1, 2.9.1, 4.4.1, 4.4.2, 4.4.3.

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

3. Filming location: Will the filming need to take place in multiple locations? **yes**

Ground floor (two adjacent room) and 2nd floor.

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 27

Number of Shots: 53

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Sophie Bass Ruchi Komal:** Our research focuses on how light regulates the circadian system, particularly how retinal signals influence the brain's master circadian clock, the suprachiasmatic nucleus or SCN.

- 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Samer Hattar:** Existing methods fail to induce daytime circadian phase resetting because light input to the SCN is limited; this protocol uses ipRGC activation and violet light stimulation that overcome this limitation.

~~Existing methods poorly induce daytime circadian phase resetting because of weak light responsiveness; this protocol uses ipRGC activation and violet light stimulation.~~ **NOTE: The author has changed it.**

- 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.3.2*

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.3. **Samer Hattar:** This protocol can be applied broadly in circadian and neuroscience research to study mechanisms of light entrainment and circadian plasticity.

- 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.4. **Ruchi Komal:** This protocol enables reliable induction and measurement of circadian phase shifts during subjective day, facilitating investigation of photic entrainment, SCN responsiveness, and circadian plasticity.

- 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Samer Hattar:** Future studies can build on this work by exploring whether wavelength-specific daytime light stimulation can be adapted for use in humans.
 - 1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

~~What problem in your field of research does your JoVE article address?~~

- 1.6. **Samer Hattar, Principal Investigator, SLCR**: (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How does the video format of your article help you to share your work with the broader scientific community?~~

- 1.7. **Ruchi Komal, Research Fellow, NIMH**: (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the National Institute of Mental Health, National Institutes of Health

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

NOTE: SCREEN timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sri Lakshmi priya) for queries regarding lab media.

2. Intravitreal Injection of Adeno-Associated Virus in Opn4^{Cre/+} Mice

Demonstrator: Hui Wang

- 2.1. To begin, clean and sterilize the stereotaxic stage and all surgical instruments using appropriate sterilization procedures [1]. Set up the injection apparatus [2] and attach a glass capillary injection needle [3].
 - 2.1.1. WIDE: Talent cleaning and sterilizing the stereotaxic stage and surgical instruments. ***Videographer:** Please obtain a CU shot as well for this shot.*
 - 2.1.2. Talent setting up the injection apparatus.
 - 2.1.3. Talent attaching a glass capillary injection needle to the injection apparatus.
- 2.2. After anesthetizing the mouse, set the oxygen flow rate to approximately 1.5 liters per minute [1-TXT] and maintain 1 to 3% isoflurane throughout the procedure [2].
 - 2.2.1. Talent setting the oxygen flow rate. **TXT: Anesthesia: 3-5% Isoflurane**
 - 2.2.2. Talent adjusting the isoflurane settings.
- 2.3. Using a toe pinch, verify that the animal is fully anesthetized by confirming the absence of withdrawal or flinching reflexes [1].
 - 2.3.1. Talent performing a toe pinch and mouse showing no withdrawal or flinching reflex.
- 2.4. Place the mouse on a heating pad to maintain body temperature throughout the procedure [1].
 - 2.4.1. Talent placing the mouse on a heating pad.
- 2.5. Then, pull glass capillary needles [1] and mark the needle to indicate a penetration depth of approximately 1.0 to 1.5 millimeters to ensure consistent intravitreal delivery [2].
 - 2.5.1. Talent pulling glass capillary needles.
 - 2.5.2. SCOPE: Talent marking the needle. **Videographer's NOTE: IMG_2530.mov and IMG_2531.mov are 2 takes of steps 2.5.2 through 2.9.1 (the majority of the shots labeled SCOPE in the script) Reviewed the footage with talent and they**

preferred the second take.

- 2.6. Using blunt-tipped forceps, gently apply pressure to the inferior eyelid to slightly protrude the eyeball and expose the dorsal limbus [1]. Penetrate the sclera 0.5 to 1 millimeter posterior to the limbus while avoiding vascularized regions [2].
 - 2.6.1. SCOPE: Shot of blunt-tipped forceps applying pressure to the inferior eyelid and exposing the dorsal limbus.
 - 2.6.2. SCOPE: Talent penetrating the sclera posterior to the limbus while avoiding vascularized regions.
- 2.7. Insert the needle into the vitreous chamber to the predetermined depth [1]. Withdraw slightly to relieve intraocular pressure and prevent blockage of the needle tip by tissue [2].
 - 2.7.1. SCOPE: Shot of needle being inserted into the vitreous chamber.
 - 2.7.2. SCOPE: Shot of needle being withdrawn slightly.
- 2.8. Inject 1.5 microliters adeno-associated virus as five 300-nanoliter sequential injections, pausing 15 seconds between injections to minimize reflux [1].
 - 2.8.1. SCOPE: Shot of adeno-associated virus solution being injected.
- 2.9. After administering the final injection, wait for 30 seconds and slowly withdraw the needle [1].
 - 2.9.1. SCOPE: Shot of needle slowly being withdrawn.
- 2.10. Administer 0.1 milligrams per kilogram ketoprofen subcutaneously immediately following surgery [1].
 - 2.10.1. Talent administering ketoprofen subcutaneously.
- 2.11. After at least 2 weeks of recovery and adequate viral expression, transfer the mice to the behavioral facility [1].
 - 2.11.1. Talent transferring the mice to the behavioral facility.

3. Wheel-Running Activity Monitoring and Chemogenetic Phase-Shift Analysis

Demonstrator: Sophie Bass

- 3.1. House mice individually in cages equipped with running wheels [1]. Record wheel-running activity using an automated data acquisition system [2].
 - 3.1.1. Talent placing a mouse in a cage equipped with a running wheel.
 - 3.1.2. SCREEN: 71594_screenshot_1.mp4: 00:00-00:05
- 3.2. After entraining mice to a 12 to 12-hour light-dark cycle for 4 to 7 days, confirm stable entrainment [1] and transition animals to constant darkness or DD (D-D) [2].

- 3.2.1. SCREEN: 71594_UPDATED_screenshot_2.mp4: 00:00-00:03
- 3.2.2. Shot of the animal room lights switching off.
- 3.3. Using analysis software, determine activity onset [1]. Once the activity onset in nocturnal rodents is defined as circadian time or CT (C-T) 12, calculate CT4 accordingly [2].
 - 3.3.1. SCREEN: 71594_screenshot_3.mp4: 00:00-00:08
 - 3.3.2. SCREEN: 71594_screenshot_3.mp4: 00:00-00:08 NOTE: 3.3.1 and 3.3.2 are combined in one shot. Show the 71594_screenshot_3.mp4: 00:00-00:08 only once.
- 3.4. Administer 1 milligram per kilogram clozapine N-oxide or CNO (C-N-O) intraperitoneally at CT 4 and control animals with an equivalent saline volume [1].
 - 3.4.1. Talent administering CNO intraperitoneally.
- 3.5. Return animals to their home cages and maintain them under DD [1]. Record locomotor activity continuously for 8 days [2].
 - 3.5.1. Talent returning animals to their home cages.
 - 3.5.2. SCREEN: 71594_screenshot_4.mp4: 00:00-00:19
- 3.6. Determine activity onsets [1] and perform regression analysis on pre-injection and post-injection periods [2]. Calculate phase shifts by comparing the projected and observed activity onsets [3].
 - 3.6.1. SCREEN: 71594_screenshot_4.mp4: 00:00-00:19
 - 3.6.2. SCREEN: 71594_screenshot_4.mp4: 00:00-00:19
 - 3.6.3. SCREEN: 71594_screenshot_4.mp4: 00:00-00:19 NOTE: 3.5.2 to 3.6.3 are combined in one shot. Show the 71594_screenshot_4.mp4: 00:00-00:19 only once.
- 4. SCN c-Fos Immunohistochemistry Following Chemogenetic Activation
Demonstrators: Michelle Sun
 - 4.1. Entrain mice to a 12-to-12-hour light-dark cycle [1] and release them into DD [2]. On the second day in DD, administer CNO at CT6 [3]. Perfuse animals 30 minutes after injection [4].
 - 4.1.1. Shot of the animal room lights turning on and off over the mouse cages.
 - 4.1.2. Talent releasing mice into a dark room.
 - 4.1.3. Talent administering CNO at CT6 in DD.
 - 4.1.4. Talent perfusing animals 30 minutes after injection.
 - 4.2. Dissect the brain [1] and post-fix it in 4 percent paraformaldehyde overnight at 4 degrees Celsius [2]. Cryoprotect the brain in 30 percent sucrose until the tissue sinks [3]. Section the tissue coronally at 40 micrometers [4].
 - 4.2.1. Talent dissecting the brain.

- 4.2.2. Talent placing the brain in 4 percent paraformaldehyde and keeping it inside the refrigerator.
- 4.2.3. Talent placing the brain in 30 percent sucrose for cryoprotection.
- 4.2.4. Talent sectioning the tissue coronally at 40 micrometers.
- 4.3. Incubate sections in blocking solution [1]. Subsequently, incubate the sections with the primary antibody overnight at 4 degrees Celsius [2]. After washing the sections, incubate them with secondary antibodies [3]. Counterstain with 4-prime,6-diamidino-2-phenylindole or DAPI (*DA-PI*) and mount the sections [4].
 - 4.3.1. Talent placing sections in the blocking solution.
 - 4.3.2. Talent placing sections in the primary antibody solution and keeping it inside the refrigerator.
 - 4.3.3. Talent placing sections in secondary antibody solution.
 - 4.3.4. Talent counterstaining with DAPI and mounting the sections.
- 4.4. Acquire images using consistent microscope settings [1]. Process images using analysis software [2]. Identify adeno-associated virus-infected regions by mCherry-positive (*M-cherry-positive*) cells [3].
 - 4.4.1. SCREEN: 71594_screenshot_5.mp4: 00:00–00:09 NOTE: This scope shot is changed to a SCREEN capture
 - 4.4.2. SCREEN: 71594_screenshot_6.mp4: 00:46–01:09
 - 4.4.3. SCREEN: 71594_screenshot_6.mp4: 01:23–01:57

5. Spectral Strategy

Demonstrators: Eve Gold

- 5.1. House the wild-type mice individually in cages equipped with running wheels at the behavioral facility [1]. Maintain the animals under DD to monitor free-running circadian activity [2].
 - 5.1.1. Shot of an individual wild-type mouse housed in a cage equipped with a running wheel.
 - 5.1.2. Shot of the animal room maintained under DD.
- 5.2. On day 8 in DD at CT6, remove the cage from the light-tight cabinet [1].
 - 5.2.1. Talent removing the mouse cage from the light-tight cabinet at CT6.
- 5.3. Keep mice in their home cages with the cage tops removed [1]. Position the cages directly beneath a fixed violet light source, followed by a blue light source [2]. Align the center of the light beam with the center of the cage to ensure uniform illumination [3]. Expose the mice to 15-minute light pulses [4]. NOTE: The VO was edited to align with the revised shots
 - 5.3.1. Shot of the mouse remaining in its home cage with the cage tops removed.

- 5.3.2. Talent positioning the cage directly beneath the fixed **violet** light source, followed by a **blue** source.
- 5.3.3. Close-up shot showing the light beam aligned with the center of the cage.
- 5.3.4. Shot of the mouse exposed to the light pulse.
- 5.4. Immediately return the cages to their original position under DD [1]. Continue wheel-running activity recording uninterrupted for phase-shift analysis [2].
 - 5.4.1. Talent returning the cages to the light-tight cabinet under DD.
 - 5.4.2. SCREEN: 71594_screenshot_7.mp4: 00:00-00:14

Results

6. Results

- 6.1. Opn4Cre/+ (*Melanopsin-cre*) mice expressing adeno-associated virus-designer receptors exclusively activated by designer drugs or AAV-DREADD (*A-A-V-Dee-Red*) that received saline at CT4 did not exhibit a significant phase shift in wheel-running activity [1].
 - 6.1.1. LAB MEDIA: Figure 1B. *Video editor: Emphasize the orange trend line.*
- 6.2. CNO administration at CT4 produced a significant phase delay in activity onset [1].
 - 6.2.1. LAB MEDIA: Figure 1C, D. *Video editor: In figure 1C, emphasize the orange trend and in fig 1D, emphasize the bar labeled CNO along with the black dots and the T shaped line and the 4 * with the horizontal line.*
- 6.3. CNO administration increased c-Fos (*C-Foss*) expression in the suprachiasmatic nucleus 30 minutes after injection [1] compared to saline controls [2].
 - 6.3.1. LAB MEDIA: Figure 2C. *Video editor: Emphasize the green cells inside the white outlined regions*
 - 6.3.2. LAB MEDIA: Figure 2B. *Video editor: Emphasize the green cells inside the white outlined regions*
- 6.4. Blue light stimulation with a maximum wavelength of 470 nanometers did not induce a measurable phase shift [1].
 - 6.4.1. LAB MEDIA: Figure 3B, D. *Video editor: In figure 3B, emphasize the blue points around days 8,9 and in figure 3D, emphasize the blue dots near 0 in the graph.*
- 6.5. A 15-minute pulse of violet light with a maximum wavelength of 385 nanometers delivered at CT6 produced a significant phase delay [1].
 - 6.5.1. LAB MEDIA: Figure 3C, D. *Video editor: In figure 3C, emphasize the violet points around days 8 and in figure 3D, emphasize the violet dots positioned below -0.5 hours on the y-axis.*

Pronunciation guides:

1. Stereotaxic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/stereotaxic>
 IPA: /ˌster.i.ooˈtæk.sɪk/
 Phonetic Spelling: steh·ree·oh·tak·sik

2. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/dictionary/isoflurane>
IPA: /ˌaɪ.sʊʊˈflʊr.eɪn/
Phonetic Spelling: eye·soh·floor·ayn
3. Intravitreal
Pronunciation link: <https://www.merriam-webster.com/medical/intravitreal>
IPA: /ˌɪn.trə.viˈtriː.əl/
Phonetic Spelling: in·truh·vih·tree·uhl
4. Limbus
Pronunciation link: <https://www.merriam-webster.com/medical/limbus>
IPA: /ˈlɪm.bəs/
Phonetic Spelling: lim·buhs
5. Sclera
Pronunciation link: <https://www.merriam-webster.com/medical/sclera>
IPA: /ˈsklɪr.ə/, /ˈskler.ə/
Phonetic Spelling: skleer·uh, skler·uh
6. Vitreous
Pronunciation link: <https://www.merriam-webster.com/dictionary/vitreous>
IPA: /ˈvɪt.ri.əs/
Phonetic Spelling: vih·tree·uhs
7. Adeno-associated
Pronunciation link: <https://www.howtopronounce.com/adeno-associated>
IPA: /əˌdiː.nʊʊ əˈsoʊ.si.eɪ.tɪd/
Phonetic Spelling: uh·dee·noh uh·soh·see·ay·tuhd
8. Ketoprofen
Pronunciation link: <https://www.merriam-webster.com/medical/ketoprofen>
IPA: /ˌke.tʊʊˈprʊʊ.fən/
Phonetic Spelling: keh·toh·proh·fuhn
9. Chemogenetic
Pronunciation link: <https://www.howtopronounce.com/chemogenetic>
IPA: /ˌkiː.moʊ.dʒəˈneɪ.tɪk/
Phonetic Spelling: kee·moh·juh·neh·tik
10. Circadian
Pronunciation link: <https://www.merriam-webster.com/dictionary/circadian>
IPA: /sərˈkeɪ.di.ən/
Phonetic Spelling: sur·kay·dee·uhn
11. Clozapine
Pronunciation link: <https://www.merriam-webster.com/medical/clozapine>
IPA: /ˈkloʊ.zə.piːn/
Phonetic Spelling: kloh·zuh·peen
12. Intraperitoneally
Pronunciation link: <https://www.howtopronounce.com/intraperitoneally>

IPA: /ˌɪn.trəˌper.ɪ.təˈniː.ə.li/

Phonetic Spelling: in·truh·peh·ruh·tuh·nee·uh·lee

13. Immunohistochemistry

Pronunciation link: <https://www.merriam-webster.com/medical/immunohistochemistry>

IPA: /ɪˌmjuː.noʊˌhɪs.toʊˈkem.i.stri/

Phonetic Spelling: ih·myoo·noh·his·toh·keh·muh·stree

14. Paraformaldehyde

Pronunciation link: <https://www.merriam-webster.com/medical/paraformaldehyde>

IPA: /ˌper.ə.fərˈmæl.dəˌhaɪd/

Phonetic Spelling: peh·ruh·for·mal·duh·hyde

15. Cryoprotect

Pronunciation link: <https://www.howtopronounce.com/cryoprotect>

IPA: /ˌkraɪ.oʊ.prəˈtekt/

Phonetic Spelling: kry·oh·pruh·tekt

16. Coronal

Pronunciation link: <https://www.merriam-webster.com/dictionary/coronal>

IPA: /kəˈroʊ.nəl/

Phonetic Spelling: kuh·roh·nuhl

17. Diamidino-2-phenylindole

Pronunciation link: <https://www.howtopronounce.com/diamidino-2-phenylindole>

IPA: /ˌdaɪ.æm.iˈdiː.noʊˌfen.əlˈɪn.doʊl/

Phonetic Spelling: dye·am·ih·dee·noh feh·nuhl·in·dohl

18. Suprachiasmatic

Pronunciation link: <https://www.merriam-webster.com/medical/suprachiasmatic>

IPA: /ˌsuː.prəˌkaɪ.əzˈmæt.ɪk/

Phonetic Spelling: soo·pruh·kye·az·mat·ik

19. Melanopsin

Pronunciation link: <https://www.merriam-webster.com/medical/melanopsin>

IPA: /ˌmel.əˈnɑːp.sɪn/

Phonetic Spelling: meh·luh·nop·sin

20. Clozapine N-oxide

Pronunciation link: <https://www.howtopronounce.com/clozapine-n-oxide>

IPA: /ˈkloʊ.zəˌpiːn en ˈɑːk.səɪd/

Phonetic Spelling: kloh·zuh·peen en ok·side