Please cite this article as: Celeste *et. al.*, (2018). Three-chamber Social Approach Task with Optogenetic Stimulation (Mice), *Bio-protocol* 8 (24): e3120. DOI: 10.21769/BioProtoc.3120.

bio-protocol www.bio-protocol.org/e3120 Vol 8, Iss 24, Dec 20, 2018 DOI:10.21769/BioProtoc.3120

Three-chamber Social Approach Task with Optogenetic Stimulation (Mice)

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[Abstract] The formation of social relationships via social interactions and memory are essential for one's physical and mental health. To date, rodent studies have used the three-chamber social approach test to measure social approach, social novelty, and social memory. In recent years, techniques including optogenetics have been developed to acutely control the activity of genetically defined populations of neurons. Recent studies have even combined optogenetics with advanced temporal gene expression control systems to label certain populations of neurons during learning and subsequently reactivated for memory testing. We combined optogenetic targeting with the three-chamber social approach test to examine particular neural circuits of interest during social memory encoding or retrieval. First, we stereotaxically infected specific brain areas with viral-encoding opsins that acutely activate or inhibit the firing of the neurons. Next, we subjected the mice to the three-chamber behavioral paradigm while delivering light during social memory encoding or retrieval. Lastly, the mice were tested with the delivery of light in a counter-balanced manner which allows each subject to be its own internal control. Thus, the optogenetic stimulation coupled with the three-chamber social approach test is a well-validated paradigm to explore the contribution of diverse brain circuits in various social cognition processes.

Keywords: Social approach, Social recognition, Social memory, Memory encoding, Memory retrieval, Optogenetics

[Background] Social cognition is essential to our mental health. Deficits in social interaction and memory are hallmark characteristics in numerous brain disorders. Social working memory is an extremely dynamic process that is often unpredictable and requires constant adaptability to the changing stimuli (Lieberman, 2007). Specifically, it is a cognitive process that involves the encoding, storage, and retrieval of socially salient information. Traditional human and primate studies using imaging and lesion experiments, have implicated the medial temporal lobe in social cognitive tasks including social recognition and context evaluation (Insel and Fernad, 2004; Olson *et al.*, 2013; Sandi and Haller, 2015). However, these previous studies employed toxins which caused extensive damage to broad areas in the brain and have limited the control of targeted cell types and subcellular compartments. Recent studies employing acute optogenetic approaches have implicated various brain regions and circuits, including the amygdala, hippocampus, and ventral tegmental area to play a critical role in the different facets of social behavior, including social interactions, approach, and discrimination memory (Felix-Ortiz and Tye, 2014; Gunaydin *et al.*, 2014; Hitti and Siegelbaum, 2014; Okuyama *et al.*, 2016).

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To investigate the behavioral significance of a particular brain region in social memory processing, subject mice can be evaluated on their performance in a well-validated three-chamber social approach paradigm (Nadler *et al.*, 2004). In this test, the subject mouse is habituated to the apparatus (stage 1). Then it is first introduced to a novel stranger mouse (S1) to evaluate memory formation and sociability (stage 2), and subsequently presented to a second novel stranger mouse (S2) to test social recognition memory and retrieval (stage 3). The amount of time the mouse spends investigating the stranger mice is recorded. Wild-type mice spend more time exploring a novel stranger mouse compared to a familiar conspecific. Therefore, combined with optogenetic targeting, specific neural circuits can be activated or inhibited during the various stages including memory formation (encoding) or retrieval (discrimination). Furthermore, the use of optogenetics can also identify particular genes in the neural circuits that may be involved in the regulation of sociability and/or social recognition memory in mice (Leung *et al.*, 2018).

Materials and Reagents

1. Filter paper
2. Multimode fiber (0.39 NA, high OH, 200 µm Core, Wavelength range: 300-1,200 nm; ThorLabs, catalog number: FT200UMT)
3. Materials for optogenetic experiments (Table 1)
   a. Multimode fiber (0.39 NA, high OH, 200 µm Core, Wavelength range: 300-1,200 nm; ThorLabs, catalog number: FT200UMT)
   b. 1.25 mm Ceramic Stick Ferrule, 230 µm (Precision Fiber Products, catalog number: MM-FER2007C-2000)

Table 1. Summary of the main characteristics of materials used for optogenetic experiments

Materials	Characteristics
Multimode fiber (0.39 NA)	- Large operating wavelength range with TECS cladding which increases fiber's strength, reduces static fatigue, and protects the fiber
1.25 mm Ceramic Stick Ferrule	- Low insertion loss and back reflection that allows for high precision and reliability

4. Injection cannula (5 mm, 26 gauge; Plastics One, catalog number: C315GS-5/SPC)
5. 1 ml Syringe (BD Sciences, catalog number: 309628)
6. Tygon tubing 1/16" I.D. 1/8" O.D. and 1/32" wall (US Plastics, catalog number: 57102)
7. Aluminum foil (Alcan)

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Note: Ceramic ferrules was chosen over metal ferrules, to ensure accurate fiber alignment.

8. 1.25 mm SM Ceramic split sleeve, 6.60 mm Length (Precision Fiber Products, catalog number: SM-CS125S)
   Note: Ceramic split sleeves were chosen over metal split sleeves, to ensure accurate fiber alignment.
9. 1.25 mm Ferrule dust cap, white (Precision Fiber Products, catalog number: BCDC-1300-W)
10. Parafilm M (Pechiney Plastic Packaging, catalog number: PM-996)
11. Cotton swab (The Lab Depot, catalog number: 394305)
12. C57BL/6 mice (The Jackson Laboratory, catalog number: 000664)
    Note: Male and female subjects (4 weeks+ old) and strangers (< 3 weeks old) have been successfully trained in this paradigm using this protocol.
13. AAV Viruses (stored at -80 °C until use with a titer of ~10^{11} to 10^{13} pfu/ml)
    For example: AAV-CaMKIIα: eArchT3.0-EYFP (University of North Carolina GTC Vector Core) AAV-CaMKIIa-hChR2 (H134R)-mCherry-WFRE-PA (University of North Carolina GTC Vector Core).
14. Dental cement (Stoelting Co., catalog number: 51458)
15. Speed set instant mix epoxy (LePage, https://www.lepage.ca/en/lepage-products/build-things/epoxies/speed_set_instantmixepoxy.html)
16. Suture kit (Ethicon, catalog number: JJ489)
17. Tear gel (Novartis, https://well.ca/products/tear-gel-liquid-eye-gel_17561.html?gclid=CjwKCAjw6-_eBRBXEiwA-5zHaVuKltJxR7GYDuMwgeSM5yh_3J6FSiTehp0ekoUOvym2ICm3-YjZTxoCIkoQAvD_BwE)
18. Betadine
19. Saline
20. 70% ethanol
21. Hydrogen peroxide
22. Ice
23. Analgesics: Metacam (CDMV, 5 mg/ml one daily injection for three consecutive days)

Equipment

1. Cleaver (Doric Lenses, catalog number: B600-0002)
2. Fiber stripping tool (ThorLabs, catalog number: T12S21)
3. 10 µl Pipette (SARSTEDT, catalog number: 90.1771.002)
4. Digital Caliper (ULINE Canada)
5. Mouse stereotaxic frame (Neurostar)
6. Anesthesia system for isoflurane (Kent Scientific Corporation, catalog number: SOMNO-MSEKIT)
7. Temperature controller (Sunbeam)
8. Heating pad

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9. Surgical tools including scissors, forceps, scalpels (Fine Science Tools)
10. 0.6 mm Drill bit connected to the stereotaxic frame (RWD Life Science, catalog number: 78001)
11. Two-syringe infusion pump (World Precision Instruments, catalog number: SP200iZ)
12. 10 µl Hamilton Gastight syringe (Hamilton, catalog number: 84875)
13. White noise machine (Marpac)
14. Fiber cable, MM, 200 µm, 0.39 NA, FC/PC-FC/PC (Thorlabs, catalog number: M72L02)
15. Equipment for optogenetic experiments (Table 2)
    a. 473 nm DPSS Laser system (Laserglow, catalog number: R471003GX)
    b. 532 nm DPSS Laser system (Laserglow, catalog number: R531003GX)
    c. Function/arbitrary waveform generator (BK Precision, catalog number: 4052)

Table 2. Summary of the main characteristics of the equipment used for optogenetic experiments

Equipment	Characteristics
473 nm Diode-Pumped Solid-State (DPSS) Laser System	- Ideal for 1 mW to 1,500 mW with adjustable output power - Long-term output power stability
532 nm DPSS Laser System	- Ideal for < 5 mW to > 20 W with adjustable output power - Long-term output power stability
Function/arbitrary Waveform Generator	- Capable of generating stable and precise sine, square, triangle, pulse, and arbitrary waveforms - 14 bit, 125 MSa/s, 16 k point arbitrary waveform generator

16. Power meter, Si sensor, 400-1,100 nm, 500 nW-500 mW (ThorLabs, catalog number: PM121D)
17. Laser Glasses, 180-532 nm (ThorLabs, catalog number: LG3)
18. Doric mini cube (Doric Lenses, catalog number: B340-0204)
19. 2 Mono fiberoptic patch cords (Doric Lenses, catalog number: D202-2302)
20. 45 cm wide x 20 cm long x 30 cm high three-chamber apparatus (ANY-maze)
21. 8 cm diameter x 17 cm high cylindrical wire cage (ANY-maze)
22. Autoclave

Software

1. ANY-maze tracking software (Stoelting Co., http://www.anymaze.co.uk/)

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Procedure

A. Optic fiber generation

1. Strip a ~5 cm segment of the multimode optic fiber (200 µm diameter, 0.39 NA) using the fiber stripping tool (Figure 1).

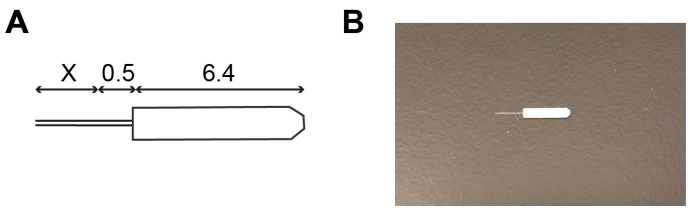

Figure 1. Optic fiber measurements. A. Schematic of optic fiber thread through a ceramic ferrule. The length (in mm) of the optic fiber depends on the desired targeting region. Add the length of the optic fiber (6.4 mm) and the gap between the ferrule and the skull (0.5 mm) to the Z coordinate of the desired targeting region (denoted as X mm). B. Example of a finished optic fiber.

2. Use the cleaver to cut a segment of the stripped multimode fiber that is of the appropriate target length as measured using the digital caliper [For example, length = 6.4 mm (ceramic ferrule) + 0.5 mm (gap between ferrule and skull) + desired target depth as determined from a reference atlas in mm] (Figure 2).

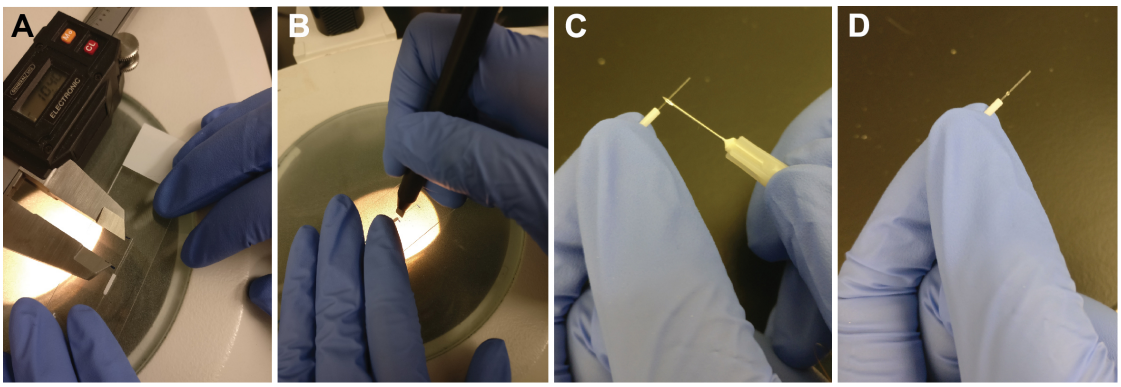

Figure 2. Optic fiber construction. A. Use the digital caliper to measure length of interest. In this example, the Z coordinate of the target region is 3.5 mm. Thus, the total length of the optic fiber is 10.4 mm (3.5 mm + 0.5 mm + 6.4 mm). B. Use the cleaver to cut the appropriate length. C. To secure the optic fiber to the ceramic ferrule, add a drop of epoxy to the middle of the cut optic fiber. D. Push the ferrule over the drop of epoxy to secure it to the optic fiber.

3. Secure one end of the cut fiber to a ceramic ferrule (230 µm bore size, 1.25 mm outer diameter) using epoxy.
4. Allow optic fibers to dry overnight.

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5. Autoclave optic fibers prior to implantation.
6. Confirm the transmittance of each optic fiber. Compare the laser power measured from the power meter directly from the laser box connected to the fiberoptic patchcord to the laser box connected to the fiberoptic patchcord and the optic fiber. The laser transmittance of each optic fiber must be > 75% to be used for implantation.

B. Preparation for surgery
1. Connect the injection cannula to one end of the Tygon tubing while the other end is attached to a 1 ml syringe.
2. Pipette a desired volume (2 µl/mouse) onto a piece of parafilm.
3. Fill the injection cannula with the virus by slowly aspirating using the 1 ml syringe. Upon completion, detach the syringe from the Tygon tubing.
4. Keep the injection cannula and Tygon tubing with the virus on ice, covered with aluminum foil. *Note: The injection cannula should be filled with the virus prior to the start of each mouse. The tubing with the virus should be kept on ice for a maximum of 1 h. The virus aliquot should be kept in dry ice.*

C. Viral injection
1. Remove mouse (4-6 weeks of age) for surgery from home cage and place it into an unused cage (single-housed) prior to surgery for ~15 min.
2. Place the mouse in the induction chamber of the rodent anesthesia machine (induction rate: 4% isoflurane) and wait 3 min until the respiration of the mouse is steady. Toe pinch to ensure mouse is fully anesthetized.
3. Transfer the mouse from the induction chamber onto the heating pad (set at 37 °C) and connect the anesthesia mask to the nose (maintenance rate: 2.5% isoflurane). Ensure that the flow rate of anesthesia is steady and the mouse is breathing naturally.
4. Insert the ear bar of the stereotaxic frame to ensure mouse is parallel to the base panel of the frame. Add tear gel to both eyes (Figure 3A).
5. Apply 70% ethanol with a cotton swab to clean the site of incision, followed by betadine, and finally repeating with 70% ethanol.
6. Make a horizontal incision, from anterior to posterior, with the scalpel to expose the skull and mark Bregma (Figure 3A).
7. Use hydrogen peroxide on a cotton swab to dry the surface of the exposed skull.
8. Mark the skull based on the desired bilateral coordinates (AP/ML) for the viral injection sites using the drill.
9. Position the injection cannula containing the virus into the stereotaxic frame and ensure it is connected to the Hamilton syringe.
10. Prior to the injection, program the infusion pump to continuously infuse at 0.1 µl/min until a drop of the virus can be observed by eye.

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11. Use filter paper to remove the small drop and program the infusion pump to withdraw 0.1 µl of air at 0.1 µl/min (to form an air bubble between the tip of the infusion cannula and the virus).
12. Once set up on the stereotaxic frame, re-measure the desired coordinates (AP/ML) with the injection cannula (the injection cannula should sit directly above the mark on the skull generated by the drill).
13. Lower the infusion cannula until its tip touches the surface of the skull.
14. Program the infusion pump to infuse 0.1 µl at a rate of 0.1 µl/min. During the infusion (of the air bubble that was previously created from Step C11), gently lower the infusion cannula to the appropriate DV coordinate.
15. Wait 2 min prior to infusing the virus and program the infusion pump to infuse 0.5 µl of the virus at a rate of 0.1 µl/min. Once infused, leave the internal cannula in place for an additional 5 min prior to raising.
16. Raise cannula slowly (~0.1 mm/s). Once removed, clean the tip of the internal cannula with ethanol. Ensure that tip is not blocked and repeat Steps C10-C15 for bilateral injections.

D. Fiber implantation
    1. Following viral injection, remove the injection cannula from the stereotaxic frame. Ensure the injection cannula and tubing with the virus is stored on ice and covered with foil.
    2. Mark the skull based on the desired bilateral coordinates (AP/ML) for the optic fibers using the drill.
    3. Connect the removable arm to the stereotaxic frame and secure a single optic fiber at the tip of the arm using parafilm (Figure 3B).
    4. Once set-up, re-measure the desired coordinates (AP/ML) with the optic fiber (the optic fiber should sit directly above the mark on the skull generated by the drill) (Figure 3B).
    5. Lower the optic fiber to the appropriate DV coordinate. Gently peel off the parafilm that is connecting the optic fiber to the arm using forceps.
    6. Move the stereotaxic arm away from the optic fiber.
    7. Repeat Steps D3-D6 for the bilateral implantation of the optic fibers (Figure 3C).
    8. Once both optic fibers are implanted, secure both optic fibers with dental cement (Figures 3D and 3E).

       Note: Gently apply dental cement around the base of the optic fibers. Ensure optic fibers remain in place using the arm until the dental cement dries.

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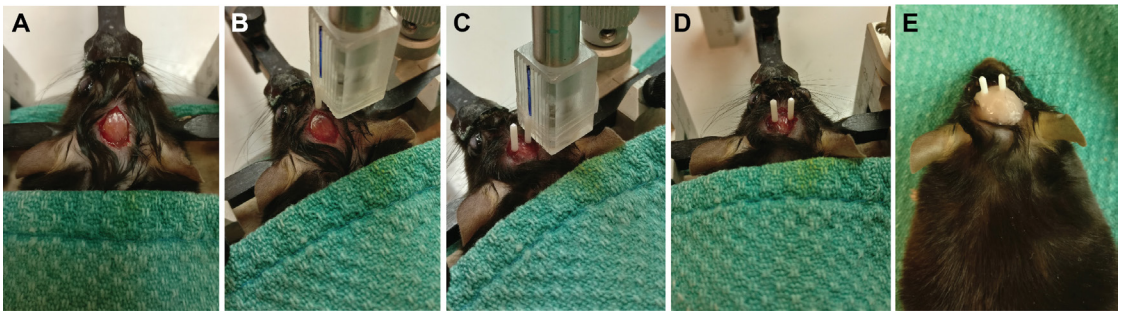

Figure 3. Stereotaxic surgery for bilateral optic fiber implantation. A. Horizontal incision to expose the skull. B. A single optic fiber secured on the removable arm with parafilm at the AP/ML coordinate, prior to being implanted. C. The second optic fiber bilaterally implanted using the removable arm. D. Optic fibers implanted bilaterally. E. Bilateral optic fibers secured with dental cement.

Note: Anesthesia mask is not displayed in these figures.

9. Suture the skin and clean surgery site with betadine. Inject appropriate analgesics and place mouse on a separate heating pad for 1 h. Ensure mice are fully recovered prior to returning it to a new cage. Mice are single-housed following surgery to prevent fighting. Monitor post-surgical health (Body weight, hydration status, posture/appearance, grimace [Matsumiya *et al.*, 2012] and behavior) for at least 7 days.

E. Three-chamber social approach test

1. Following 7 days of post-surgical recovery, handle mice for 10 min over 3 days in the testing room prior to experimentation. Ensure mice are comfortable with being scruffed and connected the patch cords to the implanted optic fibers via the ceramic sleeves in their home cage.
2. Prepare 2 juvenile wild-type stranger mice (3-4 weeks of age, to prevent fighting) that are matched by sex to the subject mice. Single house each stranger mouse, 48 h prior to testing.
3. Set up camera, lighting (20 lux), and white noise generator (65 db).
4. Clean the three-chamber apparatus thoroughly using 70% ethanol. Set up the three-chamber apparatus by placing the two empty cages in the outer chambers, while leaving the middle chamber empty.
5. Twenty-four hours prior to testing, connect each mouse to the patch cords and allow a 3-min home cage habituation period, followed by 10 min of exploration within the three-chamber apparatus. Following both habituation periods, return mouse to home cage.
6. Twenty-four hours prior to testing, habituate the stranger mice in the stranger cages (Figure 4A) for 10 min.
7. On the day of experimentation, use the power meter to test the strength for both 532 nm and 473 nm lasers (Ensure the power output is consistent between both patch cords).

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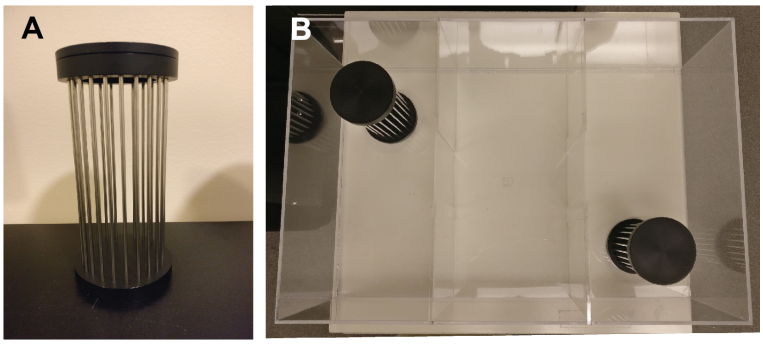

Figure 4. Apparatus for the three-chamber social approach test. A. The cage used to place the juvenile strangers for the test. B. The three-chamber social approach apparatus with two empty cages placed on the two outer chambers.

8. When ready for testing, connect mouse to the patch cords (which is in turn connected to the waveform generator) and allow a 3-min home cage habituation period, followed by an additional 3-min habituation period in the middle chamber of the three-chamber apparatus.
9. Remove the partitions and allow the mouse to freely explore the three-chambers (with 2 empty stranger cages on outer chambers) for 10 min.
10. Replace partitions following the habituation stage, while ensuring the subject mouse is back in the center chamber for 1 min.
11. Place the first stranger mouse into one of the two empty stranger cages (the placement of the stranger in the empty cages can be counter-balanced between different subjects, ensure this information is recorded through the software).
12. Remove the partitions once again and allow the mouse to freely explore the three-chambers for 5 min. The time spent sniffing the stranger mouse in the cage versus the empty cage in the other chamber is recorded manually and through the video tracking system. During this sociability stage, optogenetic manipulations can be applied to test the role of the circuit of interest in social memory encoding. For example:
    a. For mice expressing ArchT: Bilaterally deliver 532 nm (15 mW and ~119.43 mW/mm^2) green-light continuously for 5 min.
    Note: Power can be adjusted accordingly for different regions.
    b. For mice expressing ChR2: Bilaterally deliver 473 nm (20 Hz, 5 ms pulse width, 6.5 mW and ~51.75 mW/mm^2) blue-light 30 s light-on followed by 30 s light-off pattern for 5 min.
    Note: Pulse frequency/duration can be adjusted accordingly for different regions.
13. Replace partitions following the sociability stage, while ensuring the subject mouse is back in the center chamber for 1 min.
14. Place the second stranger mouse into the empty stranger cage.
15. Remove the partitions and allow the mouse to freely explore the three-chambers once again for 5 min. The time spent sniffing the new stranger mouse versus the familiar stranger mouse is recorded manually and through the video tracking system. During this social recognition

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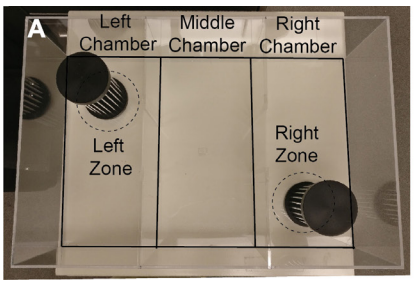
memory stage, optogenetic manipulations can be applied to test for memory retrieval (refer to the ArchT and ChR2 conditions in Step E12).

16. Following testing, lead mice to the middle chamber prior to returning to a new home cage.
17. Forty-eight hours (minimum) following testing, conduct the counter-balanced test (with/without light delivery) for the same set of mice following Steps E7-E16.
    Note: The counter-balanced behavioral test is conducted 2-7 days after the first test. Previous studies have shown that this paradigm elicits memory only within 24 h following testing (Okuyama et al., 2016). Thus, 48 h is sufficient for the mice for re-testing.

Data analysis

Please refer to Supplemental information from https://doi.org/10.1016/j.celrep.2018.04.073.

1. All stages were recorded and automatically analyzed using an overhead camera with ANY-maze. The amount of interaction was measured by manually scoring the sniffing time/direct contact when the subject animal oriented its nose or initiated physical contact within 2 cm of the stranger mouse contained in the wired cage (the 2 cm zone surrounding the wired cage was defined as a zone using the ANY-maze software). Climbing on the wire cage was manually excluded from scoring. Data were presented as a Preference (%), which was calculated based on the percentage time spent investigating the target cage over the entire time spent engaged in investigation of either cage (Figure 5).
   Note: Preference (%) calculated through the manually scored interaction time was validated with the Preference (%) determined through the ANY-maze camera software tracking system. Furthermore, this validation helps to address the potential issue of "blind spots" (i.e., top left or bottom right corners); as manual scoring can compensate for these potential issues.



B

$$\text{Preference (\%)} = \frac{\text{Interaction in Zone}}{\text{Interaction in Left Zone + Interaction in Right Zone}} \times 100$$

Figure 5. Zones defined for analysis. A. Zones defined in the three-chamber social approach apparatus. B. Calculation of percentage time spent investigating the target in either the left or right zone, expressed as a sum of both zones.
Note: Wild-type mice will typically demonstrate a greater Preference (%) for a novel mouse compared to a mouse that they have recently encountered (familiar).

2. All the data in the graphs were presented as mean ± SEM and statistically evaluated by independent-samples *t*-tests, paired-samples *t*-tests or ANOVA (one-way, two-way or repeated

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measures-RM, wherever appropriate) followed by post-hoc Holm-Sidak's multiple comparisons. $P < 0.05$ was considered as significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Notes

Please refer to the original publication for examples of the expected results and more applications of this protocol https://doi.org/10.1016/j.celrep.2018.04.073. Although this protocol focuses on the inhibition or activation of neuronal populations, with ArchT or ChR2, it is important to note that alternative systems such as halorhodopsins (NpHR) for inhibition are also effective.

Acknowledgments

This project was supported by grants from the Canadian Institutes of Health Research (CIHR) (MOP119421 and PJT-155959, Canadian Natural Science and Engineering Research Council (NSERC) (RGPIN341498 and RGPIN-2017-06295), and the Hospital for Sick Children. The authors thank Feng Cao for her technical assistance in the protocol images.

Competing interests

The authors declare no conflicts of interest or competing interests.

Ethics

All experimental procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care (CCAC) and approved by the Animal Care Committees at the Hospital for Sick Children and the University of Toronto.

References

1. Felix-Ortiz, A. C. and Tye, K. M. (2014). Amygdala inputs to the ventral hippocampus bidirectionally modulate social behavior. *J Neurosci* 34(2): 586-595.
2. Gunaydin, L. A., Grosenick, L., Finkelstein, J. C., Kauvar, I. V., Fenno, L. E., Adhikari, A., Lammel, S., Mirzabekov, J. J., Airan, R. D., Zalocusky, K. A., Tye, K. M., Anikeeva, P., Malenka, R. C. and Deisseroth, K. (2014). Natural neural projection dynamics underlying social behavior. *Cell* 157(7): 1535-1551.
3. Hitti, F. L. and Siegelbaum, S. A. (2014). The hippocampal CA2 region is essential for social memory. *Nature* 508(7494): 88-92.
4. Insel, T. R. and Fernald, R. D. (2004). How the brain processes social information: searching for the social brain. *Annu Rev Neurosci* 27: 697-722.

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5. Leung, C., Cao, F., Nguyen, R., Joshi, K., Aqrabawi, A. J., Xia, S., Cortez, M. A., Snead, O. C., 3rd, Kim, J. C. and Jia, Z. (2018). Activation of entorhinal cortical projections to the dentate gyrus underlies social memory retrieval. *Cell Rep* 23(8): 2379-2391.
6. Lieberman, M. D. (2007). Social cognitive neuroscience: a review of core processes. *Annu Rev Psychol* 58: 259-289.
7. Matsumiya, L. C., Sorge, R. E., Sotocinal, S. G., Tabaka, J. M., Wieskopf, J. S., Zaloum, A., King, O. D. and Mogil, J. S. (2012). Using the Mouse Grimace Scale to reevaluate the efficacy of postoperative analgesics in laboratory mice. *J Am Assoc Lab Anim Sci* 51(1): 42-49.
8. Nadler, J. J., Moy, S. S., Dold, G., Trang, D., Simmons, N., Perez, A., Young, N. B., Barbaro, R. P., Piven, J., Magnuson, T. R. and Crawley, J. N. (2004). Automated apparatus for quantitation of social approach behaviors in mice. *Genes Brain Behav* 3(5): 303-314.
9. Okuyama, T., Kitamura, T., Roy, D. S., Itohara, S. and Tonegawa, S. (2016). Ventral CA1 neurons store social memory. *Science* 353(6307): 1536-1541.
10. Olson, I. R., McCoy, D., Klobusicky, E. and Ross, L. A. (2013). Social cognition and the anterior temporal lobes: a review and theoretical framework. *Soc Cogn Affect Neurosci* 8(2): 123-133.
11. Sandi, C. and Haller, J. (2015). Stress and the social brain: behavioural effects and neurobiological mechanisms. *Nat Rev Neurosci* 16(5): 290-304.

