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An operant social self-administration and choice model in rats

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It is difficult to translate results from animal research on addiction to an understanding of the behavior of human drug users. Despite decades of basic research on neurobiological mechanisms of drug addiction, treatment options remain largely unchanged. A potential reason for this is that mechanistic studies using rodent models do not incorporate a critical facet of human addiction: volitional choices between drug use and non-drug social rewards (e.g., employment and family). Recently, we developed an operant model in which rats press a lever for rewarding social interaction with a peer and then choose between an addictive drug (heroin or methamphetamine) and social interaction. Using this model, we showed that rewarding social interaction suppresses drug self-administration, relapse to drug seeking, and brain responses to drug-associated cues. Here, we describe a protocol for operant social interaction using a discrete-trial choice between drugs and social interaction that causes voluntary abstinence from the drug and tests for incubation of drug craving (the time-dependent increase in drug seeking during abstinence). This protocol is flexible but generally requires 8–9 weeks for completion. We also provide a detailed description of the technical requirements and procedures for building the social self-administration and choice apparatus. Our protocol provides a reliable way to study the role of operant social reward in addiction and addiction vulnerability in the context of choices. We propose that this protocol can be used to study brain mechanisms of operant social reward and potentially impairments in social reward in animal models of psychiatric disorders and pain.

Introduction

Decades of preclinical research on the pharmacological, circuit, and molecular mechanisms of opioid and psychostimulant addiction[1–3] have yet to be translated to successful clinical treatments[4,5]. Heilig et al.[5] suggested that incorporating social factors, which play a critical role in human drug addiction[6], into mechanistic studies using animal addiction models will improve their predictive validity. On the basis of these considerations, we recently introduced a rat model[7] that mimics features of a clinical behavioral treatment known as the community reinforcement approach (CRA)[8,9]. The CRA is based on principles of operant conditioning by substitution of positive social reinforcers (e.g., family support, employment adherence) for drug use, contingent in part on cessation of drug use[10–12].

Our rat 'CRA model' is based on studies of choice between food and drug reward in rats and monkeys[13–18]. This model is also an extension of our studies on relapse to drug seeking after voluntary abstinence induced by providing rats mutually exclusive choices between a self-administered drug and palatable food[19–22]. Using the rat CRA model, we showed that rewarding social interaction suppresses methamphetamine and heroin self-administration in established addiction models[23–25], incubation of methamphetamine craving (the time-dependent increases in drug seeking that occur during home-cage forced abstinence or food choice–induced voluntary abstinence)[26], and brain responses to methamphetamine-associated cues[7]. We also showed that social choice–induced voluntary abstinence decreases incubation of heroin craving[27].

Here, we describe how to use our rat CRA model, which provides a standardized protocol for operant social self-administration, discrete-trial choice between drugs and social interaction that causes voluntary abstinence from the drug and tests for incubation of drug craving. In addition, we demonstrate potential applications of the protocol to study individual variability in the context of the choice between drug and social reward, as well as the motivation to seek operant social reward. We also provide a detailed description of the technical requirements, procedures, and troubleshooting

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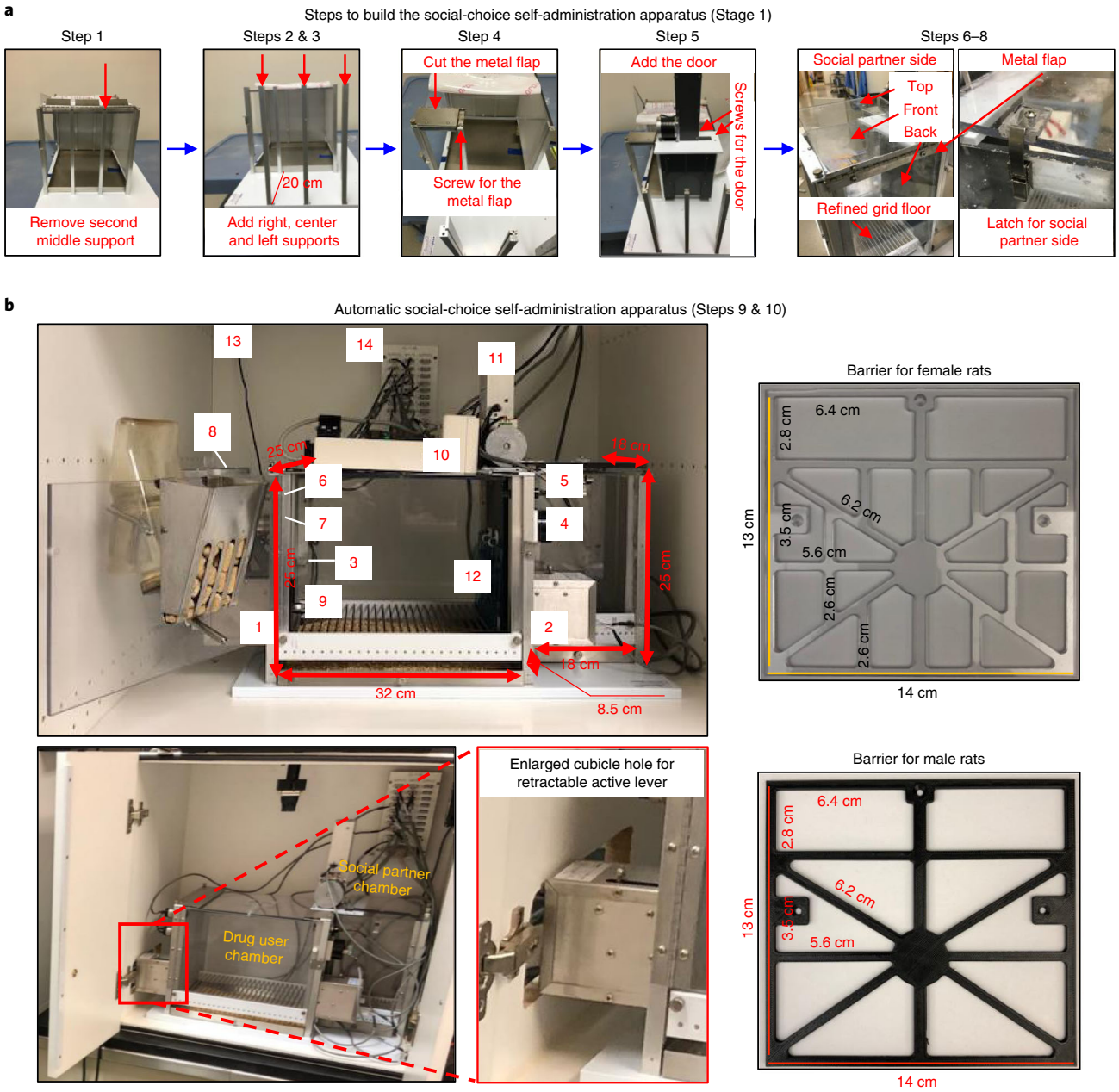

Fig. 1 | Social-choice self-administration apparatus. a, Steps for building the apparatus (Stage 1). The base of the apparatus is a standard modular operant test chamber with a modified top for rat (Med-Associates). Image reproduced from ref. [7], Springer Nature. **b**, Automatic social-choice self-administration apparatus. Top left, the apparatus with the configuration described in this protocol and detailed measurements. Food and water are available during the self-administration and choice sessions and are attached to the front door. (1) Retractable lever 1; (2) retractable lever 2; (3) inactive lever; (4) Sonalert module; (5) house light, white; (6) house light, red; (7) cue light, white; (8) food magazine (optional); (9) food receptacle (optional); (10) pump; (11) door; (12) barrier; (13) fan; (14) SmartCtrl Connection Panel (8 in/16 out). Bottom left, social apparatus enclosed in the Med-Associates cubicle. Bottom center, magnification of the modified cubicle for a easy access to the left retractable active lever. Right, different barrier types. Top, barrier for female rats; bottom, barrier for male rats. All measurements are in centimeters. Image reproduced from ref. [27], Elsevier.

advice for building a fully automatic social self-administration and social- versus drug-choice apparatus (Fig. 1). Our protocol provides a reliable way to study the role of social reward in rat addiction models, as well as addiction vulnerability. We propose that the protocol can be used to investigate the brain mechanisms of volitional social reward and, potentially, impairments in social reward in animal models of pain, autism, depression, anxiety, PTSD, and schizophrenia.

Overview of the social-choice self-administration protocol

The timeline of the protocol is depicted in Fig. 2. Below we provide details of the experimental procedures, including a description of the steps required and a list of materials needed to build our fully automatic social self-administration and choice apparatus (Stage 1). After receiving male and female rats, we house them two per cage by sex for 2 weeks and then switch to individual housing, starting 1 week before social self-administration. We randomly assign the rats to resident ('drug user') and social partner (drug-naive) conditions (Stage 2). In our protocol, we use male and female rats, with male partners for male rats and female partners for female rats. After an acclimation period, we train the rats to self-administer for access to their social partner during daily sessions using a discrete-trial design (see below for details). During this phase, each resident rat presses a lever for access to its previously paired partner (Stage 3, social self-administration). After stable social self-administration is achieved, we insert a Silastic catheter into each resident rat's jugular vein. We first allow the rats to recover from surgery for several days and then train them to press a lever for intravenous drug infusions (Stages 4 and 5, drug self-administration). The social- and drug-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' section). Subsequently, we allow the rats to choose between the drug- and social-paired levers, using a discrete-trial procedure (Stage 7, voluntary abstinence). We take advantage of the rats' strong preference for social interaction over drugs to achieve voluntary drug abstinence[7,27]. Finally, we test the rats for drug seeking on abstinence day 1 (the day after the last day of drug self-administration) and on abstinence day 15 (Stages 6 and 8, relapse or incubation tests); only the drug-paired lever is available during testing. The two tests at different time points enable us to determine the effects of social choice–induced voluntary abstinence on incubation of drug craving (the time-dependent increase in non-reinforced drug seeking abstinence[28]). Active lever presses on the previously drug-paired lever during testing, the operational measure of drug seeking in incubation of craving and relapse studies[26,29], cause contingent presentations of the light cue previously paired with drug infusions but no drug is delivered.

Comparison with other models

The protective effects of social interaction on addiction-related behaviors were previously demonstrated in rodent models[30]. Experimenter-imposed social interaction either outside or inside the testing cage decreases drug conditioned place preference (CPP), self-administration, and reinstatement (relapse) of drug seeking[31–34]. Group-housed rats exposed to an enriched environment show decreased drug CPP, drug self-administration, and reinstatement[31,33,34]. Similarly, pairing a peer rat with a non-drug context decreases both cocaine CPP and drug priming–induced reinstatement of CPP[35,36]. Other studies focused on social facilitation and social inhibition of drug intake in rats and showed that drug self-administration was facilitated in socially housed rats if both members of the pair had access to drug and, conversely, drug self-administration was inhibited if only one rat of the pair had access to drug[32,37–40].

However, these previous models do not incorporate the volitional choice between social interaction and drug use that occurs in human drug users. In addition, in monkeys and rodents, drug self-administration is reliably decreased by operant availability of other non-drug rewards such as palatable food[13]. And most rats choose sucrose or saccharin over heroin or cocaine, even after a long history of drug self-administration[15].

Advantages

Our protocol enables researchers to study whether drug self-administration is reduced by providing rats volitional or subject-controlled operant choice between drug and social reward, a setup that more closely models the human condition[5]. The fully automatic social-choice apparatus[27] enables researchers to simultaneously train many rats, while eliminating the intense experimenter workload and confounds related to rat–human interactions of the semi-automatic model we introduced in our original study[7]. The fully automatic model makes it feasible to incorporate both classic and cutting-edge techniques in behaving rats (e.g., in vivo electrophysiology, calcium imaging) to study mechanisms underlying complex and ethologically relevant volitional social behaviors. In addition, our social-choice procedure has long-lasting effects on drug relapse and craving, even when the social reward is discontinued. We showed that social choice–induced voluntary abstinence prevents incubation of methamphetamine craving (Fig. 3a; ref. [7]) and decreases

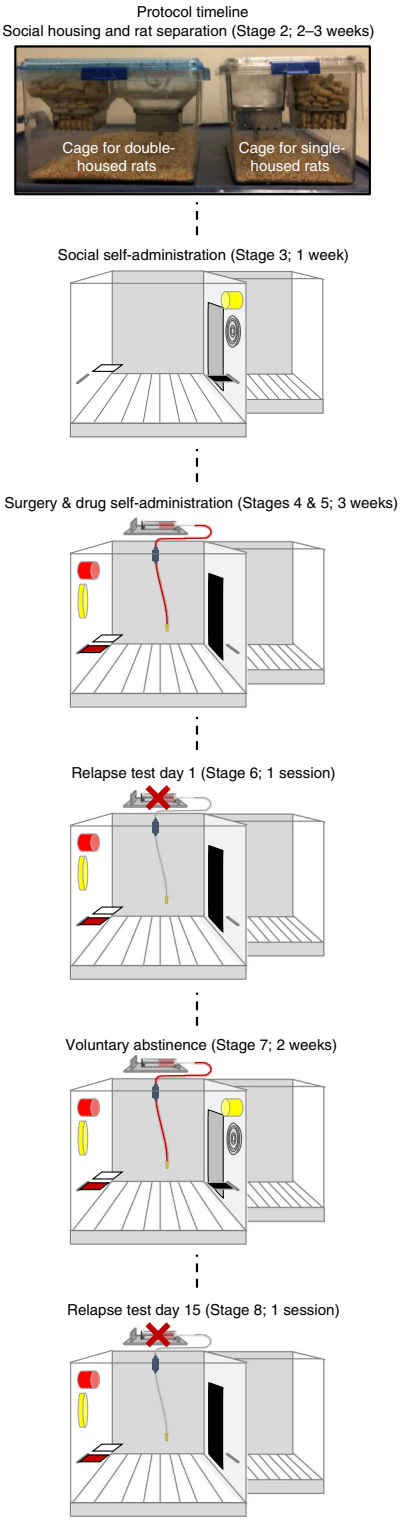

Fig. 2 | Protocol timeline. Stage 1—Steps 1–10 (Fig. 1), building the apparatus: 3–6 d; Stage 2—Steps 11 and 12, social housing and rat separation: ~2 weeks for acclimation to the new colony and social housing and 1 week of single housing; Stage 3—Steps 13–15, social self-administration: 1 week; Stage 4—Step 16, surgery: 1 d plus ~3–4 d of recovery; Stage 5—Steps 17–19, drug self-administration: 12 d; Stage 6—Steps 20 and 21, relapse or incubation test on day 1: 1 d; Stage 7—Steps 22–24, discrete-choice procedure: 10 sessions over 2 weeks; Stage 8—Step 25, relapse or incubation test on day 15: 1 d. This protocol is flexible but most commonly requires 8–9 weeks for completion.

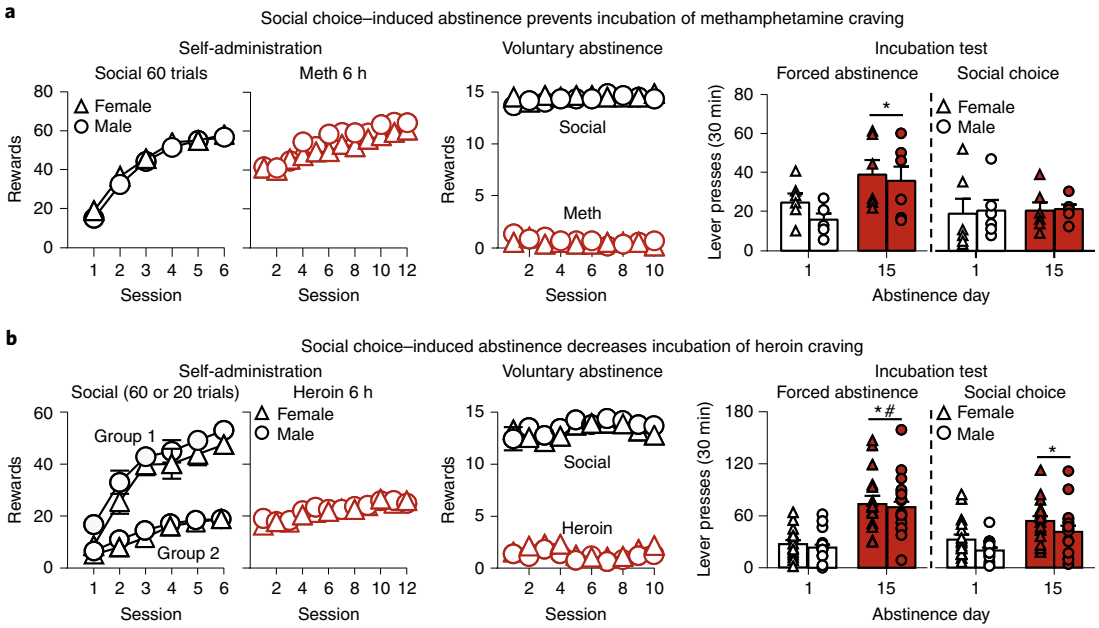

Fig. 3 | Using the protocol to study incubation of drug craving. a, Effect of social choice–induced voluntary abstinence on incubation of methamphetamine craving. Left, self-administration training: numbers of social rewards (60 trials) and methamphetamine infusions (6 h) in male and female rats. Center, voluntary abstinence: number of social rewards and methamphetamine infusions earned during the 10 discrete-choice sessions. Right, relapse or incubation tests: active lever presses during the 30-min test sessions; left: forced abstinence; right: social-choice abstinence. During testing, active lever presses led to contingent presentation of the light cue previously paired with methamphetamine infusions during training but not to methamphetamine delivery (extinction conditions). Data are mean ± SEM; *different from test day 1. Image adapted from ref. [7], Springer Nature. **b**, Effect of social choice–induced voluntary abstinence on incubation of heroin craving. Left, self-administration training: number of social rewards (60 or 20 trials) and heroin infusions (6 h) in male and female rats. Center, voluntary abstinence: number of social rewards and heroin infusions earned during the 10 discrete-choice sessions. Right, relapse or incubation tests: active lever presses during the 30-min test sessions; left: forced abstinence; right: social-choice abstinence. Data are mean ± SEM; *different from test day 1; #different from social-choice abstinence. Adapted from ref. [27], Elsevier. This study was approved by the NIDA IRP Animal Care and Use Committee.

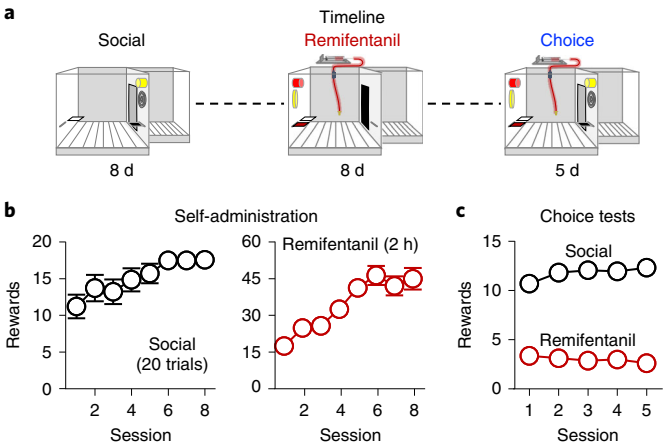

Fig. 4 | Generalization of the protocol to Long-Evans rats and remifentanil. a, Timeline of the experiment. **b**, Self-administration training: number of social rewards (20 trials) and remifentanil infusions (2 h), $n = 8$ Long-Evans male rats (plus $n = 8$ social partners). **c**, Choice tests: number of social rewards and remifentanil infusions earned during the 5 discrete-choice sessions. Data are mean ± SEM. This study was approved by the NIDA IRP Animal Care and Use Committee.

incubation of heroin craving (Fig. 3b; ref. [27]). The fully automatic procedure is generalizable to different rat strains (Fig. 4).

From a translational perspective, our choice procedure models the human treatment in which abstinence is rewarded with alternative social-based non-drug incentives[41,42]. Although almost

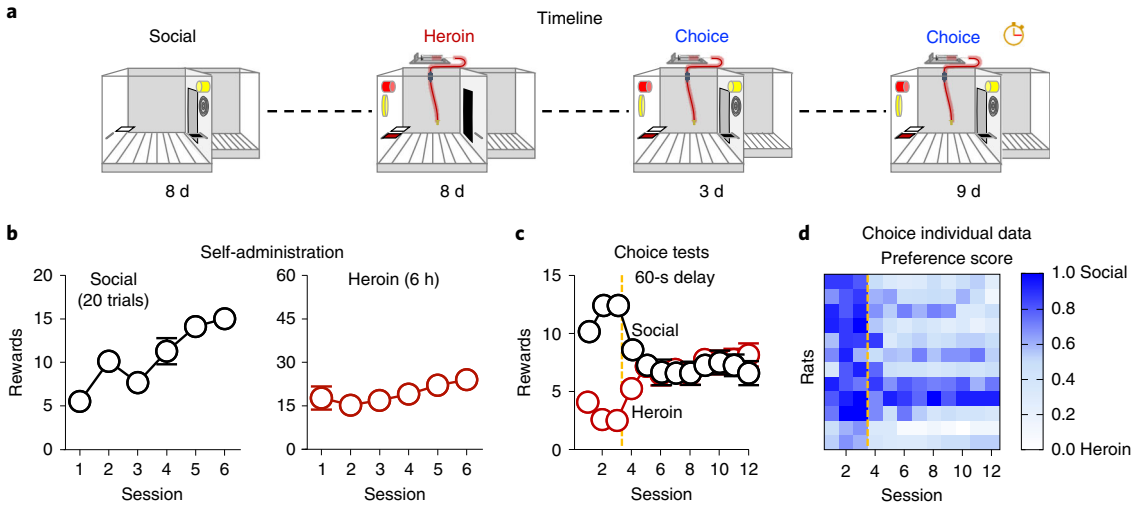

Fig. 5 | Using the protocol to study addiction vulnerability. a, Timeline of the experiment. **b**, Self-administration training: number of social rewards (20 trials) and heroin infusions (6 h), $n = 12$ Sprague Dawley female rats (plus $n = 12$ social partners). **c**, Choice tests: number of social rewards and heroin infusions earned during the 12 discrete-choice sessions. After the first three choice sessions (yellow dashed line), we introduced a 60-s delay for the social reward. **d**, Individual data: heatmap of the preference score (number of social rewards/[number of social rewards + number of drug infusions]) for each rat during the 12 discrete-choice sessions. 1.0 (dark blue) indicates preference for social reward and 0.0 (white) indicates preference for heroin. Data are mean ± SEM. This study was approved by the NIDA IRP Animal Care and Use Committee.

100% of our rats prefer to interact with a social partner rather than self-administer a drug, some human drug users will continue to use drugs despite the availability of social support[43]. The difference between the clinical scenario and our rats is related to the time of social reward delivery: in our rats it is immediate upon pressing a lever, whereas in humans social reward is often delayed[44]. However, when we introduced a delay between the lever press and getting access to a social partner, the abstinence rate decreased to ~50% (Experiment (Exp.) 2; Fig. 5), consistent with findings in humans[45]. Thus, our model can be adapted to study addiction vulnerability and potentially model addiction of people who appear to benefit less from the protective effects of social support.

More broadly, the role of social interaction is not only critical in drug addiction but also plays a major role in the etiology of several human psychiatric disorders. Therefore, here we show that our protocol can be adapted to study motivational aspects of social reward (Exp. 3; Fig. 6), using the progressive ratio reinforcement schedule, commonly used to study the motivation to seek drug and food rewards[46], and a social seeking test analogous to the one routinely used in drug studies[26] (Exp. 4; Fig. 7). These procedures provide a platform for future studies on behavioral, pharmacological, and circuit mechanisms of social reward and social seeking. We propose that progressive ratio responses to social reward and social seeking tests at different times after cessation of operant social self-administration can be used to study disruption of social reward in animal models of pain, autism, depression, anxiety, PTSD, and schizophrenia.

Finally, although it is now possible to buy the social-choice apparatus directly from Med-Associates (note that the authors do not have any commercial agreement with this company), it is substantially cheaper to build our custom-made automatic apparatus (Fig. 1). As described below, it requires principal components purchased from Med-Associates and then additional inexpensive materials (primarily plastic and Plexiglas). This represents a cheaper solution, and therefore our custom-made apparatus is potentially affordable for any laboratory.

Limitations

Our protocol requires double the number of rats for any given experiment (as resident rats and social partners are required). This increases the overall cost of the experiment and requires more animal facility space (and therefore has higher associated per diem costs). Another limitation is that the protocol is sensitive to the sizes of the barrier holes that separate the two sides of the chamber (Fig. 1). It is critical to allow the rats to at least touch each other's faces during social interactions.

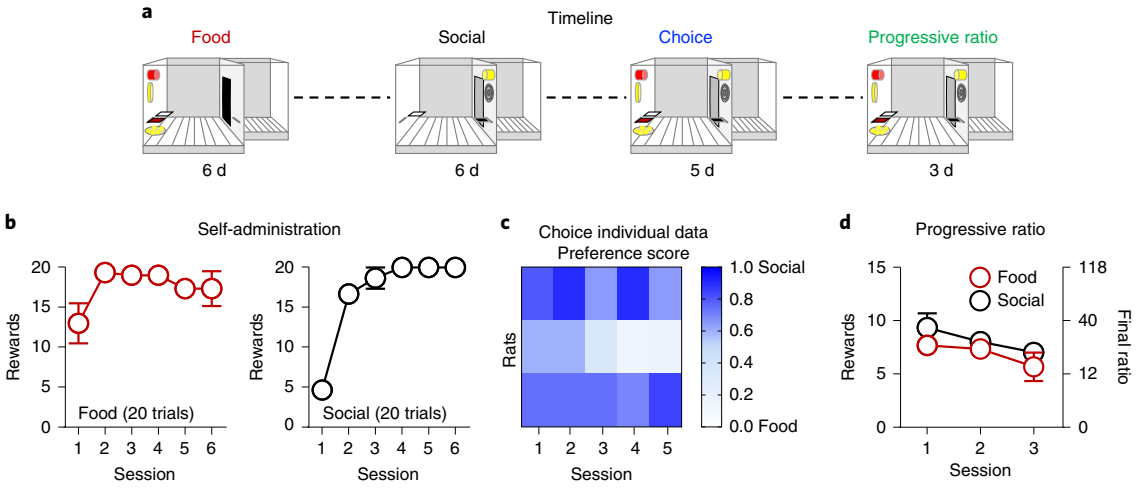

Fig. 6 | Using the protocol to study the motivation to seek operant social rewards. a, Timeline of the experiment. **b**, Self-administration training: number of palatable food pellets (20 trials) and social rewards (20 trials); $n = 3$ male Sprague Dawley rats (plus $n = 3$ social partners). **c**. Choice individual data: heatmap of the preference score (number of social rewards/[number of social rewards + number of palatable pellet rewards]) for each rat during the 5 discrete-choice sessions. 1.0 (dark blue) indicates preference for social reward and 0.0 (white) indicates preference for palatable food. **d**, Progressive ratio test: number of palatable food pellets or social rewards earned during progressive ratio tests. Data are mean ± SEM. This study was approved by the NIDA IRP Animal Care and Use Committee.

Fig. 7 | Using the protocol to study social seeking. a, Timeline of the experiment. **b**, Self-administration training: number of social rewards (60 trials); $n = 12$ (6 male and 6 female) Sprague Dawley rats (plus $n = 12$ (6 male and 6 female) social partners). **c**, Social seeking test: active and inactive lever presses during the 30-min test sessions performed 1 d after the last social self-administration session. During testing, active lever presses led to contingent presentation of the light cue previously paired with social reward during training but not to social interaction (extinction conditions). Data are mean ± SEM. This study was approved by the NIDA IRP Animal Care and Use Committee.

We recommend using a barrier with the dimensions described below; if the holes are too small, the rats will not maintain stable social self-administration and social preference behaviors (see Troubleshooting section).

Experimental design

Obtainment of appropriate permissions

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 (NIDA) Intramural Research Program (IRP) Animal Care and Use Committee. Obtaining appropriate permissions and conforming to regulations is required for experiments involving animals and restricted drugs.

Experimental timing and organization of the protocol

This protocol is flexible, but generally requires 8–9 weeks for completion. Typically, we run 6 d of social self-administration, 12 d of drug self-administration (which varies on the basis of the drug procedure used for each experiment (see ref. [7])), 10 sessions of social choice–induced voluntary abstinence over 14 d, and 2 sessions of relapse/incubation tests (on abstinence days 1 and 15). We often run experiments in cohorts ranging from 8 to 32 rats (plus social partners), with runs occurring once daily for each rat in the different cohorts. We run our experiments throughout the entire day, and we have not observed any differences related to the time of day of testing. We advise that experimenters new to this model start with cohorts of no more than 8 rats (plus 8 social partners). We also advise that social self-administration sessions include ~60 trials (60-s interaction) per session (~2 h) to give the rats 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 rats after each trial. However, we recommend checking on the rats periodically to make sure that none cross the barrier (particularly with small female rats).

Effects of modifications to the procedure

In our published papers[7,27] and Fig. 3, we used male and female Sprague Dawley rats. We also used our protocol with Long–Evans rats (Exp.1; Fig. 4). Thus, we speculate that our protocol can be used to train a different strains of rats. We observed no sex differences, and the protocol is adaptable to both male and female rats.

Typically, we start the protocol with rats weighing 150–175 g (~40–60 d old) on arrival. In our experience, a rat's age (within 2–6 months[7]) is not a critical feature for maintaining stable social self-administration or social preference. However, we recommend keeping the residents and their social partners at the same body weight range or age range in the different experiments. In addition, although partner rats can be socially housed two per cage, resident rats should be single housed to prevent catheter damage.

We have shown that the rats' strong social preference over drug persists even if the rats are socially housed or if they are pressing a lever to gain access to an unfamiliar rat[7]. In a modified version of this protocol, we trained the rats first to self-administer methamphetamine and then for social interaction, followed by a series of choice tests. We investigated whether the most 'addicted' rats (selected using an established rat model of individual differences in addiction vulnerability[25]) would prefer drug over social interaction. We found that independent of the addiction score and independent of the sequence of the training procedure, the rats preferred social interaction over drug[7].

Materials

Biological materials

Male and female Sprague Dawley or Long–Evans rats (Charles River Laboratories, strain codes 400 and 006), weighing 150–175 g on arrival **! CAUTION** Experiments must follow all governmental and institutional guidelines for the care and use of laboratory animals. Moreover, it is critical to report any excluded rats (e.g., due to catheter failure or lack of reliable self-administration) **▲ CRITICAL** We house the rats two per cage by sex for 2 weeks and then house them individually, starting 1 week before social self-administration. We randomly assign rats to resident (drug user) and social partner (drug-naive) conditions.

Reagents

- Drugs (we used methamphetamine, heroin, and remifentanil provided by the NIDA Pharmacy; these drugs can also be purchased from commercial suppliers) **! CAUTION** We recommend using all laboratory safety precautions when handling drugs to avoid potential contact with the syringe needles or contact of the solutions with the experimenter's eyes. Dispose of all the sharp materials in a designated sharps container to avoid exposure of the other users of the lab space to hazardous materials **▲ CRITICAL** In our protocol we have used different classes of drugs: methamphetamine (Fig. 3a; ref. [7]), heroin (Fig. 3b; ref. [7,27]), and remifentanil (Exp. 1, Fig. 4). Thus, it is highly likely that

our protocol can be used to study the effect of social reward on self-administration and relapse to other drugs ▲ CRITICAL For any new drugs, we recommend using standard doses reported in the literature and validating the selected drug dose with a proper dose–effect curve[7] ▲ CRITICAL For any drug, report in the drug laboratory book the exact amount of drug removed from the original container.

- Gentamicin (Fresenius Kabi)
- Ketoprofen (Butler Schein)
- Saline (sterile)
- Isoflurane
- Heparin

Equipment

Parts for custom-made social-choice apparatus

▲ CRITICAL All parts can be found at Med-Associates.

- Standard modular operant test chamber with modified top for rat (Med-Associates, cat. no. ENV-008CT)
- SmartCtrl interface module (8 input/16 output; Med-Associates, cat. no. DIG-716B)
- SmartCtrl connection panel (8 input/16 output; Med-Associates, cat. no. SG-716B)
- SmartCtrl cable (M/F, 25 feet (7.6 m); Med-Associates, cat. no. SG-210CB–DB25)
- Power cable (25 feet (7.6 m); Med-Associates, cat. no. SG-210CP-25)
- Auto guillotine door (Med-Associates, cat. no. ENV-010BS)
- Aluminum mesh for guillotine door (plus thumbscrew (HAR-Thumb-4-40 × 5/16-LowPro); Med-Associates, cat. no. FAB-ENV-008-32)
- Stainless-steel grid floor (white front and back) for ENV-307A (Med-Associates, cat. no. ENV-307A-GFW)
- Waste pan for ENV-307A chamber (Med-Associates, cat. no. ENV-307-07)
- Replacement 28 VDC fan with cable (Med-Associates, cat. no. ENV-025F28)
- Retractable lever (n=2; Med-Associates, cat. no. ENV-112CM)
- Standard fixed lever (inactive; Med-Associates, cat. no. ENV-110M)
- Sonalert module with volume control for rat chamber (2,900 Hz; Med-Associates, cat. no. ENV-223AM)
- House light, hooded (100 mA, 28 V, DC; Med-Associates, cat. no. ENV-215M)
- Right/left/center front supports (one each; Med-Associates, cat. no. FAB-ENV-008-07)
- Stimulus lights (1 inch; 1 white and 1 red lens; Med-Associates, cat. no. ENV-221M)
- Infusion pump (Med-Associates, cat. no. PHM-100)
- Sound-attenuating cubicle (Med-Associates, cat. no. ENV-017M)

Other equipment

- Clear impact-resistant polycarbonate (12 × 12 × 1/4-inch sheet; McMaster-Carr, cat. no. 8574K28)
- Clear rectangular cage for double-housed rats (34 cm (w) × 40 cm (d) × 20 cm (h)) with plastic cover top and lid for food and water (Fig. 2); (Lab Products, cat. no. Super Rat 1400)
- Clear rectangular cage for singly housed rats (23 cm (w) x 35 cm (d) x 20 cm (h)) with plastic cover top and lid for food and water (Fig. 2); (Lab Products, cat. no. One Cage 2100)
- Hard woodchip bedding (Supplier Envigo. cat. no. 7086G)
- Catheters (Silastic silicone laboratory tubing, 0.020-inch i.d., 0.037-inch o.d., 0.009-inch wall thickness; VWR, cat. no. 62999-075)
- Cannula (22-gauge; Plastics One, cat. no. C313G-5up)
- Polypropylene mesh (Small Parts, Amazon.com)
- Cabinet frame (IKEA, cat. no. 802.653.98)
- Syringes (20 ml)
- Velcro

Software

- Med-Associates software, written using Med-PC code, to automatically run social and drug self-administration, social versus drug choice, and relapse tests (Med-PC programs are available from the authors upon request)
- Excel software (https://products.office.com/en-us/excel)
- SPSS v25 (IBM, https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-25)

(Optional) Equipment for palatable food versus social-choice studies

▲ **CRITICAL** Our apparatus can also be used for palatable food versus social-choice studies

- Modular pellet dispenser, magazine type (45 mg; Med-Associates, cat. no. ENV-203M-45)
- Pellet receptacle + plastic tube (Med-Associates, cat. no. ENV-200xxx)

Equipment setup

Surgery

We anesthetize the rats with isoflurane (5% (vol/vol) induction; 2–3% (vol/vol) maintenance). We then insert Silastic catheters into the jugular vein, which we pass subcutaneously to the midscapular region and attach to a modified 22-gauge cannula cemented to polypropylene mesh ▲ **CRITICAL** We inject ketoprofen (2.5 mg/kg, s.c.) after surgery to relieve pain and decrease inflammation. We allow the rats to recover from surgery for 3–4 d ▲ **CRITICAL** We flush the catheters daily with sterile saline containing gentamicin (4.25 mg/mL) during the recovery, training, and voluntary abstinence phases **! CAUTION** We recommend using all applicable laboratory safety precautions for handling gentamicin and ketoprofen to avoid potential contact with the syringe needles or contact of the solutions with the experimenter's eyes. Dispose of all the sharp materials in the designated sharps container to avoid exposure of the other users of the lab space to hazard materials.

Socially and singly housed rats

Once we receive the rats, we group-house them two per cage by sex. Figure 2 depicts a standard cage for double-housed rats. We provide free access to water and food during the entire duration of the experiments ▲ **CRITICAL** We advise allowing the rats to acclimate to the facility for 1 week and then handling them twice a week for the following 2 weeks before separation. After this period, we randomly assign rats to resident (drug user) and social partner (drug-naive) conditions. Figure 2 also depicts a standard cage for singly housed rats ▲ **CRITICAL** Both double-housed and singly housed cages should be cleaned two or three times per week.

Custom-made social-choice self-administration apparatus

The standard modular operant test chamber for rats is the base for the custom-made social-choice self-administration apparatus. The apparatus can be enclosed in a regular Med-Associates sound-attenuating cubicle with a small modification in the lateral side (Fig. 1b; we enlarged the already-existing hole on the left side of the cubicle for easy passage of the left retractable lever). Alternatively, the apparatus can be housed within an inexpensive IKEA cabinet frame. All the parts listed above are necessary to build one apparatus (Fig. 1).

A standard modular operant test chamber is combined with a custom-made social-partner chamber that is separated by a guillotine door. Each chamber should have a discriminative cue on the right panel (white house light) 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 (red house light) to signal the insertion and subsequent availability of the drug-paired active (retractable) lever located on the left side. Locate the levers 6 cm above the grid floors and a white discrete light (white light) above the drug-paired lever and a discrete Sonalert module (for the tone cue) above the social reward–paired lever. The left side can also be equipped with a pellet dispenser, pellet receptacle (our apparatus can also be used for palatable food versus social-choice studies), and an inactive (stationary) lever. Attach a fan to the back of the cubicle for background noise. Include a bottle of water and a food hopper to provide food and water during the self-administration and choice sessions (Fig. 1b) **! CAUTION** We recommend periodically checking the rats and making sure that the programs are working properly, that the rats are not crossing the barrier during social self-administration, and that they remain connected to the drug lines for the entire duration of the drug self-administration sessions ▲ **CRITICAL** Before the beginning of each drug self-administration session, check whether each line and swivel is properly working (we manually move the infusion pump and flush the line that delivers drug from the syringe to the rats' catheter). Prepare 20-ml syringes of drug and change them once they are below ~9 ml to prevent the rats from emptying the syringes during the self-administration sessions ▲ **CRITICAL** At the end of each session, change the bedding of both the resident and the social partner sides of each chamber. Also, wipe the stainless-steel grid floors with water once a week and clean the

entire apparatus before each relapse test to avoid any confounds due to the previous presence of the social partners.

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 (v.25, GLM procedure; see Anticipated results).

Procedure

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

! CAUTION For any cutting and assembling, we recommend taking the appropriate safety measures (e.g., wearing goggles, cut-resistant gloves, coats).

▲ CRITICAL We recommend building the apparatus far from any behavioral/facility rooms because the noise of a Dremel or drill can affect rats' behavior in nearby rooms.

▲ CRITICAL The timing of this phase can vary depending on the experimenter's experience. Each step is described in Fig. 1.

▲ CRITICAL As an alternative to self-building this apparatus, it can be obtained from Med-Associates Inc.

1 Remove the second center front support from a standard modular operant chamber.
2 Make three holes on the white plastic base 20 cm from the support removed in Step 1.
3 Add the right/center/left front supports.
4 Cut the right metal flap. One screw is attached to the center support, but this still leaves space for the door; this will allow easy access to levers and cues in that side.
5 Attach the auto guillotine door to the top of the modular chamber.
6 Cut two rectangular pieces (20 cm × 15 cm) from the clear impact-resistant polycarbonate sheet; attach them to the front and back of the partner side of the chamber (between the auto guillotine door and the metal supports).
7 Cut a third piece of polycarbonate to create the top of the partner chamber (18 cm × 18 cm). Prepare a metal flap (18 cm long) and attach it to the back of the partner chamber and the polycarbonate piece to allow the top part to open and close easily. Complete the top part with a latch to keep the chamber closed when the social partner is inside.
8 Trim the white plastic back part of the stainless-steel grid floor to make it ~5 cm long.
9 Add all the other components (e.g., levers, cues).
10 Remove the SmartCtrl connection panel (8 in/16 out) from the white base and attach it to the back of either the IKEA cabinet or the sound-attenuating cubicle. Test the apparatus to make sure that it works properly.
? TROUBLESHOOTING

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

11 Purchase male and female Sprague Dawley or Long–Evans rats (Charles River Laboratories), weighing 150–175 g. House the rats two per cage by sex, with free access to food and water, and allow the rats to habituate to their new colony facility for a minimum of 1 week before handling.
▲ CRITICAL STEP Our protocol is likely to work with other strain of rats.
12 Two weeks later, randomly assign the rats to resident (drug user) or social partner (drug-naive) conditions.
▲ CRITICAL STEP Mark the rat tails to keep track of the different social pairs.

Stage 3: social self-administration ● Timing 1 week

13 Train rats to self-administer for access to their social partner during daily 120-min sessions (60 trials/session, 60-s social interaction) using a discrete-trial design. Each resident rat presses for its previously paired partner. First bring the rats from the facility and move them from their home cage to their assigned side of the operant chamber (residents 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; we allow resident rats, via the Med-PC social self-administration program, 60 s to press the

active lever (fixed-ratio-1 reinforcement schedule) before the lever retracts and the house light turns off.

▲ CRITICAL STEP Successful lever presses by the resident rats cause the retraction of the active lever, a discrete 20-s tone cue, and opening of the guillotine sliding door. Resident rats are subsequently allowed to interact with their social partner for 60 s, when the house light turns off, at which point the guillotine door closes. Record the number of successful trials and inactive lever presses (presses of the standard fixed lever).

▲ CRITICAL STEP Check whether the rats are crossing the barrier.

? TROUBLESHOOTING

14 At the end of the session, remove both the resident and the partner rats and bring them back to the facility in their respective home cages.

15 During this training phase, run the social self-administration procedure for 6 sessions by repeating Steps 13 and 14 daily.

Stage 4: surgery and recovery ● Timing ~1 day plus 3–4 days of recovery

16 The day after the last day of social self-administration, anesthetize the rats with isoflurane, insert a Silastic catheter attached to a modified 22-gauge cannula cemented to polypropylene mesh into the jugular vein and pass it subcutaneously to the midscapular region. Inject ketoprofen (2.5 mg/kg, s.c.) after surgery to relieve pain and decrease inflammation. Allow the rats to recover from surgery for 5–7 d.

▲ CRITICAL STEP Flush the catheters daily with sterile saline containing gentamicin during the entire duration of the experiment.

? TROUBLESHOOTING

Stage 5: drug self-administration ● Timing 12 d

17 After recovery from surgery, train the resident rats to self-administer drug. First bring the resident rats (i.e., drug users) from the facility and move them from their home cage to the operant chamber. Start the self-administration sessions at the onset of the dark cycle; start each session by uploading the Med-PC drug self-administration program that, once initiated, will automatically start the session with the presentation of the discriminative cue (red house light) and 10 s later the insertion of the drug-paired lever; the red house light will remain on for the duration of the session.

▲ CRITICAL STEP Successful lever presses (fixed-ratio-1, 20-s time-out reinforcement schedule) by the drug user rats cause the presentation of a discrete 20-s light cue, and the automatic delivery of a drug infusion via the infusion pump (active for 3.5 s). The Med-PC drug self-administration program will run for six 1-h sessions separated by 10-min off periods. At the end of each 1-h session, the red house light is automatically turned off and the active lever is retracted. The program will also automatically limit the number of drug infusions to 15 per h to avoid overdose.

▲ CRITICAL STEP Check whether the rats are connected to the drug lines.

▲ CRITICAL STEP Record the number of drug infusions and active and inactive lever presses.

? TROUBLESHOOTING

18 At the end of the session, remove the drug user rats and bring them back to the facility in their home cages.

19 Run rats for drug self-administration for 12 sessions, by repeating Steps 17 and 18 for 12 consecutive d. Note that the timing of this phase can vary depending on the self-administration procedure used and can extend up to 3 months or more; in this scenario, repeat Steps 17 and 18 for 5–6 d per week for 3 months (provide some off days over the weekends).

Stage 6: relapse or incubation test ● Timing 1 d

▲ CRITICAL The relapse test in the presence of drug cues usually consists of 30-, 60-, or 90-min (or longer) sessions on abstinence day 1 (the day after the last day of drug self-administration) and day 15 (the day after the last day of voluntary abstinence; see Stage 8 below).

20 Bring the resident rats from the facility and move them from their home cage to the operant chamber. Start the session by uploading the Med-PC relapse program that, once initiated, will automatically start the session with the presentation of the red house light, followed 10 s later by the insertion of the drug-paired lever; the red house light will remain on for the duration of the session. Active lever presses during testing, the operational measure of drug seeking in incubation of drug craving and relapse[26,29], will automatically result in contingent presentations of the light cue previously paired with drug infusions but not drug delivery. At the end of the session, the active lever is automatically retracted, and the red house light is turned off.

▲ **CRITICAL STEP** Before the beginning of the session, remove the syringes from the infusion pumps.

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

21 At the end of the session, remove the drug user rats and bring them back to the facility in home cages.

Stage 7: discrete-choice procedure ● Timing 2 weeks

22 The day after the day 1 relapse test, bring both resident and partner rats from the facility and move them from their home cages to their assigned side of the operant chamber (residents and partners). Start the session by uploading the Med-PC choice program that, once initiated, will automatically start the session with the presentation of the discriminative cues for social interaction (house light) and drug (red light), followed 10 s later by the insertion of the levers paired with both rewards. Rats can then select one of the two levers. If the rats respond within 6 min, they receive only the reward that corresponds to the selected lever (60-s social interaction for the social reward–paired lever and one drug infusion for the drug-paired lever). Thus, in a given trial, the rat can earn either the social reward or the drug reward, but not both. Each reward delivery is signaled by the social (20-s tone cue and opening of the guillotine-style sliding door) or drug-associated (20-s light cue and activation of infusion pump) discrete cue, the retraction of both levers, and the extinguishing of both discriminative cues. If the rat fails to respond by pressing either active lever within 6 min, both levers are retracted and their related discriminative cues are extinguished with no reward delivery. The Med-PC choice program allows rats to choose between the social reward– and drug-paired levers in a discrete-trial choice procedure; it divides each 120-min choice session into 15 discrete trials separated by 8 min.

▲ **CRITICAL STEP** Record the number of social and drug rewards, as well as inactive lever presses. **? TROUBLESHOOTING**

23 At the end of the session, remove the drug user rats and bring them back to the facility in their home cages.

24 During this voluntary abstinence phase, run the choice procedure for 10 sessions over 14 d (provide some off days over the weekends) by repeating Steps 20 and 21.

Stage 8: relapse or incubation test ● Timing 1 d

25 On the day after the last day of voluntary abstinence, repeat Steps 20 and 21 to test the rats' drug seeking at the late abstinence phase.

Troubleshooting

Our protocol has been used and tested in >600 rats (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. However, on the basis of our experience, we provide below a list of solutions to potential problems that experimenters may encounter while running the protocol as described here (Table 1). In addition, we provide examples of scenarios in which the experiment went wrong to facilitate future troubleshooting (Table 2). For any unanticipated issues, experimenters can contact the corresponding authors for advice.

Table 1 | Troubleshooting table

Step	Problem	Possible reason	Solution
10	Apparatus not working	Problem with electrical or Med-PC interface	Check that all the cables and wires are properly connected and the SmartCtrl card pins interface with the chamber
	Social partners escaping from the chamber	Open space between the back wall and the stainless-steel grid floor	Add a polycarbonate panel to the back of the chamber
	Apparatus does not fit inside the Med-Associates cubicle	Not enough space for the left retractable lever	Enlarge the already-existing hole on the left side of the cubicle for easy passage of the left active retractable lever (Fig. 1b)
	Difficult to access to the SmartCtrl connection panel for plugging in all cables	Not enough space for the panel	Attach the panel (using Velcro) to the back of either the IKEA cabinet or the sound-attenuating cubicle
13	Low number of rewards earned during the session	Rats cross the barrier that separates the two sides of the social-choice self-administration chamber	Re-create the barrier with smaller holes and increase the number of social self-administration sessions
		The holes of the barrier are too small to allow enough social interaction	Re-create a barrier with larger holes and increase the number of social self-administration sessions
16	Rats lose weight during recovery	Infection close to the catheter site	Provide antibiotics and contact the facility veterinary staff
	Rats in discomfort, clean fluids coming out of the rats back, or tension in the catheter during flushing	Malfunction or blocked catheter	Flush the catheter with saline and heparin. If the problem persists, implant the catheter in the other vein. If this strategy does not work, remove the rat form the experiment
17	Rats do not reliably self-administer drug	Wrong dose of drug or catheter malfunction	Prepare new drug and check catheter patency
	Low number of rewards but high number of active lever presses	Drug syringe is empty, or rats are disconnected from the drug lines	Change the syringes each time they fall to below ~9 ml. Flush the catheter and reconnect the rats
22	Missing trials during the session	Rats crossing the two sides of the chamber or not enough space for social interaction	Re-create the barrier (as described above)
		Levers, cues, or door malfunction	Change levers, cues, or door

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 3 cm in diameter. Some of the rats (especially males) would become stuck in the holes. We had to manually free the rats by breaking the plastic screen	The rats did not reliably perform the operant task. Therefore, we had missed sessions/trials during social self-administration (in one case) and choice (in another case) experiments. This is because the experience of being stuck in the holes was extremely stressful for the rats and they stopped pressing the lever
On one occasion the guillotine door was not properly working. The gears inside were spinning and the door was not opening, resulting in extremely loud noise for the duration of the experiment	The rat was too scared to perform the task for the next 3 d

Timing

The duration of an entire social-choice self-administration protocol, including acclimation and separation of drug users and social partners, is ~8–9 weeks.

Stage 1—Steps 1–10, construction of social-choice self-administration apparatus: 3–6 d per chamber, depending on experience

Stage 2—Steps 11 and 12, social housing and separation of rats: ~2 weeks for acclimation to the new colony and social housing, and 1 week of single housing
Stage 3—Steps 13–15, social self-administration: 6 d
Stage 4—Step 16, surgery: 1 d plus 3–4 days of recovery
Stage 5—Steps 17–19, drug self-administration 12 d
Stage 6—Steps 20 and 21, relapse or incubation test on day 1: 1 d
Stage 7—Steps 22–24, voluntary abstinence: 10 sessions over 14 d
Stage 8—Step 25, relapse or incubation test on day 15: 1 d

Anticipated results

We report the social self-administration data as number of rewards (equal to successful trials) that rats earn during the 120-min daily sessions. We then report the number of drug infusions earned by the rats during the 6-h self-administration sessions. For the voluntary abstinence phase, we report the number of social rewards and drug infusions earned during the 10 discrete-choice sessions. Finally, for the relapse or incubation tests, we report the number of previously active lever presses during the sessions. We use factorial ANOVA and *t*-tests using SPSS (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's protected least significant difference (PLSD)). For choice data, the statistical analyses are performed on a social preference ratio score (number of social rewards/[number of social rewards + number of drug (or palatable pellet rewards; see below) infusions])[27]. Usually, we do not present the inactive lever data in figures, because response on this lever during the any stage of the protocol is very low (although we typically report the range of inactive lever presses for each experiment). Moreover, in our studies we do not observe sex differences during social or drug self-administration, social preference over drug during the voluntary abstinence phase, or for the relapse/incubation tests[7,27]. We do not use statistical methods to predetermine sample sizes, and our sample sizes are similar to those reported in previous publications[7,27].

Using this protocol, we have shown that social choice–induced voluntary abstinence prevents incubation of methamphetamine craving[7] (Fig. 3a) and reduces incubation of heroin craving[27] (Fig. 3b).

Here, we also provide unpublished data (M.V. and Y.S.) to show generalization of our protocol to Long–Evans rats (Exp. 1, $n = 8$ male drug users and $n = 8$ male social partners, weighing 150–175 g upon arrival) and the opioid drug remifentanil. A timeline of the experiment is reported in Fig. 4a. The rats reliably pressed a lever for social interaction (Fig. 4b). The analysis of the number of operant social interactions showed a significant main effect of session ($F_{7,49} = 5.1$, $P < 0.001$; partial eta$^2 = 0.4$). The rats also reliably pressed a lever for remifentanil infusions (Fig. 4b). The analysis of the number of infusions showed a significant effect of session ($F_{7,49} = 15.8$, $P < 0.001$; partial eta$^2 = 0.7$). We then tested the rats during 10 discrete-choice sessions, and they showed strong preference for social interaction over remifentanil (Fig. 4c). The analysis of the social preference score showed a significant effect of session ($F_{4,28} = 0.4$, $P = 0.8$; partial eta$^2 = 0.05$).

Our protocol can be adapted to study addiction vulnerability. On the basis of our original findings with methamphetamine[7], in Exp. 2, we tested whether rats would reverse their social preference over heroin by introducing a delay of the social reward during the choice sessions. A timeline of the experiment is shown in Fig. 5a. The rats ($n = 12$ female drug users and $n = 12$ female social partners, weighing 150–175 g upon arrival), increased the number of social rewards and heroin infusions over time (Fig. 5b). The analysis of the number of social-reward and heroin infusions showed a significant effect of session ($F_{5,55} = 16.6$, $P < 0.001$; partial eta$^2 = 0.6$; and $F_{5,55} = 2.8$, $P = 0.03$; partial eta$^2 = 0.2$, respectively). We then tested the rats during 12 discrete-choice sessions. After the first three choice sessions, we introduced a 60-s delay for the social reward but not for heroin. This manipulation decreased rats' preference to ~50% (Fig. 5c). The analysis of the social preference score showed a significant effect of session ($F_{11,121} = 13.2$, $P < 0.001$; partial eta$^2 = 0.6$). However, this reversal occurred with different magnitude in different rats, showing individual variability in delayed social choice (Fig. 5d).

Our protocol can also be adapted to study motivational aspects of social reward. In Exp. 3, we directly compared the rats' motivation to seek two natural rewards (e.g., palatable food and social interaction) using our choice procedure and progressive ratio reinforcement schedule. A timeline of the experiment is shown in Fig. 6a. Non-food-deprived rats ($n = 3$ male reward users and $n = 3$ male

social partners, weighing 250–275 g upon arrival) increased both the number of palatable food pellet rewards and the number of social rewards over time (Fig. 6b; Session: $F_{5,10} = 3.9$, $P = 0.03$; partial eta$^2 = 0.7$; and $F_{5,10} = 50.2$, $P < 0.001$; partial eta$^2 = 0.9$, respectively). We then tested the rats during five discrete-choice sessions showing that two out of three rats preferred social interaction over palatable food pellets (Fig. 6c). Then we tested the rats' motivation to seek the two rewards using a progressive ratio reinforcement schedule and we found no differences between the two reward types ($P > 0.05$; Fig. 6d).

Our protocol can also be adapted to study more broadly social reward and non-reinforced social seeking. In Exp. 4, we tested whether rats would perform stable social self-administration for longer periods (12 d) than the usual 6–8 sessions and if they would show social seeking after a short abstinence period (1 d). A timeline of the experiment is reported in Fig. 7a. The rats ($n = 12$ drug users (6 females and 6 males) and $n = 12$ social partners (6 females and 6 males), weighing 150–175 g upon arrival), increased the number of social rewards over time (Fig. 7b; Session: $F_{11,110} = 9.0$, $P < 0.001$; partial eta$^2 = 0.5$). One day after social self-administration training, the rats showed robust non-reinforced social seeking (Fig. 7c). The analysis showed a main effect of Lever (inactive, active): $F_{1,10} = 53.7$, $P < 0.001$; partial eta$^2 = 0.9$. During this phase, we presented the cues and the lever previously paired with social self-administration, but there was no social interaction and the door remained closed during the test. For both social self-administration and non-reinforced social seeking, we did not observe sex differences ($P > 0.05$).

Taken together, these data show that our protocol is flexible and can be extended to different strains of rats and different drugs of abuse. Our data also show that the protocol can also be used to study addiction vulnerability and the mechanisms of social reward and social reward seeking.

Reporting Summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data and code availability

The Med-Associated programs are available from the corresponding authors (M.V. and Y.S.) upon request.

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Author contributions

M.V. and Y.S. designed the experiments; M.V. ran the experiments and collected the data; M.V. and Y.S. analyzed the data; M.V. and Y.S. wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	med-associated; excel;GraphPad Prism
Data analysis	SPSS

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Materials, datasets, and protocols are available upon request from Marco Venniro (venniro.marco@nih.gov) or Yavin Shaham (yshaham@intra.nida.nih.gov).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size is based on previous behavioral studies in our lab. We also report post-hoc power values (Eta2).
Data exclusions	N/A
Replication	We replicated the main findings in previous publications (Venniro et al., 2018 Nat. Neurosci. and Venniro et al., 2019 Bio. Psych.) and also with the new data added in the current manuscript.
Randomization	Initially, we randomly assigned rats to the "Resident (drug user)" and "Social partner (drug naïve)" groups. For testing, We matched the rats in the different groups for drug intake during the training phase.
Blinding	N/A

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	We reported data from published manuscripts (Venniro et al., 2018 Nat. Neurosci. and Venniro et al., 2019 Bio. Psych.). For the new data reported here we used: Exp. 1: 16 male Long Evans rats (Charles River) (8 drug users and 8 social partners), weighing 150–175 g upon arrival; Exp. 2: 24 female Sprague Dawley rats (Charles River) (12 drug users and 12 social partners), weighing 150–175 g upon arrival; Exp. 3: 6 male Sprague Dawley rats (Charles River) (3 rewards users and 3 social partners, weighing 250–275 g upon arrival. Exp. 4: 24 male and female Sprague Dawley rats (Charles River) (12 drug users - 6 males and 6 females - and 12 social partners - 6 males and 6 females), weighing 150–175 g upon arrival. Our procedures followed 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). This study has been approved by the NIDA IRP Animal Care and Use Committee.
Wild animals	No wild animals were used in the study.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Our procedures followed 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). This study has been approved by the NIDA IRP Animal Care and Use Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.