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TITLE:

Monocular Visual Deprivation and Ocular Dominance Plasticity Measurement in the Mouse Primary Visual Cortex

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KEYWORDS:

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SUMMARY:

Here, we present detailed protocols for monocular visual deprivation and ocular dominance plasticity analysis, which are important methods for studying the neural mechanisms of visual plasticity during the critical period and the effects of specific genes on visual development.

ABSTRACT:

Monocular visual deprivation is an excellent experimental paradigm to induce primary visual cortical response plasticity. In general, the response of the cortex to the contralateral eye to a stimulus is much stronger than the response of the ipsilateral eye in the binocular segment of the

mouse primary visual cortex (V1). During the mammalian critical period, suturing the contralateral eye will result in a rapid loss of responsiveness of V1 cells to contralateral eye stimulation. With the continuing development of transgenic technologies, more and more studies are using transgenic mice as experimental models to examine the effects of specific genes on ocular dominance (OD) plasticity. In this study, we introduce detailed protocols for monocular visual deprivation and calculate the change in OD plasticity in mouse V1. After monocular deprivation (MD) for 4 days during the critical period, the orientation tuning curves of each neuron are measured, and the tuning curves of layer four neurons in V1 are compared between stimulation of the ipsilateral and contralateral eyes. The contralateral bias index (CBI) can be calculated using each cell's ocular OD score to indicate the degree of OD plasticity. This experimental technique is important for studying the neural mechanisms of OD plasticity during the critical period and for surveying the roles of specific genes in neural development. The major limitation is that the acute study cannot investigate the change in neural plasticity of the same mouse at a different time.

INTRODUCTION:

Monocular visual deprivation is an excellent experimental paradigm to examine V1 plasticity. To study the importance of visual experience in neural development, David Hubel and Torsten Wiesel[1-2] deprived kittens of normal vision in one eye at various time points and for varying periods of time. They then observed the changes in response intensity in V1 for the deprived and nondeprived eyes. Their results showed an abnormally low number of neurons reacting to the eye that had been sutured shut in the first three months. However, the responses from the neurons in the kittens remained identical in all respects to those of a normal adult cat's eye that was sutured shut for a year, and the kittens did not recover. MD in adult cats cannot induce OD plasticity. Therefore, the impact of visual experience on V1 wiring is strong during a brief, well-defined phase of development, before and after which the same stimuli have less influence. Such a phase of increased susceptibility to visual input is known as the critical period in visual cortex.

Although the mouse is a nocturnal animal, individual neurons in mouse V1 have similar properties to neurons found in cats[3-5]. In recent years, with the rapid development of transgenic technology, an increasing number of studies in visual neuroscience have used mice as an experimental model[6-8]. In mouse visual studies, neuroscientists use mutants and knockout mouse lines, which allow control over the genetic makeup of the mice. Although mice V1 lack OD columns, single neurons in the V1 binocular zone show significant OD properties. For example, most cells respond more strongly to contralateral stimulation than to ipsilateral stimulation. Temporary closure of one eye during the critical period induces a significant shift in the OD index distribution[9-11]. Therefore, MD can be used to establish an OD plasticity model to investigate how genes involved in neural developmental disorders influence cortical plasticity in vivo.

Here, we introduce an experimental method for MD and suggest a commonly used method (electrophysiological recording) to analyze the change in OD plasticity during monocular visual deprivation. The method has been widely used in many laboratories for more than 20 years[12-16]. There are other methods used in measuring the OD plasticity as well, such as chronic visual evoked potential (VEP) recording[17], and intrinsic optical imaging (IOI)[18]. The significant advantage

of this acute method is that it is easy to follow, and the results are remarkably reliable.

PROTOCOL:

In this protocol, male C57Bl/6 mice were obtained from the Institute of Laboratory Animals of Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee, University of Electronic Science and Technology of China.

1. Monocular deprivation (MD) at postnatal day 28 in mice

1.1. Put the surgical tools, the suture needle (0.25 mm diameter, string diameter 0.07 mm) and cotton swabs in an aluminum box and autoclave them at 120 °C for 0.5 h. Sterilize the hood with 75% ethanol. Dry the surgical tools in a drying oven.

1.2. Prepare a 2% agarose solution, put it in a water bath at 75 °C to avoid solidifying.

1.3. Use isoflurane mixed with oxygen to anesthetize the mouse (2% induction and 1.2–1.5% maintenance). Fix the mouse on the stereotaxic apparatus and use a heat regulating device to maintain the mouse body temperature at 37 °C and prevent hypothermia.

1.4. Apply a thin layer of petroleum-based eye ointment to both eyes.

1.5. Under the anatomical microscope with illumination, suture the eyelid on one eye. Make the needle pass though both sides of the eyelid 2x (**Figure 1A**) and make about four stitches.

1.6. Knot the thread 2–3x and then trim the thread. Apply 3 µL of instant drying glue on the knot to increase its stability. Then cut the extra suturing thread.

1.7. Provide an intraperitoneal injection of buprenorphine (1 mg/kg) to the mouse.

1.8. Transfer the mouse onto a heating pad to maintain its body temperature at 37 °C and prevent hypothermia and monitor it until it regains consciousness**.**

1.9. When the mouse is fully awake place it into a separate holding cage.

1.10. Check the eyelids daily to ensure that they remain shut and uninfected. Exclude the mouse if an eyelid opening is found.

1.11. Before electrophysiological recording, use isoflurane mixed with oxygen to anesthetize the mouse (2% induction and 1.2–1.5% maintenance).

1.12. Remove the stitches with eye scissors to expose the eyeball. Carefully trim the eye lids.

1.13. Flush the eye with lens solution and check the eye under a microscope for clarity. Exclude mice with corneal opacities or signs of infection.

2. Craniotomy in the mouse V1 binocular region after monocular deprivation on the 4th day

2.1. After anesthetizing the mouse, check for the depth of anesthesia by the lack of response to a toe pinch.

2.2. Place and fix the mouse on the stereotaxic apparatus. Adjust the height of the ear bar and tooth rod to keep the brain flat and stable.

2.3. Use a heating pad to maintain the body temperature.

2.4. Apply a petroleum-based eye ointment on the surface of the eyes to keep them moist.

2.5. Remove the hair on the mouse's head to expose its skin. Rub the skin with alternating scrubs of iodine and 70% ethanol 3x.

2.6. Incise an 8 x 8 mm area of the skin between the ears to expose the skull and remove the scalp tissue. Then remove the overlying connective tissue with 30% hydrogen peroxide.

2.6. Drill a 1 x 1 mm hole in the skull above the cerebellum. Affix a small bone screw in the hole as a reference.

2.7. Perform a small craniotomy of 1 mm in diameter in the V1 binocular region from the contralateral hemisphere to the deprived eye (**Figure 1B**, A-P: lambda -0.51–lambda +1.67 mm; M-L: -2.6– -3.0 mm; D-V: 0–1 mm). Carefully remove the skull fragment without hurting the brain.

2.8. Cover the exposed cortical surface with 75 µL of 2% agarose at 40 °C to prevent drying.

2.9. Fix a tungsten electrode on the stereotaxic frame. Place the tungsten electrode vertically on the surface of the exposed cortex, the binocular region of V1, to make sure that the cells that are recorded react to both eyes.

2.10. Use cotton swabs to remove the eye gel and apply silicon oil to the eye every 2 h.

3. Visual stimulation and electrophysiological recording

3.1. Mask the one eye with nontransparent plastic plate. Position an LCD monitor 23 cm from the mouse’s eye.

3.2. Reduce the anesthesia to 0.5–0.8% when the mouse is fully anesthetized.

3.3. Advance the microelectrode electrode slowly with an oil hydraulic micromanipulator. Stop it when a high signal-to-noise ratio is observed and the electrode is advanced to layer 4 (**Figure 1C**, approximately 250–450 μm in depth). Ensure that the amplification factor is set at 1,000, the filter at 300–100 Hz, and the sample rate at 40 Hz.

3.4. Present a full-field moving sinusoidal grating (**Figure 1D**, 12 directions, 100% contrast, 2 Hz of temporary frequency, 0.04 cycles per degree of spatial frequency) on the LED monitor.

3.5. Measure the cell's response by stimulating the ipsilateral and contralateral eye separately. Present 3–5x total.

3.6. Measure the responses of five to eight cells in each penetration. Perform four to six penetrations in each mouse.

3.7. After the recording, adjust the isoflurane flow rate to 5% or greater, continue isoflurane exposure for 1 min, and then perform the cervical dislocation.

NOTE: Separate penetrations were spaced at least 200 μm apart in the V1 binocular zone.

4. Off-line spike sorting and data analysis

4.1. Detect spikes when the raw signal crosses a threshold level. Align captured spikes on the first positive or negative peak. Use software to detect spikes from different cells.

4.2. Set two cursors: one for positive and the other for negative deflection. Set the spike template (**Figure 2A**). Set the template area to that with the most significant variation between different classes of spikes.

4.3. Use principal component analysis to separate them into clusters. Clustering methods can vary between different laboratories.

4.4. Classify the spike of a boundary by using the K-means algorithm.

4.5. Correlate the orientation with the spike firing rate and plot the orientation tuning curves for the ipsilateral and contralateral eye.

4.6. Calculate the OD index for the single unit, which represents the contralateral/ipsilateral response strength ratio:

$$OD = \frac{R_{contra} - R_{ipsi}}{R_{contra} + R_{ipsi} - 2R_{spon}} \qquad (1)$$

where R_{contra} and R_{ipsi} are the cell's optimal response for the contralateral and ipsilateral eye,

respectively, and R_{spon} is the cell's spontaneous activity.

4.7. Assign OD scores to 1–7 as follows: − 1 to −0.75 = 1; −0.75 to −0.45 = 2; −0.45 to −0.15 = 3; −0.15 to 0.15 = 4; 0.15 to 0.45 = 5; 0.45 to 0.75 = 6; and 0.75 to 1 = 7.

4.8. Calculate the contralateral bias index (CBI):

$$CBI = [(n_1 - n_7) + \frac{2}{3}(n_2 - n_6) + \frac{1}{3}(n_3 - n_5) + N] / 2N \qquad (2)$$

where N is the cell number, and n_x equals the cell number with OD scores equal to x.

REPRESENTATIVE RESULTS:

The experimental results described here enable successful MD and OD plasticity measurements from a deprived and nondeprived mouse during the critical period (P19–P32). **Figure 1** shows how to perform single unit recordings in layer 4 from V1 the binocular zone for comparing responses in the ipsilateral and contralateral eye 4 days after MD. **Figure 2** shows the spike sorting and orientation tuning measurements for stimulating the ipsilateral and contralateral eyes. For spike sorting, the spike template was established by clustering the principal component weights of the spikes. As an example in (**Figure 2A**,**B**), cell 01 and cell 02 were classified by spike sorting. The orientation tuning curves of single units were measured by stimulation of the ipsilateral and contralateral eyes. **Figures 2C**,**D** show the orientation tuning curves of four sample cells, in which two are from the mouse that underwent MD and the others from the mouse that did not. Our results show that the firing rate was relatively close by stimulating the contralateral and ipsilateral eye in the mouse 4 days after MD was performed (**Figure 2C**). However, the firing rate obtained by stimulating the contralateral eye was much stronger than that obtained by stimulating the ipsilateral eye in the nondeprived mouse (**Figure 2D**). **Figure 3A** shows the distribution of OD scores for all units and the CBI index for a mouse that underwent MD (P28, 4 days after MD). **Figure 3B** shows the OD scores for all units and the CBI index from a nondeprived C57/BL6 mouse (P26, no MD). The CBI index is 0.38 for an MD mouse and 0.67 for another one without MD.

The results show that the response of the V1 neurons to the contralateral eye to a stimulus was much stronger than the response of the ipsilateral eye in the binocular segment of the nondeprived mouse. However, 4 days after MD in the critical period, the response of most neurons to stimulation to the contralateral eye was relatively close or weaker than the response to the ipsilateral eye. Therefore, the V1 neurons in critical periods have significant OD plasticity. MD alters the relative strength of the cell's response by stimulating the contralateral and ipsilateral eye.

FIGURE AND TABLE LEGENDS:

Figure 1: Schematic of the visual deprivation experiment. (**A**) A schematic for suturing the eyelid. The needle passes through the eyelid 2x and then 2–3 knots are made. Images 1–4 show the position where the needle passes through the eyelid. (**B**) Recording schematic in an anesthetized, head-fixed mouse. An enlarged view of V1 is displayed in the gray circle, and the binocular zone

is indicated with dark gray. The recording sites within the binocular zone are shown with small circles. (**C**) The coronal plane of the mouse brain and the recording sites are shown in the layer 4 of V1. (**D**) Illustrations of the visual stimulus of different orientations. Twelve different orientations were totally used in each measurement.

Figure 2: Illustrations of data analysis procedure. (**A**) Spikes were sorted using a commercial software (see **Table of Materials**). The waveform in green shows the filtered signal (0.3–10 kHz). Two cells were sorted from the filtered data. (**B**) Example of the separation of spiking activity on a single microelectrode by spike sorting. The spike sorting method is a principal component of the analysis. (**C**) Orientation tunings from two cells to respond to the contralateral (red solid line) and ipsilateral (red dotted line) stimuli in a monocular-deprived mouse (cell 01 and cell 02). (**D**) Orientation tuning from two cells to respond to contralateral (blue solid line) and ipsilateral (blue dotted line) eye stimuli in a nondeprived mouse (cell 03 and cell 04). The error bar indicates the standard error of the mean (SEM) in each measurement. The black line indicates spontaneous activity.

Figure 3: Shift in the OD index by MD. We recorded the cell's response from the binocular zone in the contralateral brain by stimulating the ipsilateral and contralateral eyes individually. We calculated and added the OD index for the single units. (**A**) Distribution of OD scores for 38 neurons recorded from a C57/BL6 mouse that underwent MD from P28–P32. (**B**) Distribution of OD scores for 38 neurons from a nondeprived C57/BL6 mouse.

DISCUSSION:

We present a detailed protocol for MD and measuring OD plasticity by single unit recording. This protocol is widely used in visual neuroscience. Although the MD protocol is not complicated, there are some critical surgical procedures that must be followed carefully. First, there are two important details ensuring the quality of the stitching. The suture is sufficiently stable if the stitches are concentrated in the medial portion of the eyelid. Moreover, 3 µL of glue is applied to the head of the knot to increase the stability of the knot to prevent eye reopening. Second, some key steps should be taken to improve wound healing and reduce discomfort. The suture method is very important for the protocol. Previous studies have proven that a simple continuous suturing pattern has the benefits of better wound healing and shorter suturing time[19-20]. The thread should be thin and stable to avoid causing a large wound and reduce discomfort. A suture needle with a diameter of approximately 0.25 mm is suitable for suturing and two to three knots are necessary.

There are also some key points that need to be paid attention to in the recording. Control of anesthetic concentration is an important factor in electrophysiological recordings. In anesthetized animals, the experiment is very easy to control, and the results are highly stable and reliable. Many previous studies used urethane as an anesthetic. However, it is difficult to use urethane to control the depth of anesthesia in mice. At lower levels, the mice are not fully anesthetized, and at higher levels, the mice are prone to death. Isoflurane is more appropriate as an anesthetic in a mouse study. While it is almost impossible to obtain good neural activity in the neocortex of mice that are receiving over 1% isofurane[21], most V1 neurons have good visual

evoked activity at lower levels of isoflurane. Thus, start with a higher concentration of isoflurane (1%) to anesthetize the mouse, and then reduce the isoflurane (0.5–0.8%) when the mice are completely anesthetized. Besides, alternating measurements from the deprived eye and the nondeprived eye can ensure the accuracy of the experimental results. It is not appropriate to measure one eye's response many times and then measure the other eye, because the electrode may move, and the intensity of the cell's response may change during long-term recordings. Furthermore, this protocol targets neurons in layer 4, which is the primary thalamo-recipient layer in V1. But in older mice, which do exhibit OD plasticity primarily mediated by open eye potentiation, it is better to record in layers 2 or 3, which retain plasticity beyond the critical period. Therefore, it is important to determine the cortical laminar in recording.

There are still some limitations in the methods. Calculating a relatively accurate CBI index requires 4–6 penetrations and more than 30 units because recording too few samples will lead to inaccurate results. But it is not easy to obtain more than 30 high-quality units from a single mouse. A better method is to use multiunit recording, which can provide enough units in a single measurement. In addition, VEP recording and IOI can also be used to measure OD plasticity[17-18]. Single unit recording involves the activity of individual neurons, while VEP involves recording the activity of the sum of neurons near the electrode. But single unit recording data yield no information on synchronization among neurons, while VEP amplitudes depend on temporal synchronization among neurons[22]. A reversal grating is often used for VEP measurement. The most commonly used reversal frequency is 3–4 Hz. However, the exact value is determined by the computer refresh rate when presenting the grating. OD plasticity is measured by comparing the average of VEP amplitudes evoked by the deprived and nondeprived eye. The IOI technique can effectively detect the blood oxygen level-dependent signal evoked by contralateral and ipsilateral stimulation. It could show the OD plasticity of a large area in V1.

In summary, single unit recording and IOI are suitable for acute anesthesia experiments. In the future, MD and OD plasticity measurements in conjunction will be widely used in the study of neural plasticity as a experimental method.

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DISCLOSURES:

The authors declare that they have no competing financial interests.

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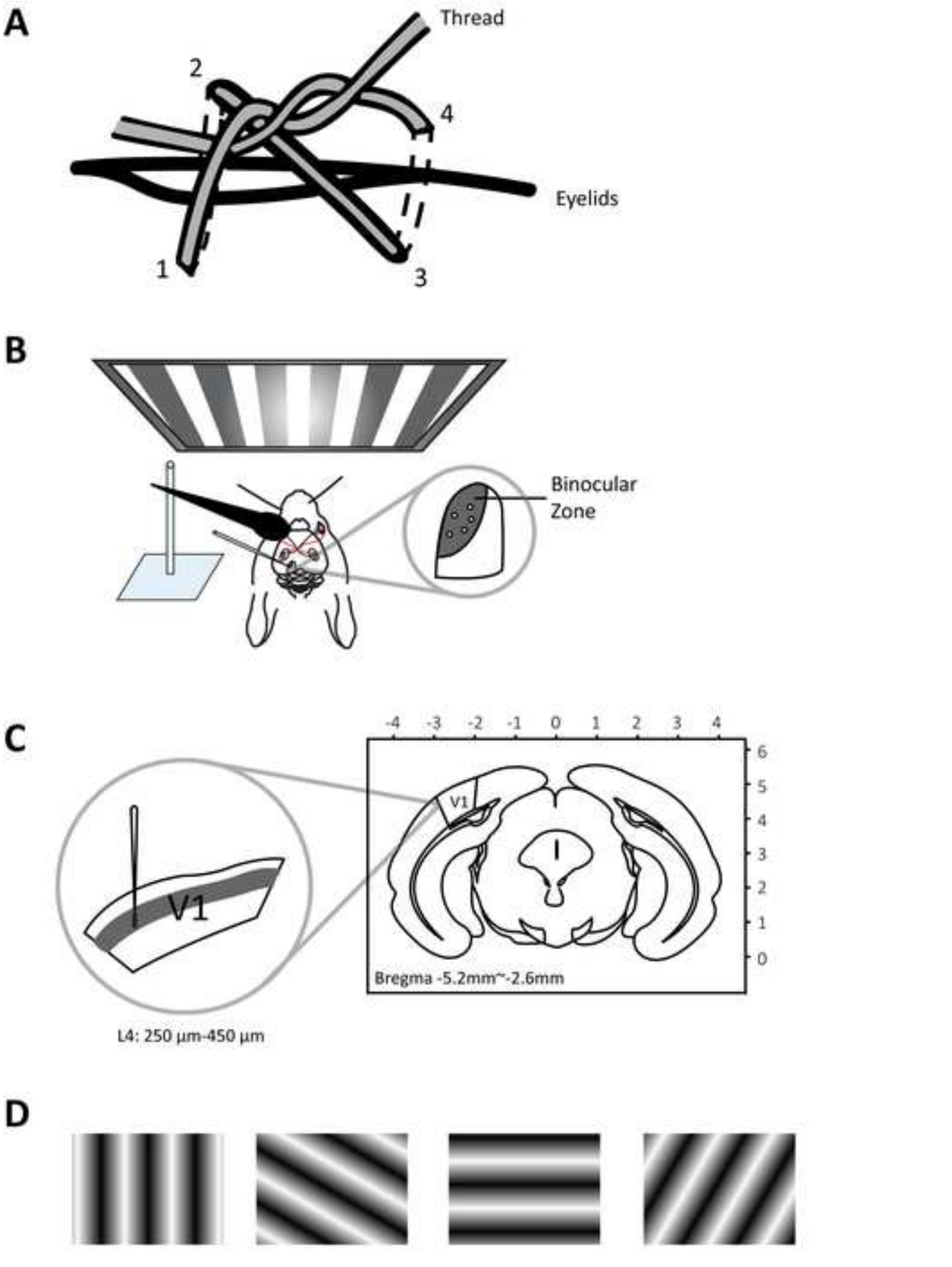
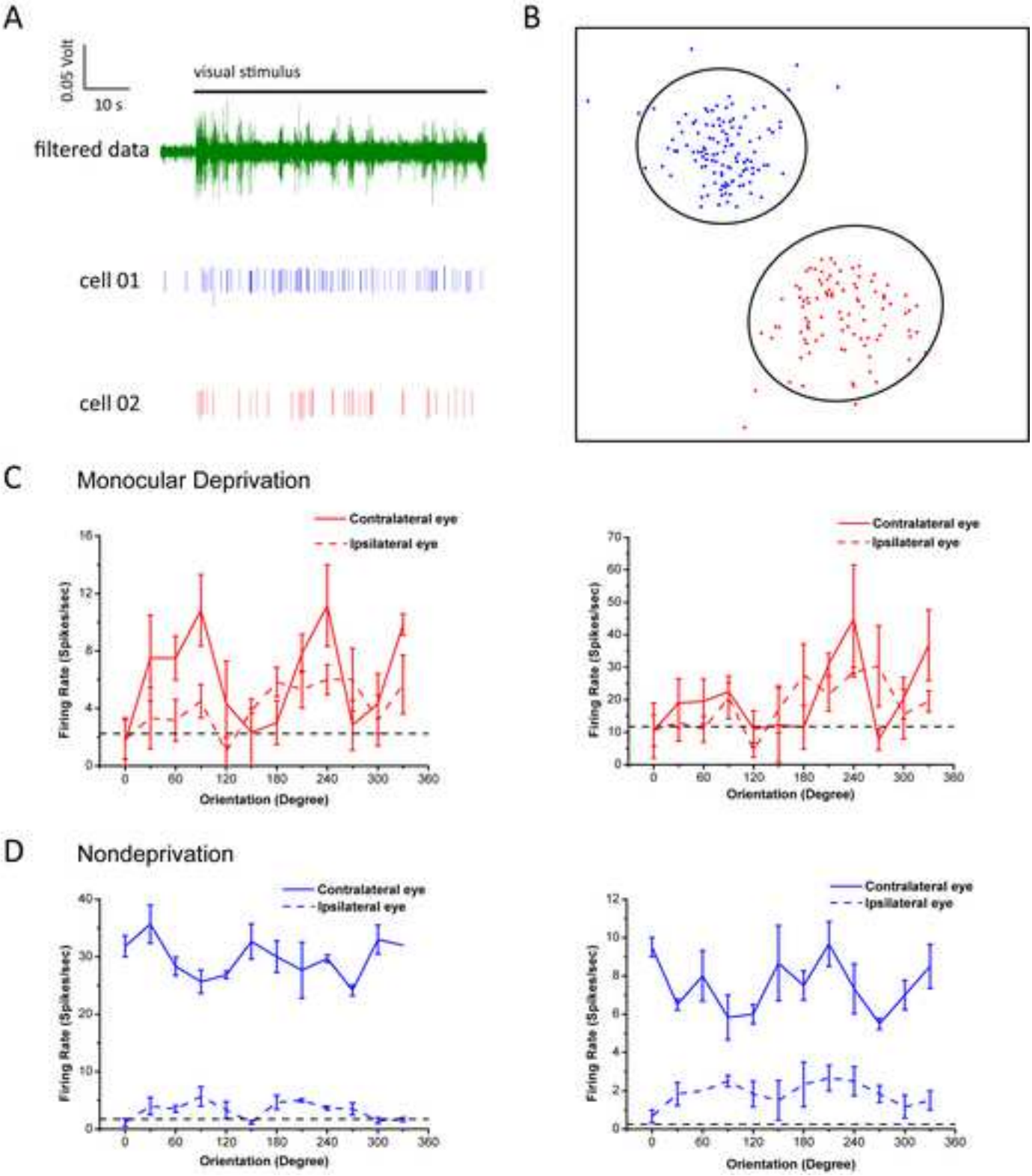


Figure



Figure

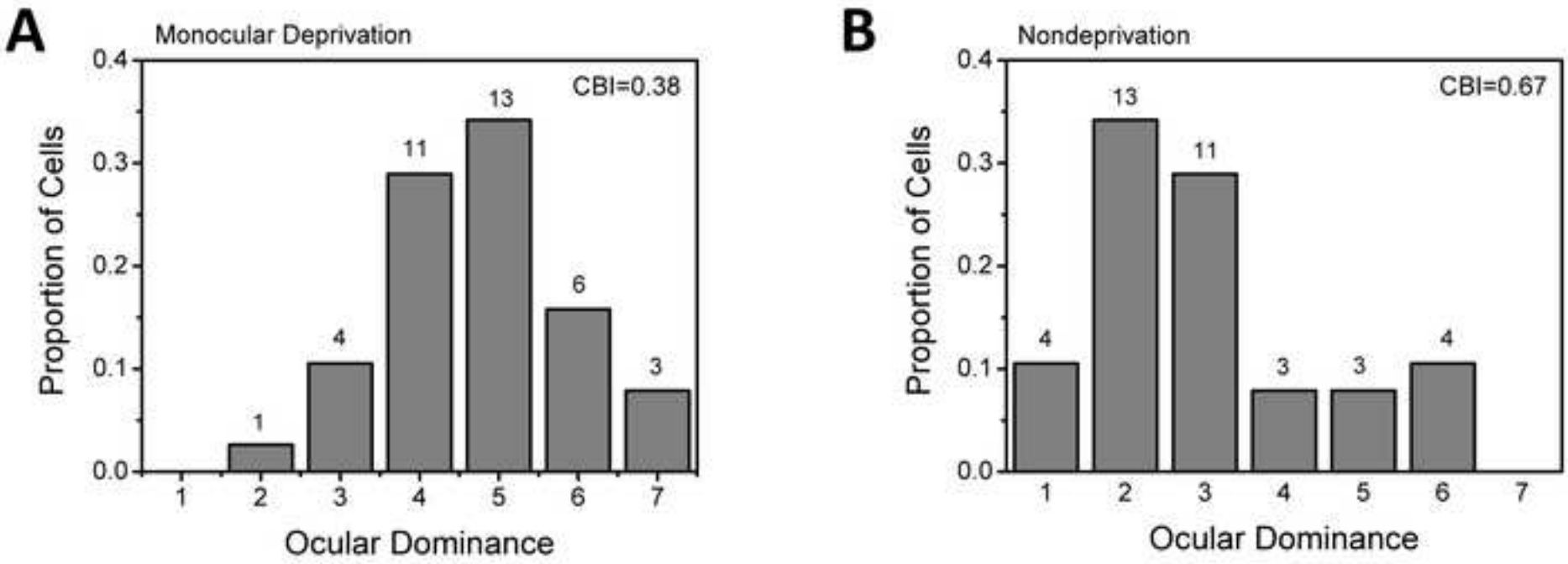


Table of Materials

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Name of Material/ Equipment	Company
502 glue	M&G Chenguang Stationery Co., Ltd.
Acquizition card	National Instument
Agarose	Biowest
Amplifier	A-M system
Atropine	Aladdin Bio-Chem Technology Co., Ltd
Brain Stereotaxic Apparatus	RWD Life Science Co.,Ltd
Cohan-Vannas spring scissors	Fine Science Tools
Contact Lenses Solutions	Beijing Dr. Lun Eye Care Products Co., Ltd.
Cotton swabs	Henan Guangderun Medical Instruments Co.,Ltd
Fine needle holder	SuZhou Stronger Medical Instruments Co.,Ltd
Forcep	66 Vision Tech Co., Ltd.
Forcep	66 Vision Tech Co., Ltd.
Forcep	66 Vision Tech Co., Ltd.
Heating pad	Stryker
Illuminator	Motic China Group Co., Ltd.
Isoflurane	RWD Life Science Co.,Ltd
LCD monitor	Philips (China) Investment Co., Ltd.
Microscope	SOPTOP
Noninvasive Vital Signs Monitor	Mouseox
Oil hydraulic micromanipulator	NARISHIGE International Ltd.
Petrolatum Eye Gel	Dezhou Yile Disinfection Technology Co., Ltd.
Spike2	Cambridge Electronic Design， Cambridge， UK
Surgical scissors	66 Vision Tech Co., Ltd.
Surgical scissors	66 Vision Tech Co., Ltd.
Suture Needle	Ningbo Medical Co.,Ltd
Tungsten Electrode	FHC, Inc
Xylocaine	Huaqing

Catalog Number	Comments/Description
AWG97028	
PCI-6250	
G-10	
Model 1800	
A135946-5	
68001	
15000-02	
GM17064	
CZQ1370	
53320A	
53072	
#5	
TP 700 T	
MLC-150C	
R510-22	
39PHF3251/T3	
SZMT1	
PC-5N06022	
17C801	
Spike2 Version 9	
54010	
54002	
3/8 arc 2.5*8	
L504-01B	

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Date:	2019.7.19

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Changes to be made by the Author(s) regarding the written manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Response: After carefully revising the spelling and grammar issues, we send the manuscript to an English editing company for a further revision.

2. Please provide an email address for each author.

Response: We have provided the email address for all authors in the manuscript.

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3. Please include a Short Abstract/Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

Response: We have included a short Summary to describe the protocol and it’s applications in the manuscript. “Here, we present detailed protocols for monocular visual deprivation and ocular dominance plasticity analysis, which are important methods of studying the neural mechanisms of visual plasticity during the critical period and the effects of specific genes on visual development”.

4. Please ensure that the long Abstract is within 150-300 word limit and clearly states the goal of the protocol.

Response: The Abstract is 238 words and we clearly states the goal of the protocol.

5. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep and note in the protocol section. Please use Calibri 12 points

Response: We have changed the format of the manuscript according to the Instructions for Authors.

6. Please remove the line header from all the pages of the manuscript.

Response: We have removed the line header from all the pages.

7. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly.

Response: We have changed the inappropriate tone and ensure that all text in the protocol section is written in the imperative tense.

8. The Protocol should contain only action items that direct the reader to do something.

Response: We have revised the protocol according to the requirement.

9. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please ensure that individual steps of the protocol should only contain 2-3 actions per step.
Response: We have revised the protocol according to the requirement.

10. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary.
Response: We've adjust the numbering of the protocol to follow the JoVE Instructions for Authors.

11. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed?

Response: We've added more details to the protocol steps in the revision including all following questions.

1.3: Do you check the depth of anesthesia?

Response: It is important to maintain a moderate anesthetic concentration in animal surgery and recording. We estimate the depth of anesthesia mainly by mouse's respiratory frequency and their response to pinch their hind legs according to our experience. In surgery and electrophysiological recording, we often check the depth of anesthesia and adjust the anesthetic concentration.

13. 2.1: What kind of deprivation is performed?
Response: The mouse are performed monocular visual deprivation. The protocol of monocular visual deprivation is in part 1 of the protocol.

14. 2.3-2.6: How is this done?

Reponse: 2.3-2.5. This steps are the most routine operations in mouse experiment. So we did not explain it in details. 2.6: We added some explanation about how to “fix a bone screw to the skull as the reference”. It included three steps. Drill a 1mm×1mm hole in the skull above the cerebellum. Affix a small bone screw in the hole as the reference electrode and seal the hole with dental cement.

15. Please describe the result with respect to your experiment, you performed an experiment, how did it help you to conclude what you wanted to and how is it in line with the title.

Response: We have reedited the result part to conclude our experiment, “The result shows the response of the V1 neurons to the contralateral eye to a stimulus was much stronger than the response of the ipsilateral eye in the binocular segment of the nondeprived mouse. However, after 4 days MD in critical period, the response of most neurons to stimuli contralateral eye was relatively close or weaker than the response to the ipsilateral eye. Therefore, the V1 neurons in critical periods have significant OD plasticity. MD alters the relative strength of cell’s response by stimulating the contralateral and ipsilateral eye”.

16. Please discuss all figures in the Representative Results. However, for figures showing the experimental set-up, please reference them in the Protocol. Please discuss figures in the order of their numbering.

Response: We have discuss all figures in the representative results.

17. Please remove the embedded figure(s) from the manuscript. All figures should be uploaded separately to your Editorial Manager account. Each figure must be accompanied by a title and a description after the Representative Results of the manuscript text.

Response: We have removed the embedded figure(s) from the manuscript. And all figures have been uploaded separately to your Editorial Manager account.

18. Please include all the Figure Legends together at the end of the Representative Results in the manuscript text.

Response: We have include all the Figure Legends together at the end of the Representative Results in the manuscript text.

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Response: The Figures we used are original, so there are no copyright issue in this manuscript.

20. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

Response: We have revised the discussion part according to the requirement including all the parts mentions above.

21. Please do not abbreviate the journal titles in the references section.

Response: We have revised the journal titles in the references section complying with the requirements of the JoVE journal.

Changes to be made by the Author(s) regarding the video:

1. The video is not publication grade. This video was delivered at a 16:9 aspect ratio, but the visible portion of the video itself is a 4:3 aspect ratio. Future submissions should be delivered at a 4:3 aspect ratio so that the video fills the entire frame. There should be no wide black borders on the left and right of the frame. This video was also encoded as anamorphic, meaning the pixel aspect ratio is not 1:1 (i.e. the true pixels are not square). This results in resolution loss, and thus quality loss in the video. Future submissions should also have a square pixel aspect ratio.

Response: We have revised and reedited the video according to the requirement of the JoVE.

2. Please use the degree symbol and do not use any superscript 0 or o in the text. Please use h for hour. Please include a single space between the number and the units.

Response: We have revised and reedited the video according to the requirement of the JoVE.

3. Please ensure that the section titles are same in the text and the video.

Response: We have checked the all section titles to ensure it.

4. For the software steps please include a click by click instruction of how to do

the procedure.

5. Please ensure that all the result figures are present in both text and the video.

6. 1:05 - There is a typo here. It should read Sterilization.

7. 5:37 onwards… please include video clips of the same.

8. 8:30 - The www.JoVE.com must be removed.

Response: We have revised and reedited the video according to the requirement.

Please upload a revised high-resolution video here: https://www.dropbox.com/request/imxx3dKGeoW0IKqY8VgX?oref=e

Response: We have uploaded a revised high-resolution video.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The authors provide a protocol for the standard method of OD experiments in mice, as it has been used for at least 23 years. There's nothing innovative in the manuscript, but apparently, "novelty is not a requirement", as the reviewer invitation informs me.

The video is concise and clear. There are just a few points that should be fixed in the paper:

Major Concerns:

For both MD and recording, the authors suggest to put the mouse into a stereotaxic apparatus. It is vital to add that non-crush ear bars must be used

(as the authors have apparently done, judging by the video). Otherwise, deafening the mouse will interfere with both ocular dominance and its plasticity, as Manuel Teichert and colleagues have recently shown in several papers.

Response: Thanks for your comments. The ear bars have two sides. We reedited the video and used the non-crush side of the ear bar to fix the mouse. And we also noticed not put so much pressure on its head.

Minor Concerns:

Introduction: The first paragraph might require a few citations, e.g. to the sentence "Many investigations have shown a critical period . . ."

Response: Thanks for your comments. We have revised and reedited the first paragraph.

After having referred to "different animals", it is imprecise to later focus on LGN layers and OD columns in the first paragraph, since they don't exist in rodents. Maybe just add "in cats" at an appropriate place.

Response: Thanks for your suggestion. We have reedited and revised the first paragraph.

Response: Thanks for your comments. We have revised and reedited the corresponding sentences and paragraph.

Protocol:

The first sentence doesn't quite make sense. The animals were 28-32 days old "on the day of recording from the Institute"? In correcting, please make sure that you clarify whether the age is given at MD or at recording.

Response: P28 is the time for MD. We revised the expression in the manuscript.

1. MD, point 4: From the description of the suturing and knotting, I couldn't do it. You might provide an illustration, given that you emphasize the point again in the discussion. Another important point: At least in Germany, it is mandatory to provide the animal with analgesia (carprofen) on a daily basis until the day of recording.

Response: We have revised the Figure 1A and protocol including the description of suturing and knotting and injecting intraperitoneal the mouse with buprenorphine SR (1mg/kg).

2. Craniotomy, point 1: Sure that you induce anaesthesia with 0.5% isoflurane? Higher concentrations are usually needed for induction, and then you reduce isoflurane.

Response: Actually we use 2% isoflurane to induce anaesthesia, and then use 1.2-1.5% isoflurane in craniotomy. Finally, we reduce the anaesthesia to 0.5%-0.8% in electrophysiological recording because V1 cells have good response in low isoflurane. We reedit the steps in anaesthesia operation. We have revised and reedited the manuscript.

point 2: "Mouse with corneal opacities or signs of infection were excluded from further study." First, it should be "mice", and second, as this is a protocol for others, it should be "must be excluded" rather than "were excluded".

Response: Thanks for your suggestion. We have revised the express.

3. Visual stimulation, point 4: Although it is self-understood, it should be added that, of course, each eye is stimulated for any recorded neuron.
Response: Thanks for your comments. We have revised the corresponding parts.

Figure 1: The legend does not correspond to the image, as the illustration of MD (supposedly Fig. 1A) is missing.

Response: Thanks for your comment. We have revised the corresponding figure and figure legend.

Discussion, line 232: "then, the lids of extra mice should be sutured." I don't understand what is meant.

Response: Thanks for your comment. We have reedited the discussion.

Line 269: "An interesting study showed . . ." Then, of course, it must be cited.

Response: Thanks for your comments. We have reedited the corresponding paragraph.

Line 291: "We could have surveyed a larger range of V1 by IOI; however, the experiment is much more complicated." Sorry, but that is most certainly nonsense.

Response: Thanks for your comments. We have revised the corresponding paragraph.

Reviewer #2:

Manuscript Summary:

The author present their protocol of monocular deprivation and in vivo single unit recording of visually evoked cortical activity.

Major Concerns:

Comment 1:

The first paragraph of the introduction need major editing. There are sentences basically discussing the same concept repeatedly thus are very redundant. The author described the experiment of Hubel and Wiesel from line 49 to 53 and then again describe the consequence of MD in line 54 to 57. Same with the LGN experiment. Line 57-59 and line 59-61 deliver the same information so the author should combine those into one sentence. The author need to properly cite other's work. Especially in sentence like "Many investigations have shown a critical period for the visual deprivation effect in different animals." and also the LGN work. The author should explain what critical period (CP) is because it is a very important concept for ODP. The authors should also pay attention to the details of previous work in the field and describe the results more accurately. When discussing the work of Hubel and Wiesel, the author state that Hubel and Wiesel deprived the animal at various time points and for varying periods of time and this deprivation resulted in loss of response to the deprived eye. But Hubel and Wiesel also pointed out MD in adult cat cannot induce ODP. Failure to mention this important information is misleading to the readers. The authors also stated "During this critical period, the deprivation of vision in one eye will reduce the intensity of the neural response driven by the deprived eye and increase the response driven by the nondeprived eye". Actually in CP animals, short term MD leads to reduction of response to the deprived eyes but no increase in nondeprived eye yet. This is actually what the author observed in their own experiment in Fig 2.

Response: Thanks for your comments. We have reedited and revised the first paragraph of the introduction according your suggestion.

Comment 2:

Fig 1 missing panel A. Fig B, lacking label of x and y axis.

Response: Thanks for your comments. We revised the Figure 1.

Comment 3:

The video of the protocol lacks some important steps, such as trim the eyelid, monitor the heart rate and degree of blood oxygen saturation. The procedure of craniotomy is also not clear enough. This part is better videotaped using microscope.

Response: Thanks for your suggestions. We reedited the video and added some important video clip, included trimming the eyelid and doing clear craniotomy. We did not monitor the heart rate and degree of blood oxygen saturation in the experiment because the anesthesia is easy to control by isoflurane. It is important to maintain a moderate anesthetic concentration in animal surgery and recording. We estimate the depth of anesthesia mainly by mouse's respiratory frequency and their response to pinch their hind legs according to our experience. In surgery and electrophysiological recording, we often check the depth of anesthesia and adjust the anesthetic concentration.

Comment 4:

The first paragraph of the discussion also need major editing. The author here mainly discussed some detail for surgical procedure but they should follow a clear logic line there. The different points are currently out of order. I suggest the author first discuss the details to ensure the quality of suture: trim the eyelid, number and position of knots and using 502 glue. Then the detail to improve wound healing and reduce discomfort: simple continues suture and size of needle. Alternating measurements actually does not belong to surgical procedures. So line 226 should not limit the following discussion to surgical procedure.

Response: Thanks for your comments. We have revised the according corresponding parts ensure a clear logic.

Response: Thanks very much for your valuable suggestion. We have reedited and revised the discussion according to your comments.

Minor Concerns:
Line 30, "the response of the contralateral eye" should be "the response of the cortex to the contralateral eye"

Line 34, "neural circuits" is not a technology

Line 40, "during" should be "between".

Response: Thanks for your comments. We have revised the corresponding parts.

Line 90, ointment to eyelid?

Line 101, sterilize the hood twice?

Response: Thanks for your comments. We have revised the corresponding parts.

Line 122, what is the temperature of the agarose gel? Will it be too hot for the mice?

Response: The temperature of the agarose gel is 40 degree centigrade. It is a right temperature for the mice.

Line 127, how to monitor the heart rate and blood oxygen saturation?
Response: We change the express in the protocols. In fact, we observe the

breathing of the mouse to see its anesthesia.

Visual Stimulation and Electrophysiological Recording part, what is the isoflurane level here?

Response: We start with a higher concentration of isoflurane (1%) to anesthetize the mouse, and then you reduce isoflurane (0.5%) when the mice were completely anesthetized.

Line 144, what is "block"?
Response: We change the “block” to “repeat”.

Line 158, how to correlate the visual stimulus with single neuron spike, more specifically, how to synchronize the recording with the visual stimulation?
Response: We use a photoelectric sensor to synchronize the recording with the visual stimulation. When the stimuli start, the corner of the screen will flash, then the photoelectric will receive the flash and transfer it to a pulse signal. The acquisition card will get the signal as a mark.

Line 161 by response strength, do the author mean spike number or spike amplitude?
Response: The response strength is mean spike number.

Fig 2, MD and nondeprivation data are mislabeled in the video
Response: We have change the mistake in the video.

Line 247, "very popular" feel strange, change to "commonly available"?
Response: Thanks for your comments. We have revised the corresponding parts.

Line257, standard cage?

Response: Enriched environment cages provide increased physical (running wheels), social (bigger housing groups), and cognitive (regularly changed labyrinths or toys) stimulation compared to the deprived rearing conditions of standard cages. In this study, we adopted enriched environment cages for MD plasticity during the critical period.

Line 262-263, is this the result of the author's experiment or previous paper?
Response: We deleted this paragraph. We think it is redundant.

Line 266-270, the sentence "It has been reported that MD in adult mice induces OD plasticity." is repeating previous sentence. "OD plasticity is equally robust in young adults and mature adults". Please be reminded that the ODP in mature adults is observed in EE mice, not normal mice, so this sentence is inaccurate and misleading.
Response: Thanks for your comments. We have remove the corresponding sentence.

Line 271, what are tracks?
Response: We change the “track” to “penetration”.

Line 293-294, the sentence feels strange here.
Response: We have deleted the sentence here.