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An operant social self-administration and choice model in mice

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Little is known about how social factors contribute to neurobiology or neuropsychiatric disorders. The use of mice allows one to probe the neurobiological bases of social interaction, offering the genetic diversity and versatility to identify cell types and neural circuits of social behavior. However, mice typically show lower social motivation compared with rats, leading to the question of whether mice should be used to model complex social behaviors displayed by humans. Studies on mouse social behavior often rely on measures such as time spent in contact with a social partner or preference for a social-paired context, but fail to assess volitional (subject-controlled) rewarding social interaction. Here, we describe a volitional social self-administration and choice model that is an extension of our previous work on rats. Using mice, we systematically compared female adolescent and adult C57BL/6 mice and outbred CD1 mice, showing that operant social self-administration, social seeking during periods of isolation and choice of social interaction over palatable food is significantly stronger in female CD1 mice than in female C57BL/6J mice, independently of age. We describe the requirements for building the social self-administration and choice apparatus and we provide guidance for studying the role of operant social reward in mice. We also discuss its use to study brain mechanisms of operant social reward, potentially extending its application to mouse models of neuropsychiatric disorders. The training commonly requires ~4 weeks for stable social self-administration and 3–4 additional weeks for tests, including social seeking and choice.

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Introduction

The field of social neuroscience has rapidly expanded over the past few decades, with more focus being placed on the role of social interactions in neuropsychiatric disorders including autism, schizophrenia and substance use disorders. Rodent models of volitional social reward are therefore critical to understand the neurobiological mechanisms mediating the protective or facilitating effects of social behavior on neuropsychiatric disorders[1–4]. Here, we introduce a protocol for studying operant social reward and choice in mice based on the version that we developed in rats[5–9]. Mice offer genetic diversity and the versatility to identify and manipulate specific cell types and neural circuits of social behavior[10–17]. However, there is debate about whether mice are an appropriate model for social behavior given their lower social motivation compared to rats[18–20]. With existing social behavior models, reliably studying social behavior has been difficult because classic animal models of social behavior have largely used measures such as contact time between social partners or time spent in an environment previously paired with social interaction to assess social motivation or preference[21,22]. Social interaction in these cases is experimenter imposed, meaning that the interaction is not volitional (subject controlled). Additionally, mechanistic studies of social behavior have primarily used either C57BL/6 mice or mice bred on a C57BL/6 genetic background[23–25] without assessing potential strain differences in operant social reward.

On the basis of this background, we developed our protocol of operant social self-administration and choice by systematically comparing adult and adolescent female C57BL/6 and CD1 mice. We demonstrated that operant social self-administration, social seeking during periods of isolation and choice of social interaction over palatable food is significantly stronger in female CD1 mice than in female C57BL/6J mice, and that these effects are almost entirely age independent. This protocol will make mechanistic studies of volitional social reward feasible by providing a low-cost, fully automatic method for studying social motivation and preference.

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Here we describe how to use our operant social learning and choice model in mice, providing a standardized protocol of operant social interaction self-administration, social seeking during isolation and discrete-trial choice between palatable food versus social interaction. We also show the potential applications of the protocol to undertake mechanistic studies by crossing the more social outbred female CD1 mice with transgenic male mice bred on a C57BL/6J background to preserve the 'social' phenotype in the F1 hybrid offspring. A similar strategy (breeding aggressive CD1 males with transgenic female mice bred on a C57BL/6J background) has been successful in preserving the 'aggressive' phenotype of hybrid F1 male mice from this breeding scheme[26–28]. We also provide detailed technical information, procedures and specifications required for building a fully automatic social self-administration and social versus food choice apparatus (Fig. 1).

Overview of the operant social self-administration and choice protocol in female mice

The timeline of the protocol is depicted in Fig. 2. We obtain female outbred CD1 and C57BL/6J mice from Charles River Laboratories. We have tested adolescent and adult CD1 and C57 mice. The protocol works well at both developmental stages in CD1 mice. C57 mice are much less socially motivated, and lever press equally for access to a social partner or an empty chamber[29]. After an acclimation period, we single house mice for 5 d before the initiation of training. We randomly assign

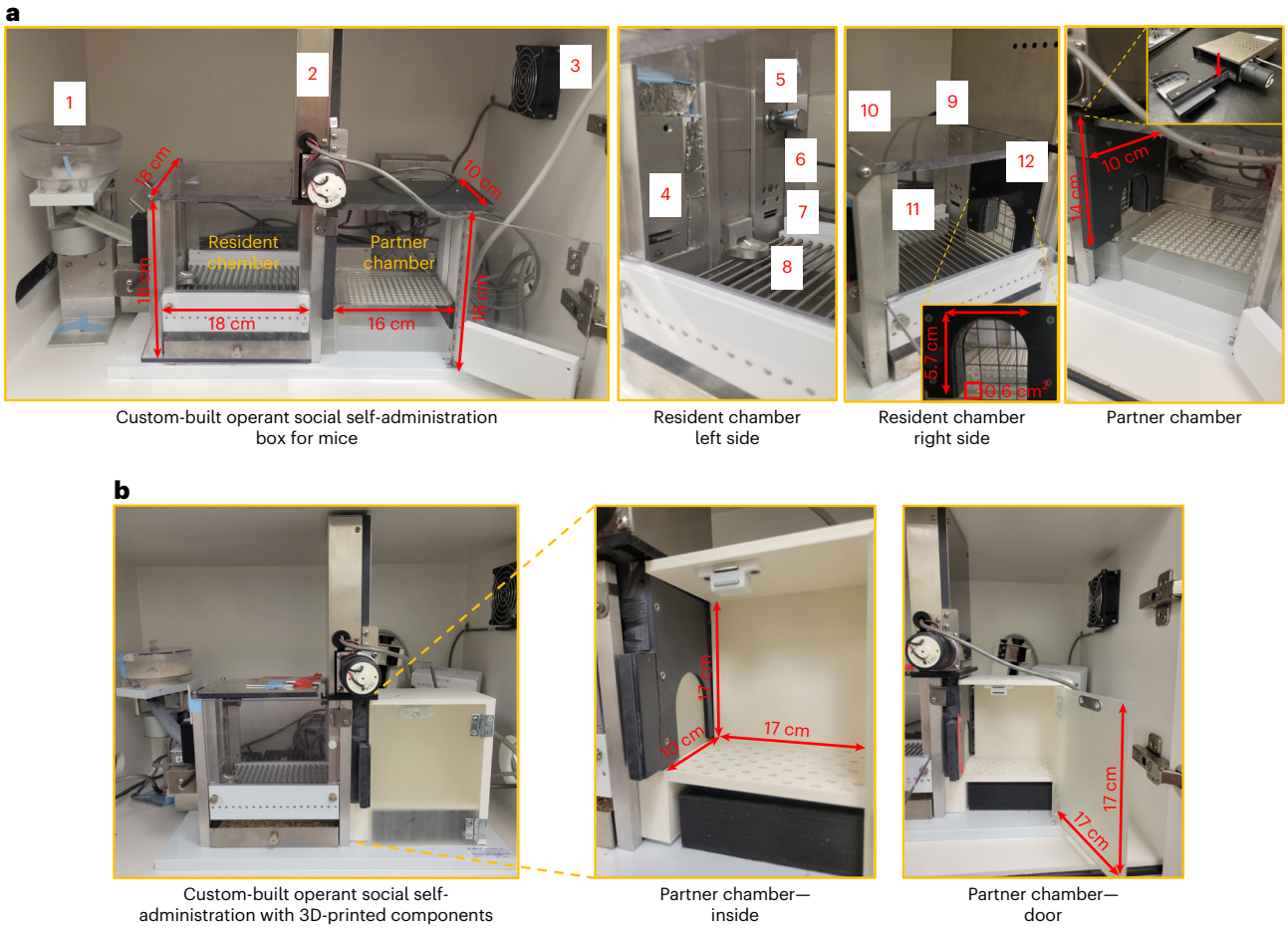

Fig. 1 | Social-choice self-administration apparatus. a, Automatic social-choice self-administration mouse apparatus (Stage 1). The base of the apparatus is a standard modular operant test Med-Associates chamber with modified top for mouse (ENV-307A-CT). Left: the picture represents the apparatus with the configuration described in this protocol and detailed measurements. Middle: magnification of the left and right sides of the resident chamber. Inset: barrier between resident and partner chambers with detailed measurements. Right: left side of partner chamber. Magnification on top right: detail of modified door to attach to the operant chamber. The arrow shows the modified insertion. (1) Food receptacle 1; (2) guillotine door; (3) fan; (4) inactive lever; (5) house light white; (6) cue light (food); (7) active lever (food); (8) food receptacle; (9) house light red; (10) cue light (social); (11) active lever (social); (12) barrier. All measurements are in cm. **b**, Social-choice self-administration apparatus using 3D printer. Left: the picture represents the social apparatus with 3D-printed partner chamber and detailed measurements. Middle: magnification of the 3D-printed partner chamber interior. Right: 3D-printed partner chamber door details and magnification of floor structure. Panel **a** adapted with permission from ref. [29], Elsevier.

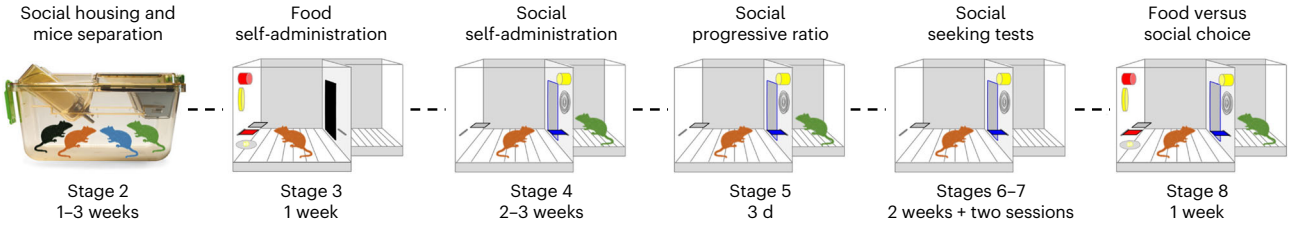

Fig. 2 | Protocol timeline. Stage 2 (Steps 75–76), social housing and mouse separation: ~1–2 weeks social housing and 1 week single housing. Stage 3 (Steps 77–82), food self-administration: 1 week food self-administration (FR1). Stage 4 (Steps 83–88), social self-administration: FR1 10 d, FR2 2 d, FR4 2 d, FR6 4 d. Stage 5 (Steps 89–91), social progressive ratio: 3 d. Stages 6 and 7 (Steps 92–95), social seeking tests: 2 d, isolation day 1 and day 15. Stage 8 (Steps 96–104), food versus social choice: six sessions over 6 d (food delivered on FR1 schedule; social delivered on FR1, FR3, FR6, FR12 and FR24 schedule). This protocol is flexible but most commonly requires 9–10 weeks for completion.

mice to presser (lever pressing test mouse) and partner conditions. We match mice for age and weight to model peer-to-peer interactions. Our protocol can be used with female or male mice, although for most studies thus far we have primarily used female mice because CD1 male mice may lever press for the opportunity to attack another male mouse[26,30]. The protocol has six phases: (1) social housing and mouse separation, (2) food self-administration, (3) social self-administration under a fixed-ratio (FR) 1-to-6 reinforcement schedule, (4) social self-administration under a progressive-ratio (PR) reinforcement schedule, (5) social seeking tests under extinction conditions after 1 or 15 isolation d, and (6) discrete choice between social interaction (FR1–FR24 schedule) versus food (FR1 schedule). We begin by housing the mice four per chamber, and single house them 1–3 weeks later. Next, we train the mice to lever press for food on the left side of the chamber for 1 week. We then train mice to lever press for access to a social partner on the right side of the box. The procedure is fully automated because interaction occurs through a wire mesh barrier. Apparatus details are shown in Fig. 1. We train mice to self-administer for access to their social partner over the course of 18 daily sessions. For the first 10 d, mice lever press on an FR1 schedule (one lever press yields one opportunity for social interaction). We increase the FR requirement over the course of 8 additional days (2 d at FR2, 2 d at FR4, 4 d at FR6). We then switch to a PR schedule in which lever pressing requirements increase within the session (3 d). We then test mice for social seeking in the absence of their social partner after either 1 or 15 d of isolation. Finally, we allow mice to choose between food and social reward using a discrete trial procedure. We increase the FR requirement for social reward and maintain food on an FR1 schedule over the course of 6 d of training. The social- and food-paired levers are on different sides of the apparatus, and we use different sets of discriminative and discrete cues for the two different rewards (Fig. 1; see 'Equipment').

Below we provide additional details of the experimental procedures, including a description of the steps required and a list of materials to build our fully automatic social self-administration and choice apparatus, 'Troubleshooting' and 'Anticipated results'.

Comparison with other models

Social behavior studies conducted in mice have primarily used measures such as contact time spent with a social partner or preference for a social-paired context (but see refs. [21,22]). In these cases, social interaction is experimenter imposed, and thus these studies are unable to assess volitional social interaction. Another major difference is that mechanistic studies of mouse social behavior typically use C57BL/6 mice or mice bred on a C57BL/6 genetic background because C57BL/6 mice are reported to be a particularly socially motivated mouse strain[23–25]. However, we and others found that C57BL/6 mice demonstrate highly variable social conditioned place preference (CPP) that is at best weakly expressed under limited experimental conditions. We did not observe social CPP under conditions where CPP for addictive drugs and food is reliably observed[25,31,32]. A recent study demonstrated operant social self-administration in adult male or female C57BL/6 mice trained to nose poke for social interaction with a novel adolescent partner[21]. An alternative interpretation of these results is that mice in this situation are lever pressing for dopamine-dependent novelty exposure[33] rather than operant social reward since a novel partner is introduced everyday during training. This contrasts with our approach of using the same familiar partner throughout training. We directly compared C57Bl/6 mice with outbred CD1 mice using our operant model of social self-administration and choice, and found that C57 mice show no greater motivation to lever press for a social partner than they do for access to an empty chamber. When given the choice between food and

social reward, CD1 mice prefer social reward, even when it requires more effort to obtain. C57 mice prefer food most of the time when effort is equivalent between the two types of reward, and quickly switch to exclusively choosing food on every trial when more effort is required to obtain social rewards. Given the discrepancy in results using subject-controlled versus experimenter-controlled social interaction in mice, our operant social self-administration and choice model will be a valuable tool for studying the neurobiological basis of social reward using the wide array of genetic tools available exclusively in mice.

Advantages

Our protocol was developed exclusively in female mice, in part to narrow the gap in biomedical and neuroscience research, in which the vast majority of studies have focused on male animals[34,35]. Reasons for excluding female animals include the erroneous dogma that female behavior is always or frequently more variable than male behavior due to estrous cycle-dependent behavioral changes[35,36]. Here, we introduced a protocol for studying social behavior developed exclusively in females of a particular strain (CD1), which is different from the standard method of developing animal models exclusively in male animals of a single strain, then relying on future studies to examine if the existing model generalizes to female animals of the same or different strains. The protocol can also be used in male mice, with the caveat that outbred CD1 male mice will lever press for the opportunity to attack a smaller male mouse of another strain under certain conditions[26,37]. Having a protocol developed for female mice is advantageous not only for the study of volitional social behavior in general, but also as a step toward closing the gap between studies done in male versus female animals in neuroscience research.

Our protocol was developed to be fully automatic, a clear advantage as we can now run multiple pairs of mice through an entire social self-administration session with minimal intervention from the experimenter. If there is a need to examine direct social interaction between presser and partner, the mesh panel can be removed to allow mice to express their full range of social behavior with their partners. This protocol is also ideally suited to study circuit mechanisms of complex volitional social interaction in CD1 mice, and potentially in other mouse strains that display high levels of social motivation. Most mouse studies on social behavior have relied on measures such as time spent in contact with a social partner or preference for a social-paired context (but see refs. [21,22]). These measures fail to assess volitional (subject-controlled) rewarding social interaction because interaction is experimenter imposed. Because of this, cellular and molecular mechanisms that underlie volitional social interaction in mice are understudied. This operant social self-administration and choice protocol will make it feasible to address this issue by directly assessing social motivation in mice.

This protocol also allows researchers the opportunity to use study social motivation using mouse models of neuropsychiatric disorders with a component of social dysfunction such as autism, schizophrenia and depression. There is also the potential for testing pharmacological interventions that mitigate or reverse deficits in social motivation in these mouse models. Finally, the model can also be used to study the protective effect of social interaction using addiction models, which has already been successfully implemented using the original rat protocol. Given the greater difficulty in maintaining stable and reliable intravenous drug self-administration in mice, another approach is to study the effects of prior drug experience via passive exposure or oral delivery on subsequent social motivation in mice using this protocol.

Finally, although it is labor intensive and expensive to build a social box, we have developed a method for 3D printing the partner chamber, enabling researchers to turn existing self-administration boxes into social self-administration boxes with the addition of a guillotine door and the 3D-printed partner chamber (Fig. 1). Thus, adding this type of study for laboratories already invested in operant behavior will be relatively easy and affordable.

Limitations

We previously outlined some limitations in the rat version of the protocol, both of which still apply here: our protocol requires double the number of mice for any given experiment (since partners and pressers are required). Furthermore, mice are sensitive to the sizes of the barrier holes that separate the two sides of the chamber (Fig. 1). In the case of mice, it is particularly difficult to choose a mesh size because if the holes are too small for the mice to physically contact each other's faces and paws during social interaction then they will not stably self-administer. However, if the holes are too big, mice can escape into the opposite side of the chamber as they are very good at squeezing through

small spaces. This is a particular concern for adolescent female mice, which are smaller than adults. Thus, we recommend using the barrier with the dimensions described below; if the holes are too small the mice do not maintain stable social self-administration and social preference (Tables 1 and 2). Another potential limitation is that most mechanistic studies of social behavior have either used C57BL/6 mice or mice bred on a C57BL/6 genetic background, and we have shown that this strain does not lever press for social reward. One potential strategy to address this limitation is to breed outbred female CD1 mice with transgenic C57BL/6J male mice to maintain the 'social' phenotype in the hybrid F1 female offspring in a manner akin to the maintenance of the 'aggressive' phenotype of the hybrid F1 males from this breeding scheme[26–28].

Experimental design

Our procedures follow the guidelines outlined in the Guide for the Care and Use of Laboratory Animals (8th edition; http://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf). The studies from which we show results here were all approved by the National Institute on Drug Abuse Intramural Resarch Program Animal Care and Use Committee. Obtaining appropriate permissions and conforming to regulations is important.

Experimental timing and organization of the protocol

This protocol is flexible, but most commonly requires 9–10 weeks for completion. Typically, after 1–3 weeks of social housing and separation, we run 8 d of food self-administration, 18 d of social self-administration on an increasing FR schedule (FR1 10 d, FR2 2 d, FR4 2 d, FR6 4 d), 3 d of social self-administration on a PR schedule, two sessions of social seeking tests (on isolation days 1 and 15) and six sessions of social versus food choice. The choice program allows mice to choose between the social- and food-paired levers in a discrete-trial choice procedure; it divides each choice session into ten discrete trials separated by 8 min. Throughout the six sessions, food will be available on an FR1 schedule. Social interaction will be available on an FR1 schedule for the first two sessions. Then, the effort level required for social interaction is increased to FR3, FR6, FR12 and FR24 (one session for each schedule). We run experiments in cohorts ranging from 6 to 24 mouse pairs (pressers plus social partners), with runs occurring once daily for each mouse in the different cohorts. The presser is paired with the same partner throughout training, with the caveat that if the presser initially seems uninterested in interacting, we will switch to a different partner mouse. We run our experiments throughout the entire day, and we have not observed any differences related to the time of day of testing. We suggest that experimenters new to this model start with cohorts of no more than eight mice (plus eight social partners). We also suggest that social self-administration sessions include ~30 trials (60 s interaction) per session (~1 h) to give the mice enough exposure to their social partners. Using the automatic social choice procedure described here, this phase does not require the presence of the experimenter to separate the two mice after each trial. However, we recommend checking on the mice periodically to make sure that none cross the barrier (this can occur with young female mice).

Effects of modifications to the procedure

In the initial characterization of the model, we tested adolescent and adult mice of two different strains, so it follows that the protocol can be used for different mouse strains and across different developmental stages. We tested female mice with female partners initially because we wanted to examine positive social interactions between peer mice. However, the model can be adapted for use in males, although operant behavior in males may be motivated by the rewarding aspects of engaging in aggressive behavior[26,37]. Housing conditions before the initiation of training had no effect on social self-administration. There was a small reduction in lever pressing for a social partner in mice housed in groups during training, but they earned the same number of social rewards overall. We train mice to lever press for access to food or social reward, but the protocol can be adapted for use with nose pokes as well. The operandum is less important than the use of proper controls (for example, if nose poke is used, both active and inactive nose poke should be included).

It is important to note that the protocol can be shortened for effective use in neurobiological studies. We have obtained stable social self-administration and social seeking behavior after omitting PR testing and choice trials. This is particularly useful for mechanistic studies of social self-administration. Additionally, the model can be combined with drug self-administration to study choice between drugs and social interaction, although we have not yet tested this directly.

Theoretically, this model can also be used in existing genetic mouse models that include a component of social dysfunction, for instance, in mouse models of autism.

Typically, we start the protocol with female CD1 mice that are approximately 7 weeks old on arrival, but the protocol works for younger mice (3–4 weeks old on arrival) or older adult mice (12–16 weeks on arrival). It is important that residents (lever pressers) and social partners are in the same body weight range and age range.

Regulatory approvals

Approval is needed from the animal care and use committee (ACUC) of the home institution before starting studies that involve the use of mice. To do this, a protocol must be written, submitted and approved by the ACUC (or equivalent body). This process can vary in length depending on how frequently the committee meets and on revisions required after the initial submission, but can last anywhere from 2–6 months.

Materials

Animals

- Female CD1 mice (Charles River Laboratories), age 4–8 weeks ▲ **CRITICAL** Male mice can be used, although thus far the majority of our experiments have been conducted in female mice. House mice in groups of three to four per cage with free access to food and water, and allow mice to habituate to their new colony facility for at least 1 week before self-administration. We maintain mice on a 12 h reverse light–dark cycle. We mildly food restricted mice (3 g chow per day) during the food self-administration training phase, with water available ad libitum. For the remainder of the experiments, food and water are freely available. ! **CAUTION** Older mice can be used but this has not yet been tested. We randomly assign mice to lever presser and social partner conditions. ▲ **CRITICAL** Experiments must follow all governmental and institutional guidelines for care and use of laboratory animals. Moreover, it is critical to report any excluded mice (due to, e.g., lack of reliable self-administration or failure to thrive). The studies described below were approved by the National Institute on Drug Abuse Intramural Research Program ACUC.

Reagents

- Palatable food pellets (Test Diet, cat. no. 1811142) ▲ **CRITICAL** Make sure to introduce mice to food pellets before the first day of food self-administration training to avoid food-related neophobia.

Equipment

Parts for custom-made social choice apparatus ▲ **CRITICAL** All parts can be found at Med-Associates (https://www.med-associates.com/)

- ENV-022MD Med-Associates sound attenuating cubicle
- ENV-307A-CT Standard modular operant test chamber with modified top for mouse
- DIG-716 SmartCtrl interface module (4 input/8 output)
- SG-716 SmartCtrl Connection Panel (4 input/8 output)
- Can be purchased as a package: DIG-716P1 SmartCtrl Package (4 input/8 output). Comes with SG-716, DIG-716 and SG-210CP-25 28v Power Cable, 25
- SG-210CB DB25 SmartCtrl cable, M/F 25′ (7.6 m)
- SG-210CP-25 Power Cable, 25′ (7.6 m)
- ENV-3015BD Auto guillotine door
- FAB-ENV-008-32 Social chamber aluminum mesh (plus Thumbscrew HAR-Thumb-4-40 × 5/16-LowPro)
- ENV-307A-GFW Stainless steel grid floor (white front and back) for ENV-307A
- ENV-307-07 Waste pan for Classic Modular Test Chamber for Mouse
- ENV-307A-GFW Grid floor for Classic Mouse Chamber
- ENV-307A-QD Quick Disconnect Grid Floor Connection Harness for Classic Mouse Chamber
- ENV-307A-FP Filler panel package for Classic Modular Test Chamber for Mouse
- ENV-025F28 Replacement 28 VDC fan with cable
- ENV-312-2M U/S Retractable lever ($n = 2$)
- ENV-310M Ultra-Sensitive Lever (inactive)
- ENV-315M LED House light for Standard Mouse Chamber
- ENV-321M LED Stimulus light, (one yellow and one red lens) for Standard Mouse Modular Chamber

- ENV-203-20 Modular pellet dispenser, magazine type 20 mg ▲ CRITICAL This is required only if using food training and food choice in the protocol
- ENV-300R1AM Pellet receptacle for Classic Mouse Chamber
- ENV-025F28 Replacement 28 VDC fan with cable
- SG-6510D 28V T/T Interface cabinet
- DIG700G Decode Card (Peripheral Component Interconnect)

Other equipment

- Easy-to-Form Stainless Steel Wire Cloth (304 stainless steel, opening size 0.453″ 3′ or 4′ sheets purchased from McMaster-Carr) ! CAUTION Make sure that mesh openings are wide enough for mice to interact, but not so big that they can squeeze through the openings
- Clear impact-resistant polycarbonate (12″ × 12″ × 1/4″ sheet; McMaster Carr (https://www.mcmaster.com/8574K28)). ! CAUTION For any cutting and assembling, we recommend applying the appropriate safety measures (goggles, cut-resistant gloves, coats, etc.)
- Clear rectangular cage for mice (17 × 31 × 13 cm) with plastic cover top and lid for food and water (Fig. 2)
- Hard woodchip bedding (Envigo 7090A Teklad Sani-Chips)
- Cleaning solution and wipes
- Med-Associates programs, written using Med-PC code to automatically run social and food self-administration, social seeking and social versus food choice (available upon request from the authors)

Optional: 3D-printed partner chamber equipment

3D printers

- Ultimaker 2+ (to print partner chamber and bedding box, polylactic acid (PLA) material)
- FormLabs Form 3 (to print partner chamber door, resin material)
- Form Wash + Form Cure (to remove uncured resin and cure printed resin parts, respectively)

Software programs

- Ultimaker Cura (for use with Ultimaker 2+)
- Preform (for use with FormLabs Form 3)
- Tinkercad (to design 3D models)

Materials

- Ultimaker 2+ series nozzle pack (5 × 0.4 mm, Dynamism, product no. 9525)
- Ultimaker 2.85 mm NFC PLA—white 750 g (Dynamism, product no. 1613, one spool makes about two partner chambers)
- Ultimaker 2.85 mm NFC PLA—black 750 g (Dynamism, product no. 1609)
- FormLabs clear resin cartridge v4 1L (Dynamism, product no. RS-F2-GPCL-04, one cartridge makes about three partner chamber doors)
- 8 GB secure digital (SD) card
- 3D printer spatula
- Everbilt 4.4 lbs. magnetic catch—white (one pack) includes plate (Home Depot, model no. 9235997)
- Everbilt 1 in. non-removable pin narrow utility hinges—satin brass or zinc-plated finish (two pack) (Home Depot, model no. 15161)
- Everbilt pan head Phillips #6 × 3/8 in. sheet metal screws—zinc 16 pack (Home Depot, model no. 805211)
- Gorilla 2 oz. original glue (Home Depot, model no. 269)
- 100% acetone 500 ml
- Kimwipes 4.5″ × 8.5″ (280 count)

Equipment setup

Mouse housing requirements

Once we receive the mice, we group house them three or four to a cage. Figure 2 depicts a standard protocol procedure. We provide free access to water and food during the duration of the experiments. ▲ CRITICAL We suggest allowing the mice to acclimate to the facility for 1 week. After the 1 week period in which mice become acclimated to the animal facility, we randomly assign mice to lever presser and social partner conditions. ▲ CRITICAL Mouse cages should be cleaned twice per week.

Custom-made social-choice self-administration apparatus

The standard modular Med-Associates operant test chamber for mouse (ENV-307A-CT) is the base for the custom-made social-choice self-administration. The apparatus can be enclosed in a regular Med-Associates sound attenuating cubicle (ENV-022MD) (Fig. 1b). All the parts listed above are necessary to build one apparatus (Fig. 1; all parts can be found at Med-Associates (https://www.med-associates.com/)).

A standard Med-Associates self-administration chamber is combined with a custom-made social-partner chamber that is separated by a guillotine door (ENV-3015BD). Each chamber should have a discriminative cue on the right panel (white house light; ENV-215M) to signal the insertion and subsequent availability of the social reward-paired active (retractable) lever located near the guillotine door and a discriminative cue on the left panel (ENV-321M, red lens) to signal the insertion and subsequent availability of the food-paired active (retractable) lever located on the left side. Locate the levers 2 cm above the grid floors and a white discrete light cue (ENV-321M, white lens) above the food-paired lever and a discrete light cue (ENV-321M) above the social-paired lever. The left side is equipped with a pellet dispenser, pellet receptacle and an inactive (stationary) lever. Attach a fan on the back of the cubicle for background noise (Fig. 1b). **! CAUTION** We recommend periodically checking that the mice are not crossing the barrier during social self-administration, and that the pellet dispenser is properly connected for the entire duration of the self-administration session. **▲ CRITICAL** Check body weight of the mice daily before the beginning of each session. Daily body weight checks for both the presser and the partner mice are a way to ensure that mice remain healthy during training. If a mouse loses more than ~20% of body weight this can be a sign of sickness or distress. Training should be paused and mice should be put under vet care if this occurs. **▲ CRITICAL** At the end of each session, we change bedding from both the resident and the social partner side of each chamber. Also, we wipe the stainless-steel grid floors with water once a week and clean the entire apparatus before each seeking test to avoid any confounds due to the previous presence of the social partners.

? TROUBLESHOOTING

Data collection

We collect behavioral data via a computer using the Med-PC program. Subsequently, we transfer the data to an Excel file and analyze the data using SPSS (IBM, version 25, GLM procedure). See 'Anticipated results'.

Procedure

Stage 1: building the social-choice self-administration apparatus ● Timing 3–6 d per chamber

Building the custom-made social-choice self-administration apparatus for mice requires steps similar to those reported for our rat operant chamber[6], with the exceptions described below.

▲ CRITICAL The timing of this phase may depend on the experimenter's experience.

1 Remove the second center front support from a standard modular operant chamber (ENV-307A-CT).
2 Make three holes on the white plastic base 18 cm from the support removed above.
3 Add the right/center/left front supports (right side of Fig. 1a, left).
▲ CRITICAL STEP only for mouse chamber.
4 Cut the Plexiglas top to create space for the auto guillotine door.
5 Disassemble the auto guillotine door (ENV-3015BD), cut top, front and back dark plastic to fit in between the right and left support (Fig. 1a).
6 Cut the external left plastic component of the door to allow the metal strap to easily open and close for the resident chamber.
7 While the door is diassembled, insert the metal mesh barrier between the dark and gray plastic and anchor it using the screws holding the two different plastic components.
8 Reassemble the door and attach it to the top of the modular chamber (Fig. 1a, right magnification).
9 Cut two rectangular pieces (16 × 10 cm) of clear impact-resistant polycarbonate sheet.
10 Attach the two rectangular pieces to the top and back of the partner side of the chamber (between the auto guillotine door and the metal supports); for the right side of the partner chamber use metal pieces between the vertical supports. Metal parts are the standard components included with

purchase of Med-Associates chambers. These parts can also be obtained from alternative sources such as McMaster-Carr.

? TROUBLESHOOTING

11 Cut a 16 × 13 cm piece of polycarbonate to create the door of the partner chamber.

12 Close the front of the chamber with a latch to keep the social partner inside.

? TROUBLESHOOTING

13 Create a floor (using preformed polycarbonate, 16 × 10 cm) and a plastic tray beneath the floor to collect waste. The floor is positioned between the plastic component dividing the two sides of the chamber (the bottom of the door) and an additional plastic piece (10 × 2 cm) anchored to the right side of the partner chamber (Fig. 1a, left). Plastic floor and tray should be removable for cleaning purposes.

3D printing partner chamber and bedding box ● Timing ~3 d to print a chamber, ~6 h for bedding box

14 As an alternative to Steps 9–13, the partner chamber can be built using 3D printers, which can improve the affordability (Fig. 1b) and customization of the approach.

▲ CRITICAL STEP We provide specific instructions for use with the Ultimaker2+ for the chamber and bedding box, and the FormLabs Form 3 for printing the resin door.

15 Design the 3D chambers: we use Tinkercad, a free online 3D modeling program. Design files are available (Supplementary Software) and compatible with the resident chamber (Steps 1–8).

? TROUBLESHOOTING

16 Load the partner chamber design file onto the SD card.

17 Turn printer on and insert the SD card.

18 Place spool on the spool holder at the back of the printer.

▲ CRITICAL STEP The coil should be counterclockwise and toward the bottom of the feeder (we use white PLA for the chamber and black PLA for the bedding box).

19 On the printer screen, select 'Materials' and then 'Change'.

20 The screen will display the following message 'Insert new material from the backside of your machine above the arrow'.

? TROUBLESHOOTING

21 Turn the printer around to access the back.

22 Lift the white side button up and gently push the filament into the bottom of the feeder until the feeder is gripping the filament.

23 The filament should now automatically move up the Bowden tube.

24 Select 'Read'.

25 The screen will display this message 'Wait till material comes out the nozzle'.

26 Once material is extruding steadily and in a straight vertical line, select 'Read→PLA→OK'.

27 Return to main menu by selecting 'Return'.

28 Select 'Print' and then scroll to find partner chamber design file.

29 Once completed, the screen will display this message: 'Print finished You can remove the print.'

30 Insert spatula underneath the print and lift up to release the adhesion.

31 Trim supports off. The supports are the bottom-most layer (where the print meets the build plate) and should come off with ease.

3D printing partner chamber door ● Timing ~1 d to print a single resin door

▲ CRITICAL Always wear nitrile gloves when handling resin.

32 Turn on printer and make sure it is connected to a computer via USB.

33 Open the design file in PreForm software on the computer.

34 Click 'File→Print→Upload Job'. Do not disconnect from printer while upload is in progress.

35 Lift the orange printer lid.

! CAUTION Only open briefly because resin hardens with light exposure.

36 Clean resin in tray by moving wiper sideways a few times.

37 Close the orange printer lid.

38 Open resin cartridge lid.

39 Follow prompts on touchscreen to print.

40 When complete, lift the black handle to release the platform and remove

! CAUTION Be careful to avoid dripping resin on printer.

41 To finish, the resin door needs to be washed and cured.

42 Open the Form Wash lid.
43 Select 'OPEN' to raise metal basket and place print inside—the top lip of the platform rests on the Form Wash platform mount arms.
44 Rotate knob to and select '15 min'.
45 Select 'START' to start the cycle.
46 Allow print to air dry for 30 min.
47 Select 'SLEEP' to lower metal basket.
48 Close Form Wash lid.
49 Use spatula to remove print from platform.
50 Wet Kimwipes with 100% acetone to clean platform and remove residual resin.
51 Replace platform in printer.
52 Open Form Cure lid and place print on circular spinning platform.
53 Close Form Cure lid.
54 Rotate knob and select '15 min' and '60 °C'.
55 Select 'START' to start the cycle.
56 Collect prints and gently cut supports off with pliers as close to print as possible.

Assembling the partner chamber and partner chamber door

57 Gather materials: partner chamber, partner chamber box, bedding box, hinges, screws, magnetic catch, Gorilla glue and water in a 20 ml disposable syringe for curing glue.
58 Attach hinges to the resin door using Everbilt pan head phillips #6 × 3/8 in. sheet metal screws—tighten slightly so that it stays in place and has some flexibility.
59 Attach door-attached hinges to the partner chamber using screws provided in hinge packaging—tighten slightly so that it stays in place and has some flexibility.
60 Tighten all screws, then test opening and closing door (it should move with slight resistance).
61 Align magnetic catch to holes on top inside of partner chamber; make sure the magnet is as far forward as possible (as close to where door will be).
62 Use flat screws in the magnetic catch packaging to tighten and hold the magnetic catch in place.
63 Place the flat magnetic plate from package on magnetic catch.
64 Close the door against the magnetic catch.
65 Use a pencil to mark holes for the magnetic plate on the resin door (outer side).
66 Open door.
67 Apply Gorilla Glue to resin door and water to magnetic plate.
68 Carefully place magnetic plate on door to adhere. Align holes on magnetic plate with pencil-marked holes.
69 Keep door open as wide and flat as possible to dry. Check after ~15 min to see whether magnetic plate moved and adjust as needed (glue may dry puffy and can be peeled off or removed with a screwdriver).
70 Remove the guillotine door and set aside. The tabs extending off the partner chamber platform slot into the metal opening.
71 Reposition the guillotine door back into place.
72 The bedding box slides in under the partner chamber platform and can be pushed to the back right corner. The bedding box is not flush with the partner chamber to allow for easy access/cleaning and removal with the attached partner chamber door.
73 Add all the other components (levers, cues, etc.).
74 Remove the SmartCtrl Connection Panel (8 in/16 out) (SG-716B) from the white base and attach it to the back of the sound attenuating cubicle.

Stage 2: social housing and separation of mice ● Timing 2–3 weeks

75 Mice are housed in groups of three or four during the period that they are acclimating to the facility (1 week minimum). They have free access to food and water during this time. One week before the start of operant training, mice are housed individually and then maintained in isolation for the remainder of training and testing. We mildly food restrict lever presser mice during this period (3 g chow per day to maintain body weight ~85–90% of standard body weight and facilitate operant food training).
▲ CRITICAL We have tested the protocol with outbred CD1 mice and inbred C57BL6/J mice; the protocol is in principle compatible with other mouse strains.
76 During the week before the start of operant food training, assign mice to lever presser and social partner conditions.

! CAUTION When pairing lever presser and partner mice, make sure they are similar age and body weight, and that the mice are familiar with each other (have been housed together) to maximize affiliative behavior.

▲ CRITICAL Use the same pairs throughout training, unless pairing is unsuccessful because mice are not interested in their social partners

▲ CRITICAL STEP Mark the mouse tails to keep track of the different social pairs.

Stage 3: food self-administration ● Timing 1–2 weeks

One day before the initiation of training, introduce mice to food pellets in their home cages to avoid a neophobic response to food during training. If mice fail to consume the pellets that are introduced in the home cage and continue to avoid them during operant training, an additional exposure session may be necessary to overcome the neophobic response.

77 Train mice to self-administer food pellets during daily 60 min sessions (FR1 schedule, 20 s timeout after active lever press) as follows.

78 First, bring mice from the facility and place them in their operant chambers.

! CAUTION Handle cages with care during transport to minimize stress during the transport process. It is a good idea to allow the mice 30 min to habituate to the room before loading them into the operant training chambers.

79 Start the session by uploading the Med-PC food self-administration program that, once loaded, will automatically start the session with illumination of the food-paired house light followed by insertion of the food-paired lever. Stationary (inactive) lever remains accessible throughout the session.

▲ CRITICAL STEP Successful food lever presses from the mouse cause the retraction of the active lever and the house light turns off.

80 Record the number of rewards received and active and inactive lever presses.

81 At the end of the session, remove mice and bring them back to the facility in their respective home cages.

82 During this training phase, run the food self-administration procedure for eight sessions (once per day) by repeating Steps 77–82.

Stage 4: social self-administration ● Timing 3–4 weeks

83 On day 9, following 8 d of food self-administration, begin training mice to lever press for access to a social partner during daily 60 min sessions (FR1 schedule, 20 s timeout after active lever press). Each test mouse lever presses for its previously paired partner.

84 First, bring the mice from the facility and move them from their home cage to their assigned side of the operant chamber (lever pressers and partners).

Start the session by uploading the Med-PC social self-administration program that, once issued, will automatically start the session with illumination of the social-paired house light followed 10 s later by insertion of the social-paired lever.

▲ CRITICAL STEP Successful lever presses from the test mice cause the retraction of the active lever, a discrete 20 s light cue, and opening of the guillotine-style sliding door. Test mice are subsequently allowed to interact with their social partner through the mesh barrier for 60 s until the house light turns off, at which point the guillotine door closes.

85 Record the number of rewards earned and active and inactive lever presses.

▲ CRITICAL STEP Younger mice (20–25 g) have on occasion escaped under the guillotine door or through the mesh barrier. Check frequently to make sure mice are not crossing the barrier or escaping from the chambers.

86 At the end of the session, remove both lever presser and partner mice and bring them back to the facility in their respective home cages.

87 During this training phase, run the social self-administration procedure for ten sessions (once per day, ideally at the same time everyday within 1–2 h of the beginning of the dark cycle, if maintaining mice on a reverse light cycle) by repeating Steps 78 and 79.

88 After establishing social self-administration on the FR1 schedule, we increase the effort required to obtain a social reward by increasing the number of lever presses required to open the guillotine door, from FR1 to FR2, FR4 and FR6. For FR1, a single active lever press opens the guillotine door. For FR2, two presses open the door, and so on. Although this can be varied according to the needs of the experiment, we have had success training for 2 d at FR2, 2 d at FR4, and 4 d at FR6.

Stage 5: PR testing ● Timing 3 d

89 Repeat Step 78, except in this case a different Med-Associates program will need to be loaded. During the PR sessions, we increase the ratio of responses per social interaction period according to the following sequence: 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, etc.) (ref. [38]). The sessions end when the mice failed to earn the social reward for 30 min after the last reinforced lever press. The final completed response ratio represents the 'breaking point' value. Record the number of social rewards earned and active and inactive lever presses.

90 At the end of the session, remove both lever presser and partner mice and bring them back to the facility in their respective home cages.

91 During this training phase, run the PR social self-administration procedure for three sessions (once per day) by repeating Steps 89–91.

Stages 6 and 7: social isolation and social seeking test ● Timing 2 weeks plus two sessions

▲ CRITICAL The social seeking test in the presence of social cues consists of 30 min sessions on isolation day 1 (the day after the last day of PR testing) and isolation day 15. The length of the test session can be varied according to the requirements of the experiment.

92 Bring the test mice (lever pressers) from the facility and move them from their home cage to the operant chamber.

▲ CRITICAL STEP The seeking test is conducted with no partner present, just social cues.

93 Start the session by uploading the Med-PC seeking program that, once issued, will automatically start the session with the presentation of the red discriminative house light, followed 10 s later by the insertion of the social-paired lever; the red house light remains on for the duration of the session. Active lever presses during testing, the operational measure of social seeking in incubation of drug craving and relapse[39–41], will automatically result in contingent presentations of the light cue previously paired with access to the social partner.

94 At the end of the session, the active lever is automatically retracted, and the red house light is turned off.

▲ CRITICAL STEP Record the number of active and inactive lever presses. During this phase, we do not present the lever or cues previously associated with food.

95 At the end of the session, remove the mice and bring them back to the facility in home cages.

Stage 8: food versus social choice procedure ● Timing 6 d

96 The day after the day 15 social seeking test, bring both test mice and partner mice from the facility and move them from their home cages to their assigned side of the operant chamber.

97 Start the session by uploading the Med-PC choice program that, once issued, will automatically start the session with the presentation of the discriminative cues for social interaction (house light) and food (red light), followed 10 s later by the insertion of the levers paired with both rewards.

98 Mice can then select one of the two levers. If the mice respond within 6 min, they only receive the reward that corresponds with the selected lever (60 s social interaction for the social-paired lever and one drug infusion for the drug-paired lever). Thus, on a given trial, the mouse can earn either the social reward or the food reward, but not both.

99 Check that each reward delivery is signaled by either the social- (20 s light cue and opening of the guillotine-style sliding door) or the food-associated (20 s light cue and food pellet delivery to the magazine) discrete cue, the retraction of both levers and the extinguishing of both discriminative cues.

100 If the mouse fails to respond on either active lever within 6 min, check that both levers are retracted, and their related discriminative cues are extinguished with no reward delivery.

101 Run six sessions using the FR1 schedule for food (ten trials separated by 8 min)

102 Progressively increase the social interaction schedule over the course of the six choice sessions as follows: 2× FR1, then 1× FR3, 1× FR6, 1× FR12 and 1× FR24.

▲ CRITICAL It is easiest to use a separate program for each different social choice FR schedule to avoid confusion in loading the wrong program and to avoid having to edit the program each day during testing.

▲ CRITICAL STEP Record the number of social and food rewards, and inactive lever presses.

103 At the end of the session, remove the mice and bring them back to the facility in home cages.

104 During this phase, run the choice procedure for six sessions over 6 d (days off over the weekends are acceptable) by repeating Steps 102–104.

Troubleshooting

Our protocol has been used and tested in more than ~200 mice (including published and unpublished data) with consistent and reliable data across experiments. Therefore, we do not anticipate critical issues at any of the stages reported above. Table 1 lists solutions to potential problems that experimenters may encounter while running the protocol as described. Table 2 provides examples of scenarios in which the experiment went wrong to facilitate future troubleshooting. For any unanticipated issues, experimenters can contact the corresponding authors for suggestions.

Table 1 | Troubleshooting table

Step	Problem	Possible reason	Solution
Equipment setup	Apparatus not working	Electrical or Med-PC interface	Check that all the cables and wires are properly connected and the SmartCtrl card pins interface with the chamber
10	Social partners escaping from the chamber, particularly smaller mice (<21 g)	Open space between the guillotine door and the stainless-steel grid floor	Add a metal panel underneath the guillotine door and the grid floor
12	Difficult to access the SmartCtrl Connection Panel (8 in/16 out) for plugging in all cables	Not enough space for the panel	Attach the panel (using Velcro) to the back of the sound-attenuating cubicle
15	Mice do not acquire stable food self-administration	Not interested in the palatable food pellet	Mild food deprivation to 85% of body weight during food self-administration training
			Press once on the active food lever to demonstrate the connection between lever press and food reward (priming). Use sparingly and record each time mouse is primed by the experimenter
		Levers, cues or door malfunction	Change levers, cues or door
		Inconsistent self-administration	Make sure mice begin training within the first 2 h of the dark cycle
20	Low number of rewards earned during the social session	Partner mouse can squeeze underneath the door to interact directly with test mouse	Prevent escape from the partner chamber by ensuring that all gaps are covered using aluminum tape or metal panels
		The holes of the barrier are too small to allow enough social interaction	Recreate a barrier with larger holes and increase the number of social self-administration sessions
		Mouse shows interest in the partner but does not lever press	If the mouse does not make the connection between lever pressing and door opening, prime the mouse by pressing the social lever once to show that this will cause the door to open revealing the partner mouse. Use this technique sparingly (no more than four times per hour of training) and account for every time the experimenter primes the mouse
		Mouse is uninterested in social partner	Switch to a different, familiar, size-matched mouse if after three operant sessions the mouse has not lever pressed for the opportunity to interact with the partner mouse
			On occasion, mice are simply not interested in social partners and will not self-administer or fail to continue when FR is increased. In this case, the animal can be excluded, but take note and report when this is done

Timing

The duration of an entire social-choice self-administration protocol, including acclimation and separation of drug users and social partners, is ~9–10 weeks.

Stage 1 (Steps 1–13; Steps 1–74 if using optional 3D printing instructions), social-choice self-administration apparatus building: 3–6 d depending on experience

Stage 2 (Steps 75–76), social housing separation of mice: ~1–2 weeks acclimation to the new colony in social housing and 1 week single housing

Stage 3 (Steps 77–82), food self-administration: 8 d

Stage 4 (Steps 83–88), social self-administration: 18 d

Stage 5 (Steps 89–91), PR testing: 3 d

Stages 6 and 7 (Steps 92–95), social seeking test: 2 weeks plus two sessions

Stage 8 (Steps 96–104), food versus social choice procedure: 6 d

Table 2 | Scenario of protocol issues

Scenario	Consequences
While we were developing the automatic social choice procedure, we tested several different screens. One of them had circular holes of 1.5 cm of diameter. Some of the mice were able to escape	Mice did not reliably perform the operant task. Therefore, during social self-administration (in one case) and choice (in another case) included missed sessions/trials
On one occasion the guillotine door was not working properly. The gears inside were spinning and the door was not opening making a loud noise for the duration of the experiment	The mouse failed to perform the task for the next 3 d

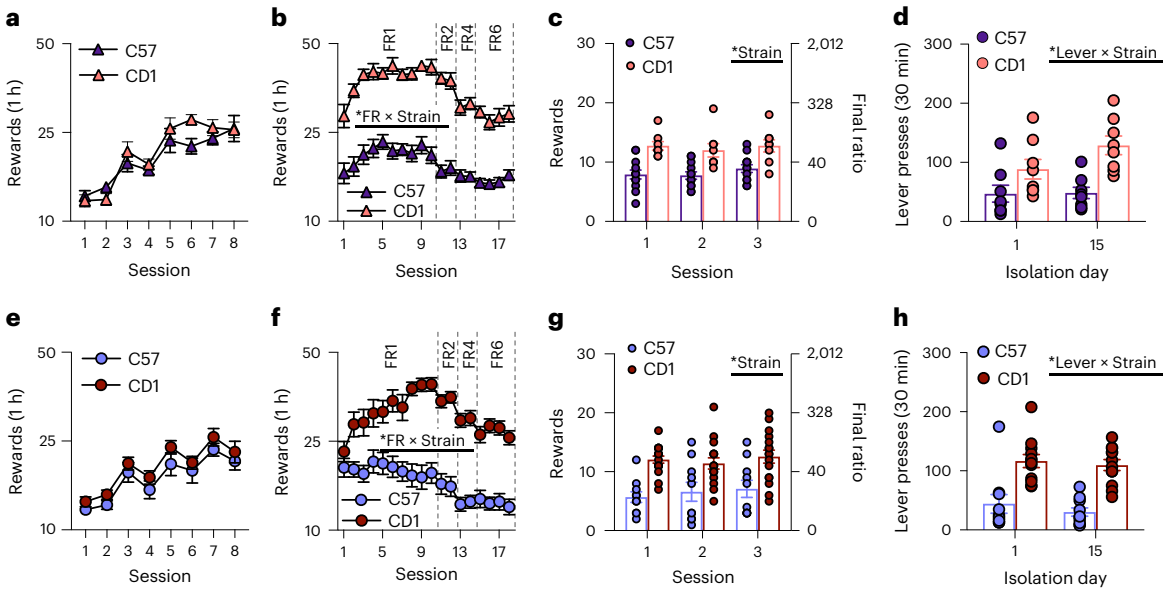

Fig. 3 | Testing strain and age effects on volitional social interaction in female mice. Adolescent and adult CD1 mice earn and seek more social rewards than C57BL/6J mice. **a**,**e**, Food self-administration training in adolescent (**a**) and adult (**e**) CD1 and C57BL/6J female mice. Number of rewards earned in 1 h session (CD1 adults: $n = 12$; CD1 adolescents: $n = 8$; C57BL/6J adults: $n = 10$; C57BL/6J adolescents: $n = 8$). **b**,**f**, Social self-administration training in adolescent (**b**) and adult (**f**) CD1 and C57BL/6J female mice. Number of rewards earned in 1 h session. *Significant FR × Strain interaction. **c**,**g**, Social PR testing in adolescent (**c**) and adult (**g**) CD1 and C57BL/6J female mice. Social rewards earned during each session (sessions ended after either 30 min of no reinforcement or 3 h (CD1 adults: $n = 16$; CD1 adolescents: $n = 8$; C57BL/6J adults: $n = 10$; C57BL/6J adolescents: $n = 12$). *Significant main effect of Strain ($P < 0.001$). **d**,**h**, Social seeking tests in adolescent (**d**) and adult (**h**) CD1 and C57BL/6J female mice. Active lever presses during 30 min test session on isolation days 1 and 15 (CD1 adults: $n = 16$; CD1 adolescents: $n = 8$; C57BL/6J adults: $n = 10$; C57BL/6J adolescents: $n = 12$). *Significant Lever × Strain interaction ($P < 0.001$). Data are mean ± s.e.m. This study has been approved by the National Institute on Drug Abuse Intramural Research Program ACUC. Figure reproduced with permission from ref. [29], Elsevier.

Anticipated results

We report the food and social self-administration data as the number of rewards that mice earn during the 60 min daily sessions, and often we also report the number of active lever presses during the sessions. For the PR testing, we report the number of rewards earned and the final ratio of responding obtained during the session, which is maximally 3 h long. For the social seeking tests, we report the number of active and inactive lever presses during the sessions. For the food versus social choice phase, we report the number of social rewards and food rewards earned during the ten discrete-choice sessions. We use factorial analysis of variance and *t*-tests using SPSS (IBM, version 25, GLM procedure) for statistical analysis of the behavioral data. When we obtain significant main effects and interaction effects ($P < 0.05$, two-tailed), we follow them with post hoc tests (Fisher protected least significant difference). For choice data, the statistical analyses are performed on a social preference ratio score (number of social rewards/(number of social rewards + number of food rewards)) (ref. [5]). Usually, we do not present the inactive lever data in figures, because responding on this lever during the any stage of the protocol is very low (though we typically report the range of inactive lever presses for each experiment). We do not use statistical methods to predetermine sample sizes, and our sample sizes are similar to those reported in previous publications[5,7].

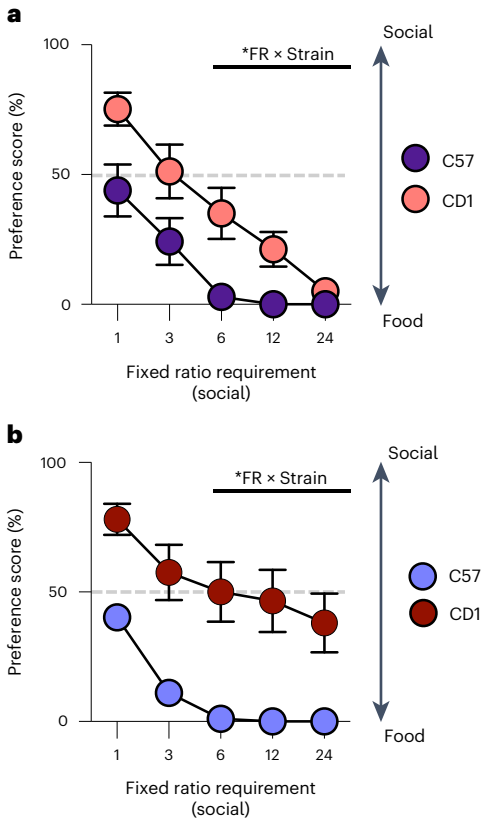

Fig. 4 | Testing the preference for social interaction versus palatable food. CD1 mice prefer social interaction over access to palatable food even when it requires more effort; C57BL/6J mice prefer food over access to social interaction regardless of the effort level required. **a**,**b**, Preference score % (number social trials/(number social trials + number food trials) × 100) in adolescent (**a**) and adult (**b**) female mice (CD1 adults: $n = 16$; CD1 adolescents: $n = 8$; C57BL/6J adults: $n = 10$; C57BL/6J adolescents: $n = 12$). Data are mean ± s.e.m. *Significant FR × Strain interaction ($P = 0.007$). This study was approved by the National Institute on Drug Abuse Intramural Research Program ACUC. Figure reproduced with permission from ref. [29], Elsevier.

Using this protocol, we have shown that operant social self-administration (Fig. 3a–c), social seeking during periods of isolation (Fig. 3d) and choice of social interaction over palatable food is significantly stronger in female CD1 mice than in female C57BL/6J mice (Fig. 4a), and that these effects are age independent (Figs. 3e–h and 4b)[29]. In addition to using the protocol to study motivational aspects of social reward, we adapted the protocol to assess the effect of prior environmental experience and social devaluation on social reward. Mice spent an extended period (60 d) housed either in isolation or in groups of three or four. Mice in all groups were then isolated for 7 additional days before the initiation of operant social self-administration. We included a group of mice that lever pressed for access to an empty chamber as an additional control in which mice experienced everything except the social component of self-administration. Isolated and group-housed mice received a similar number of social rewards during self-administration, suggesting that extended isolation housing did not alter social motivation (Fig. 5a,b). We then either regrouped mice or left them in isolation while continuing social self-administration (FR6 schedule only; Fig. 5c). Data trended toward a reduction in social rewards in the regrouped mice, but the effect was not statistically significant[29].

We tested the breeding of outbred female CD1 mice with transgenic C57BL/6J male mice to maintain the social phenotype in the hybrid F1 offspring (Fig. 6a,b), a strategy that has previously been used with male CD1 hybrids to maintain their aggressive phenotype[27]. Wild-type CD1 female mice and FosGFP × CD1 female and male F1 hybrid mice show similar operant social self-administration ($F_{22,352} = 1.5$, $P = 0.064$; partial Eta^2 0.09) (Fig. 6c). FosGFP × CD1 female and male F1 hybrid mice show reduced social seeking relative to wild-type CD1 female mice ($F_{2,32} = 12.9$, $P < 0.001$; partial Eta^2 0.5) (Fig. 6d). Given their similar performance on social self-administration, the reduced social seeking may be indicative of weaker associative learning, rather than a deficiency in social motivation. Although lever pressing may be lower in the CD1 hybrid mice, both wild-type and

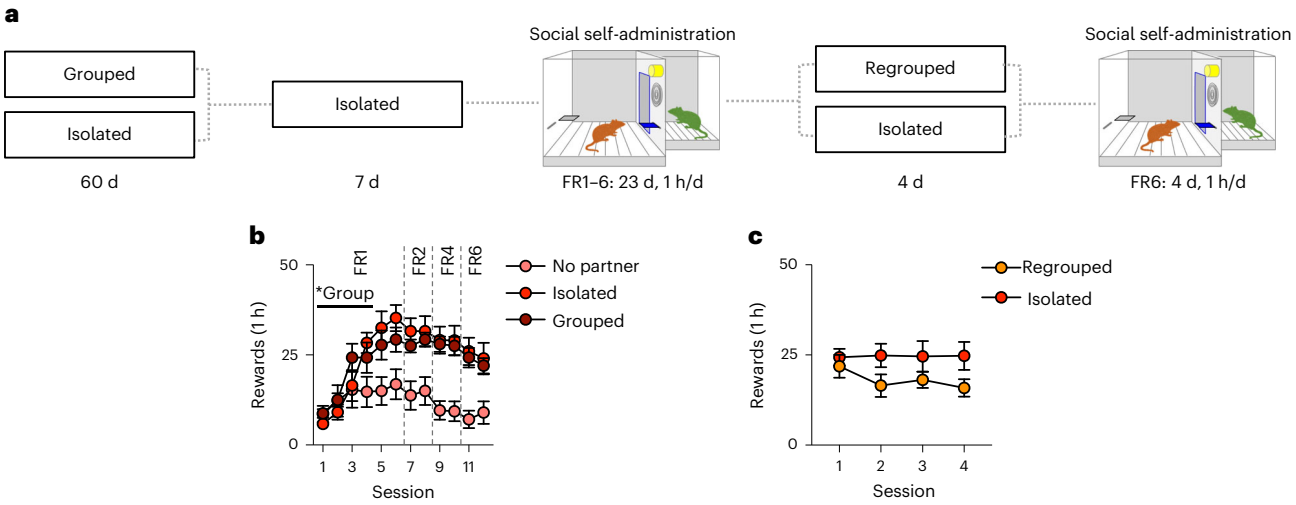

Fig. 5 | Testing the effect of housing conditions on volitional social interaction in female mice. a, Timeline of the experiment. Social self-administration training in long-term group housed or isolated mice. **b**, Number of social rewards earned (no partner control: $n = 8$; isolated: $n = 8$; grouped: $n = 8$). *Significant main effect of group ($P = 0.002$). **c**, Social self-administration (FR6 schedule) in regrouped or continuously isolated mice, number of social rewards earned (isolated: $n = 8$, regrouped: $n = 8$). Data are mean ± s.e.m (**b** and **c**). This study was approved by the National Institute on Drug Abuse Intramural Research Program ACUC. Panels **b** and **c** reproduced with permission from ref. [29], Elsevier.

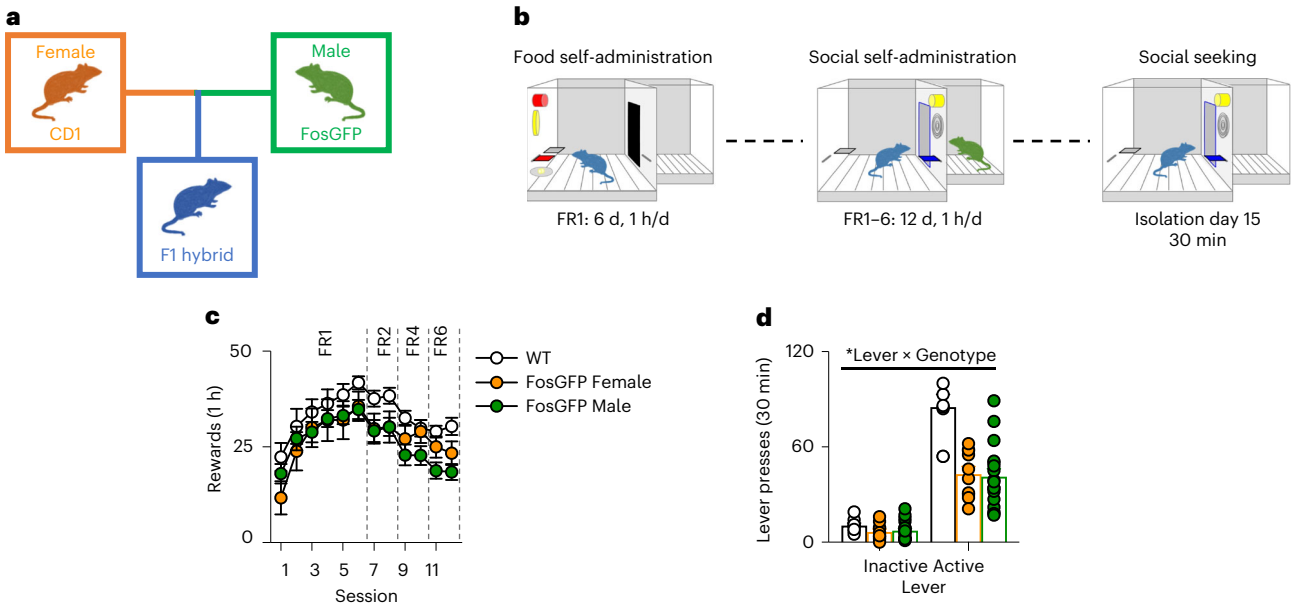

Fig. 6 | Testing activity-dependent neuronal ensembles involved in volitional social interaction using FosGFP × CD1 transgenic hybrid mice. a, Breeding scheme for FosGFP × CD1 transgenic hybrid mice. **b**, Timeline of the experiment. **c**, Social self-administration training in wild-type or transgenic mice bred on CD1 background (wild type: $n = 6$; FosGFP females: $n = 10$; FosGFP males: $n = 17$). **d**, Social seeking tests in wild-type or transgenic mice bred on CD1 background. Lever presses during 30 min test session on isolation day 15 (wild type: $n = 6$; FosGFP females: $n = 10$; FosGFP males: $n = 17$). *Significant Lever × Genotype interaction ($P < 0.001$). Data are mean ± s.e.m. This study was approved by the National Institute on Drug Abuse Intramural Research Program ACUC.

CD1 hybrid mice were able to distinguish between active and inactive levers during social seeking ($F_{1,32} = 196.7$, $P < 0.001$; partial Eta^2 0.9) (Fig. 6d).

Our protocol is versatile and can be used with different ages, strains and mouse models. Our approach of breeding CD1 females with male transgenic mice on C57 background to preserve the social phenotype in the F1 hybrid female and male mice ensured reproducible data in our laboratory. However, it is worth noting that this breeding strategy should be validated for use in other genetically more complex models.

Data availability

The main data discussed in this protocol are available in the supporting primary research paper (https://doi.org/10.1016/j.biopsych.2021.10.023)

Code availability

The Med-Associates programs are available upon request of the corresponding authors (L.A.R. and M.V.). Files necessary for 3D printing the partner chamber are provided in the Supplementary Software.

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Author contributions

L.A.R., F.M.H., S.S.L. and M.V. contributed to various aspects of the study, including the design and performance of the research and the writing of the paper.

Competing interests

The authors declare no competing interests.

Additional information

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