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Operant Self-medication for Assessment of Spontaneous Pain Relief and Drug Abuse Liability in Mouse Models of Chronic Pain

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Abstract

The search for safe and efficient chronic pain treatments is dampened by the lack of reliable models that faithfully reproduce current pharmacological treatments for chronic spontaneous pain in humans. Preclinical models often assess the antinociceptive efficacy of non-contingent pharmacological treatments evaluated in the short-term. Here, we provide a protocol of contingent operant self-medication in mice, which allows the estimation of spontaneous pain relief and drug abuse liability in models of persistent pain. This paradigm requires preliminary habituation and animal handling, followed by training of mice in operant conditioning boxes, to allow subsequent analgesic drug self-administration. After the initial acquisition of food-maintained operant behavior, a chronic pain sensitization is induced. Posterior intravenous jugular catheterization and coupling of operant conditioning boxes to perfusion pumps allow quantification of operant responding for intravenous drug self-administration. All mice show an initial operant drug self-administration behavior associated with the previous food-maintained operant training. This initial operant responding is extinguished after administration of ineffective treatments, but continues when the compounds have analgesic efficacy or intrinsic reinforcing properties. The identification of a significant drug self-administration selectively expressed in mice exposed to the chronic pain condition is indicative of analgesic drug effects, whereas persistent self-administration in control mice is indicative of abuse liability. The present protocol provides the behavioral and surgical procedures needed to assess spontaneous pain relief and potential for abuse of pharmacological treatments, through contingent analgesic self-medication in mice.

Keywords: Self-Administration, Neuropathic pain, Analgesic, Sedative, Drug abuse liability, Operant behavior, Partial sciatic nerve ligation

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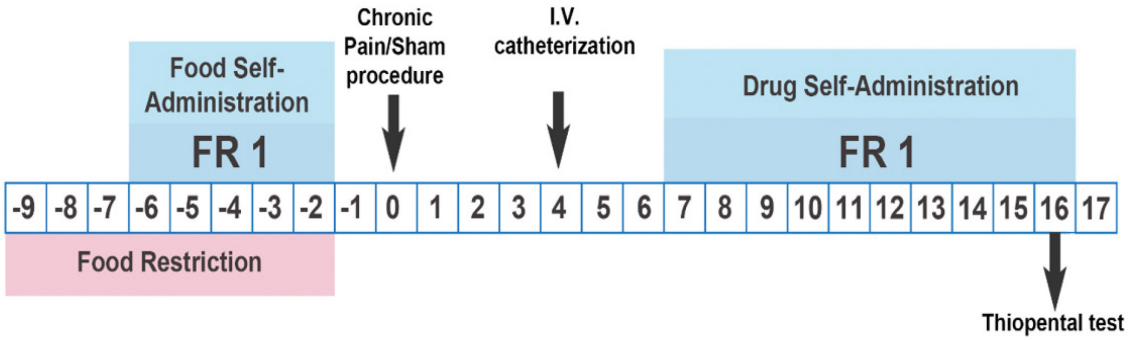
Graphic abstract:



Experimental design.
Animals are subjected to a 5-day food self-administration protocol with a fixed ratio of reinforcement of 1 (FR1, 1 interaction with the active nose-poke causes the release of 1 reinforcer/infusion), to acquire the operant behavior. After this training, mice are subjected to the chronic pain or sham procedure, and four days later an intravenous (i.v.) catheterization is performed, to allow self-administration with the selected compound or its vehicle. Three days after the catheterization, animals start the drug/vehicle self-administration protocol at FR1. The patency of the catheter is evaluated with the thiopental test after the last self-administration session. Adapted from Bura *et al*. (2018).

Background

Chronic pain comprises a complex symptomatology that can include signs of gain of function, such as hyperalgesia and allodynia, signs of loss of function, like paresthesias or dysesthesias, and emotional disturbances, which may lead to anxiety, fear, and depression. However, the cardinal symptom of chronic pain syndromes is continuous or paroxysmal spontaneous pain (Mogil, 2009). Unfortunately, widespread use of opioid and non-opioid drugs with abuse potential has created additional problems associated with tolerance and dose escalation for chronic pain patients, that may lead to drug addiction and overdose-related deaths (Wilson-Poe and Morón, 2018). Spontaneous pain has been estimated in rodent models through different approaches, such as the grimace scale for facial expression, measurement of ultrasound vocalizations, home cage monitoring, and changes in wheel running, burrowing, or nesting (Tappe-Theodor *et al*., 2019). These methods have been used for the evaluation of acute spontaneous pain, but their applicability for the study of chronic pain has been scarce and these techniques also fail to assess the abuse potential of analgesic drugs. Conditioned place preference or conditioned place aversion are useful to study context-dependent associations with drugs of abuse and also with painful or pain-relieving stimuli. However, these other paradigms involve non-contingent drug treatments and do not mimic the drug-taking behavior that currently involve chronic pain treatments. Operant drug self-administration paradigms faithfully reproduce this human behavior and, when applied to chronic pain models, can be useful to identify the ability of analgesic drugs to elicit a voluntary drug-taking behavior selectively associated with relief of spontaneous pain (Cabañero *et al*., 2020). The use of appropriate control groups not suffering from chronic pain in the same experiments allows the identification of reinforcing properties related to psychotropic drug effects at the same time (Bura *et al*, 2018), in a method that showed high translatability to humans in drug addiction research.

The present protocol describes the materials and methods needed to conduct analgesic drug self-administration in mice subjected to chronic pain models with the expected purpose of alleviating spontaneous pain, with special focus on handling methodology, surgical manipulations, and data interpretation. The procedure will be useful for models that provide a persistent and stable pain sensitization, but the data will be difficult to interpret when the pain sensitization is unstable or self-limiting. For this reason, this procedure has been useful in a model of neuropathic pain induced by partial sciatic nerve ligation (PSNL) (Bura *et al*., 2013 and 2018; Cabañero *et al*., 2020), but should also be useful in other neuropathic pain models, such as chronic constriction injury (CCI), spared-nerve ligation

(SNL), or diabetic neuropathy, in models of osteoarthritis pain and other models induced by chemicals, viruses, illnesses, or genetic alterations that promote sustained pain sensitizations. Thus, the present methodology will identify significant self-administration to alleviate chronic spontaneous pain, whenever drug-taking behavior is selectively expressed in mice subjected to the chronic painful condition. On the other hand, it will also detect abuse potential of the evaluated drugs when control mice without the chronic condition develop persistent or escalating drug self-administration. Taking into consideration the frailty of chronic pain patients, perhaps the efficacy and safety of every chronic pharmacological treatment should be screened through operant self-medication models before the translation of potential treatments to the clinic.

Materials and Reagents

Materials

1. Mice: 6–8 week-old C57BL/6J male or female mice (Charles River, France or any company that provides mice for laboratories)
2. Suture thread (black braided silk, TB10, 3/8 TRIANG 15 mm 4/0 90 cm, LorcaMarín, Spain, catalog number: 55327-50U)
3. 22 Gauge ID39 tubing OD71 (Bilaney Consultants GmbH, catalog number: C313G-5up/SPC)
4. 6.5 cm of Silastic® laboratory tubing 0.3 mm/0.64 mm (Dow Corning Corporation, USA, catalog number: 508-001) (Tube 1)
5. 1.5 cm of Silastic® laboratory tubing 0.64 mm/1.9 mm (Dow Corning Corporation, USA, catalog number: 508-003) (Tube 2)
6. Polyethilene tubing (PE-20, Plastics One, UK, catalog number: C315CT)
7. 20 Gauge 1½ inch needles (BD Microlance 3, #301300, BD, USA)
8. Ceys® acid silicone (Olivé Química, catalog number: 600F)
9. Catheter mold for mouse (CBX Corporation 5WELL, REF: CM-1)
10. Tulle net (63_08922_002, https://www.telas.es)
11. Dentalon Plus liquid resine (250 mL, Heraus Kulzer, catalog number: 65041138)
12. Dentalon Plus resine powder (100 g, Heraus Kulzer, catalog number: 65041065)
13. (R)-(+)-Limonen 97% (100 mL, Sigma-Adrich, Spain, catalog number: 183164)
14. Standard 20 mg food pellets for self-administration (Testdiet, Richmond, IN, USA, catalog number: 1811142)

Reagents

1. Distilled water
2. Ethanol 70%
3. Iodine (Betadine, 500 mL, MEDA Pharma S.A.U., Spain, catalog number: 716720)
4. Physiological saline (0.9% NaCl, 250 mL, Laboratorios ERN, Spain, catalog number: 999790.8)
5. Glucose serum (GlucosaVet 5 g/100 mL, B. Braun Vet Care, Spain, catalog number: 1248ESP)
6. Sodium heparin (Heparina Hospira 5%, Hospira, Pfizer, USA, catalog number: 654753.3)
7. Ophthalmic ointment (Xilin Night, 5 g, Visufarma, Spain, catalog number: 2919-PS-CM)
8. Blastoestimulina (1%, 30 g, Almirall, Spain, catalog number: 719385)
9. Virkon™ S (Laboratorios Zotal, catalog number: 0065-P)
10. Anesthesia reagents
    a. Ketamine hydrochloride (75 mg/kg of body weight, 10 mL, Ketamidor, Richterpharma ag, Austria, catalog number: 580393) dissolved in sterile 0.9% physiological saline
    b. Medetomidine hydrochloride (1 mg/kg of body weight, Domtor; Esteve, Spain, catalog number: 570686) dissolved in sterile 0.9% physiological saline
    c. Atipamezole hydrochloride (2.5 mg/kg of body weight, Revertor; Virbac, Spain, catalog number: 570559) dissolved in sterile 0.9% physiological saline
    d. Gentamicine (1 mg/kg of body weight, Genta-Gobens; Laboratorios Normon, Spain, catalog number: 999037) dissolved in sterile 0.9% physiological saline

e. Meloxicam (2 mg/kg of body weight, Metacam; Boheringer Ingleheim, Rhein, catalog number: 059/02/08CVFPT) dissolved in sterile glucose serum
11. Thiopental (Tiobarbital, 0.5 g, B. Braun, Barcelona, Spain)
12. Antibiotic ointment (Bactroban, GlaxoSmithKline, Madrid, Spain)
13. Heparin 0.0003 mg/mL (see Recipes)
14. Ketamine (7.5 mg/mL) + Medetomidine (0.2 mg/mL) (see Recipes)
15. Atipamezole 1 mg/mL (see Recipes)
16. Gentamicin 0.3 mg/mL (see Recipes)
17. Meloxicam 0.5 mg/mL (see Recipes)

Equipment

1. Fine scissors 105 mm-4¼ (Allgaier Instrumente, Germany, catalog number: 03-320-105)
2. Curved forceps (Allgaier Instrumente, Germany, catalog number: 08-421-100)
3. Bishop-Harman forceps serrated 0.5 mm (Agnthos, Sweden, catalog number: 11069-08)
4. Graefe forceps straight serrated 0.8 mm (Agnthos, Sweden, catalog number: 11050-10)
5. 2× Dumont #5 forceps (Fine Science Tools, Germany, catalog number: 11251-10)
6. Rolled cotton 100% (Acofarma, Acofar®, catalog number: 4957051)
7. Weighing scale
8. Mouse operant self-administration chambers (Med Associates, Georgia, VT, USA, model: ENV-307A-CT). The operant chambers (Figure 1) are equipped with two nose-poke holes (holes of 1.2 cm diameter; Figure 1C: red circles), one selected as the active one and the other as the inactive. Pressing on the active nose-poke results in the delivery of the reinforcer paired with a stimulus-light (2 s, associated-cue, #ENV-321M, Med Associates; Figure 1C: orange circles) located above the active nose-poke, while nose-poking on the inactive one has no consequences. During the acquisition of the operant behavior, the animal nose-poke on the active hole results in the delivery of a pellet (standard pellet) from a food dispenser (#ENV-303M pellet receptacle, #ENV-203M-20, modular pellet dispenser, Med Associates) equidistant between the two holes. Once acquired, the reinforcer is changed by an infusion of the studied drug. Pump noise and a stimulus-light located above the active hole are paired with the delivery of the infusion. Chambers have grid floors with a magazine with bedding below, and are housed in sound- and light-attenuated boxes equipped with fans, to provide ventilation and ambient noise. When mice respond on the reinforced hole, the stimulus light is lit and an infusion is delivered via a syringe mounted on a micro-infusion pump (PHM-100A, Med Associates, Inc., Georgia, VT, USA; Figure 1B) connected via Tygon tubing [0.96 mm outer diameter (o.d.), Portex Fine Bore Polythene Tubing, Portex Limited, Hythe, Kent, UK] to the mouse intravenous (i.v.) catheter (Figure 3F) through a single channel liquid swivel (Polysulfone 22 GA single channel disposable swivel 610014, Harvard apparatus/Panlab, Barcelona, Spain).
9. Cold light (Leica C.L.S. 150×, Leica Microsystems, Spain)
10. Heating pad (#N2P 220-230, 60W, 50Hz, Daga, Spain)
11. Anesthesia circuit, including isoflurane vaporizer (Midmark, Isoflurane 3000®-Well-Fill, #91305430), tubing, regulating valves, nose cones, and induction chamber (Midmark, #93805108)
12. Electric hair-clipper (BBraun, Aesculap®, model: ISIS, #GT241)

Software

1. Med-PC IV Software (Med Associates Inc, USA). Software for registration of operant behavior.
2. Excel (Microsoft, USA) or GraphPad Prism software (GraphPad Software, USA) for graph construction.
3. SPSS software (IBM, version 25) to conduct statistical analysis.

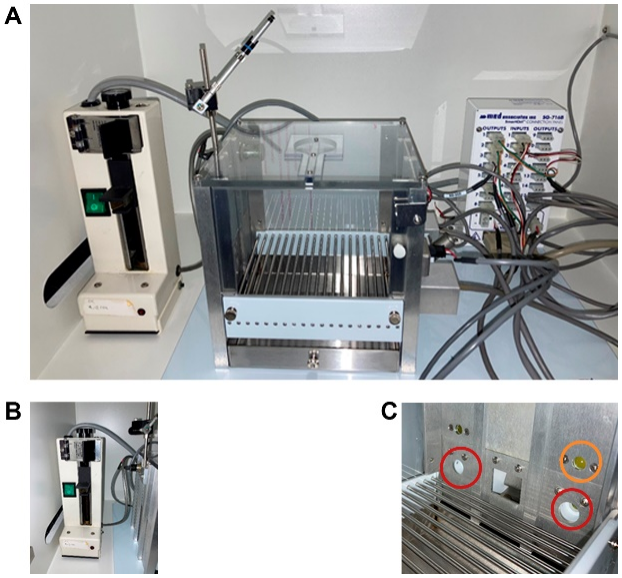


Figure 1. Operant self-administration chamber.
The operant chamber (A) is equipped with a pump (B) and a food pellet dispenser, two nose-pokes (C) (active and inactive: red circles) and two stimulus lights (orange circles).

Procedure

A. Animal Handling

1. Individualize the mice in cages with food and water available *ad libitum* and house them in a room with an inverted light cycle (light phase from 8:00 p.m. to 8:00 a.m. and dark phase from 8:00 a.m. to 8:00 p.m.). Ideally, this room will be the same as the room housing the self-administration chambers. The complete experimental procedure will take place during the dark phase.
2. For best results, keep the same experimenter during the full experimental sequence. This will facilitate habituation and will decrease stress in mice. Silence and soft movements are mandatory to decrease the alertness of the mice throughout the whole procedure.
3. Habituate the mice to the experimenter for 7 days, as follows. Every day, the experimenter will handle each mouse for 2 min, grabbing them gently by the tail with the lowest force possible and placing them on the arm that will be used to move the mice from their cage to the weighing scale and afterwards to the self-administration boxes. Aggressive behavior should decrease dramatically after the first 2 habituation sessions.

B. Food Operant Training

1. On the last day of habituation to the experimenter, mice are weighed and the standard food is removed from the home cage to achieve a 10% reduction in body weight on the following day. Three standard food self-administration pellets will be placed in the home cage to provide minimal food consumption and decrease neophobic behavior during food self-administration sessions. During the following days, pre-weighed standard pellets of 1, 1.5, 2, or 2.5 g are left together with the self-administration pellets in each home cage once a day, to maintain 90% of the initial weight. Hence, mice are weighed every day.
2. At this point, add the following manipulation before weighing the animal: Place a tissue paper over the palm of the hand. Grab the mouse by the tail with the other hand and place the mouse over the tissue paper. Close the hand holding the tissue paper, wrapping the mouse for 5 s. Release the mouse and place it in the weighing scale. This will habituate the mice to the manipulation needed to place the tube on the catheter for intravenous drug self-administration.

3. Pre-weighed standard pellets of 1, 1.5, 2, or 2.5 g are left in each home cage once a day, to maintain 90% of the initial weight for each individual mouse. Animals keep receiving 3 standard food self-administration pellets on their cage every day.
4. Three days after starting food deprivation, mice start training in the mouse operant chambers to nose-poke for food pellets. This training will consist of daily 1-h sessions and will take place at the same hour every day for 5 days. Before starting the food self-administration sessions, it is important to double check the functioning of the chambers, including home lights, cue lights, nose poke sensors, and pellet delivery systems. After the sessions, the chambers must be cleaned and the food magazine emptied before the following session. The food deprivation regime is maintained during the whole period of evaluation of the operant behavior. Water is available *ad libitum* during the whole experiment, except during the operant sessions.
5. The start of the food self-administration session is signaled with the lighting of the house light placed on the ceiling of the box during the first 3 s. This light is then turned off during the remaining duration of the session. Immediately after this event, the cue light of the active nose poke is also lighted, and one priming pellet is delivered in the food magazine to remind the functioning of the chamber.
6. Once the session is started, a fixed ratio 1 schedule of reinforcement (FR1) is used during the whole training period (one nose-poke on the active hole results in the delivery of one reinforcer, together with a light stimulus for 2 s). Thus, nose-poking the active hole results in the lighting of its corresponding cue light for 2 s and the delivery of one food self-administration pellet in the central magazine, whereas nose-poking the inactive hole has no consequences. Since the door of the chamber is placed in one side of the box, one of the two holes is closer to the door. To avoid a possible bias associated with this lack of symmetry, it is important to counterbalance the composition of the experimental groups to have similar amounts of mice nose-poking to active or inactive holes on each side of the box.
7. The delivery of reinforcers is followed by a timeout period of 10 s, where no cues are presented, and no reward is provided following active nose-pokes.
8. Food self-administration sessions last 1 h, or until the mouse nose-pokes 100 times on the active hole, whichever happens first. These sessions are carried out at the same hour for 5 consecutive days. Active and inactive nose pokes are recorded.
9. Animals are weighed after every food training session. Pre-weighed food pellets of 1, 1.5, 2, or 2.5 g are left in their home cage to maintain the 90% of the initial weight. After the last food training session, the food restriction ends and *ad libitum* access to food is provided again.

C. Catheter Preparation

1. File the cannulas (22-gauge tubing) at their long end until blunt (Figure 2A).
2. Twist the long end of the cannula with a clamp, making a 90º angle (Figure 2B).
3. Cut 6.5 cm of silastic tubing #508-001 (tube 1) and 1.5 cm of silastic tubing #508-003 (tube 2). These measurements are the appropriate ones for a 7-week-old C57BL6/J male mouse.
4. Soak tube 1 in limonene 97% and let it expand for 15 min.
5. Make a little ball (0.2–0.5 cm in diameter) with silicone and place it 1.5 cm from the tip of tube 1. This will be the edge that will allow fixing tube 1 to the jugular vein and will avoid the ejection of the catheter during implantation and afterwards.
6. Insert silastic tube 1 to cover completely the long side of the cannula and let it dry for at least 1 h.
7. Seal the cannula-tube 1 junction with silicone (Figure 2C).
8. Soak tube 2 in limonene 97% and let it expand for approximately 20 min.
9. Insert silastic tube 2 to cover the long side of the cannula, also covering the junction between the tube 1 and the cannula, and let it dry overnight. This is important to protect the tube 1 from the cement applied on the next steps.
10. After preparing the mold (Figure 2D), place the cannula with the tubes into the mold (Figure 2E), and close it tight (Figure 2F).
11. Prepare dental cement as indicated by the manufacturer and fill the mold. The amount of cement to be used

is important, since if there were excess cement it would be annoying for the mouse; for this reason, do not overfill the mold, just adjust the amount to the form of the mold.

12. Put a piece of tulle of 1 cm x 1 cm covering the bottom side of each catheter, and let it dry for 10–15 min (Figure 2G).
13. Remove the catheters from the mold.
14. Clean possible excess of dental cement.
15. The catheter should be like Figure 2H.
16. Let it dry for 2 days.

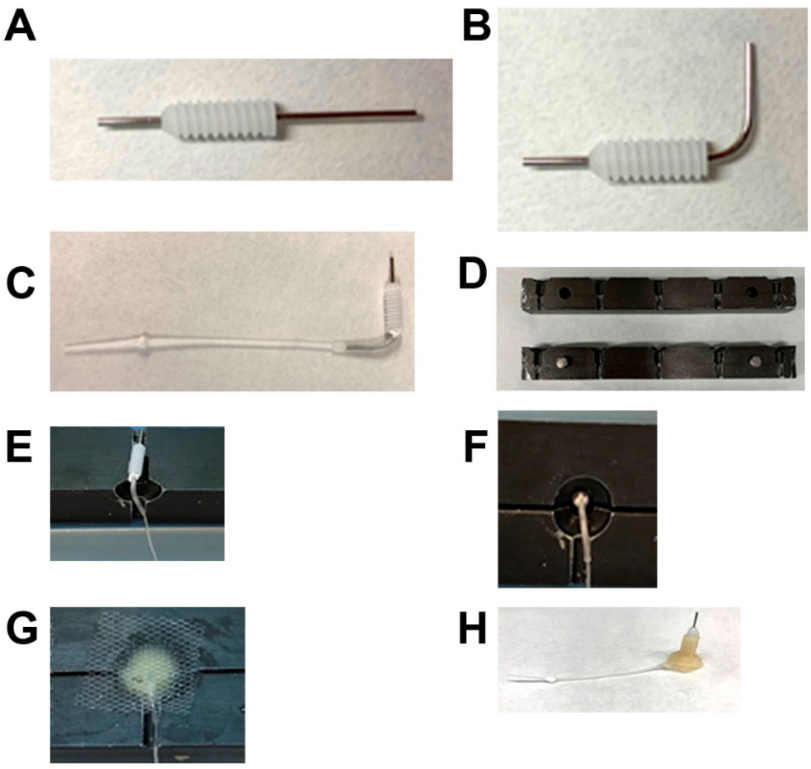


Figure 2. Cathether fabrication.
After filing the cannulas (A), twist them making a 90º angle (B) and join the two different silastic to the cannula (C). After opening the mold (D), place the cannula into the mold (E) and close the mold tight (F). Fill the mold with dental cement and place a piece of tulle in the bottom of the catheter (G). The cement should dry and the excess cleaned until the catheter looks like (H).

D. Surgery for Drug Self-administration

1. Anesthetize the mouse with ketamine/medetomidine solution 10 mL/kg *i.p.*
2. Spread xilin over the mouse's eyes to keep them moist during the surgical procedure.
3. Shave the chest and the back of the mouse, and use cotton swabs to spread ethanol over the skin, followed by application of iodine over the same skin surface.
4. Look for the interscapular zone on the back of the mouse and make a 1–2 cm incision through the skin, parallel to the spine. Use scissors to separate the skin from the underlying muscle and make room for the catheter.
5. Use the curved forceps to separate the skin from the muscle in the area where the tube 1 cannula will be placed (on the right shoulder).

6. Turn over the animal and locate the heartbeat in the chest. With the forceps, grasp the skin above the heartbeat location on the superior right side of the chest, where the jugular vein is located. Make an incision on the skin and perform blunt dissection towards the path made from the back with the curved forceps on the right shoulder.
7. Insert the forceps going through the incision of the chest until a hole in the back, and pass the catheter through the open path over the shoulder. It is important to double check that the catheter is correctly sealed by infusing heparin (0.1% in saline) through it.
8. Find and dissect the jugular vein eliminating the surrounding fat and muscle fibers.
9. Once the jugular vein is exposed, pointed forceps (Dumont forceps) are inserted under the vein from the right side of the mouse and other pointed forceps are inserted from the left side. Rotate these two forceps to separate the vein from the surrounding tissue (Figure 3A).
10. Pass a bent silk suture thread under the vein from the left to the right side. Cut the bent silk along the fold to generate two different silk segments. Tie the lower thread loosely around the right leg of the animal. In the upper thread, make a simple knot with a firm movement, to cut off the blood supply (Figure 3B).
11. Locate a straight area in the vein path to perform the catheterization and pierce the vein with a 20 G 1½ needle. Remove the needle and insert the catheter guide as far as possible into the hole made by the needle (Figure 3C).
12. Slide the catheter silastic tube into the jugular vein until reaching the silicone ball of the catheter. Check the entry of blood to the catheter to confirm correct placement (Figure 3D).
13. Tighten the lower knot to secure the catheter and administer 0.2 mL of heparin (0.1% in saline), to keep the catheter full of liquid (Figure 3E).
14. Tie with additional knots the upper and lower threads to secure the catheter. Then make two additional knots with the ends of the threads on the left and two extra knots with the ends of the threads on the right.
15. Insert the catheter tubing 1.5 cm into the right jugular vein and anchor with suture. The remaining tubing runs subcutaneously to the cannula, which exits at the midscapular region.
16. Suture the chest and coat with antibiotic ointment (Bactroban, GlaxoSmithKline, Madrid, Spain).
17. Infuse 0.025 mL of heparin (0.3 mg/kg) and close the short side of the cannula with a cap, to avoid the inclusion of air. Place the catheter tulle under the skin, suture the skin on top of the catheter to fix the tube, and close it tightly (Figure 3F).
18. Spread blastoestimulin on the sutured areas. Administer atipamezole (2 mg/kg) *i.p*. followed by gentamicin (1 mg/kg), meloxicam (2 mg/kg), and glucose serum s.c (0.9 mL).
19. After surgery, animals are allowed to recover for 3 days prior to initiation of the self-administration sessions.
20. The patency of the i.v. catheters is evaluated after the last self-administration session and whenever the behavior appears to deviate dramatically from that observed previously. This patency is assessed by infusion of thiopental (Tiobarbital 0.5 g B. Braun, Barcelona, Spain) through the catheter (1.25 mg/kg). When prominent signs of anesthesia are not apparent within 3 s of the infusion, the mouse must be removed from the experiment. The success rate for the maintenance of the patency of the catheter of a procedure with a duration of 12 days should be higher than 80%.

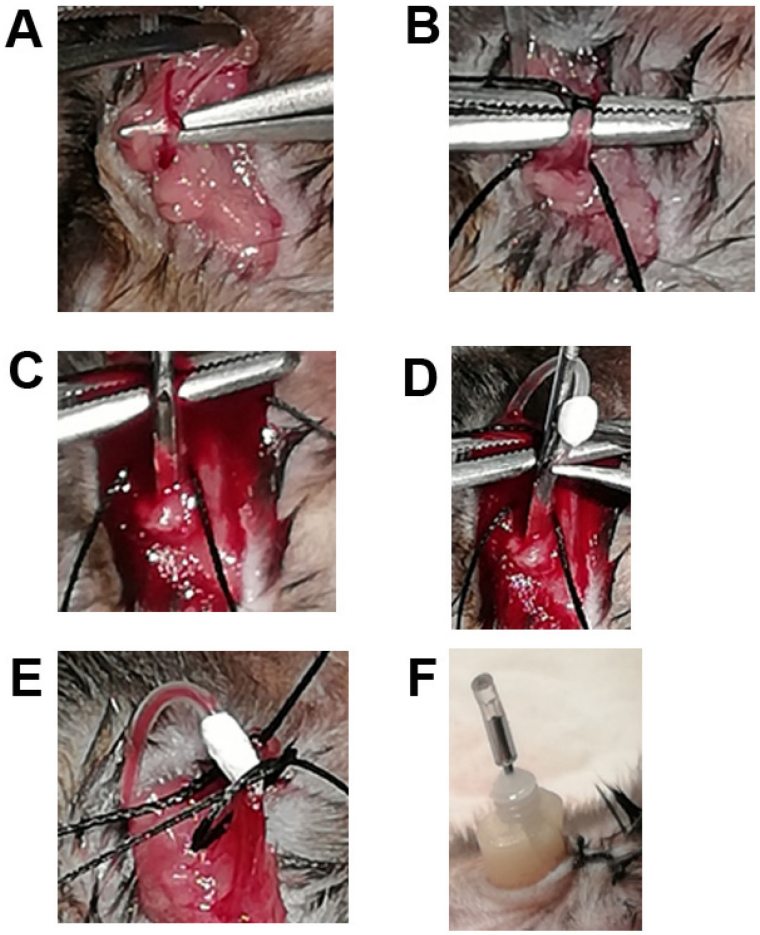


Figure 3. Surgical procedure for intravenous jugular catheterization.
Once the jugular vein is localized (A), two threads are tied around the vein (B), using the upper thread to cut blood supply. With the help of a 20 G 1½ needle the vein is pierced (C) and the silastic tube slides into the vein until reaching the silicone ball of the catheter (D). Then, after checking the entry of blood to the catheter, the lower knot is tightened to secure the catheter and heparin is administered to keep the catheter full of liquid (E). After having secured the catheter with a net of sutures, the catheter tulle is placed under the skin and the skin is sutured on top of the catheter to fix the tube (F).

E. Software Preparation before Self-medication Session

1. Open Med-PC IV Software.
2. Click on "File" and "Open Session". In this window, the experimental procedure must be selected (Procedure) (Figure 4A). The correct procedure file for each box should be selected considering the placement of the active nose-poke (right or left) (Figure 4B). Select the boxes that will be starting the self-administration session and press OK. This must be done for each box participating in the session.
   The ID of the study animal, the name of the experiment, the session, and the experimental group can be introduced, as well as a filename for the resulting data file (Figure 4B). The procedure is the programming code by which the program is governed to perform the desired actions (which are at least the active and inactive nose-poking, duration of the session, duration of the time-out, and activation of lights).
3. Click on "Configure" and "Signals". Select the box, choose the option *Issue START Command,* and press Issue (Figure 4C). This turns on the house light (Figure 4D) and, when the light turns off, the nose-pokes become active (Figure 4E).

A priming reinforcer is delivered in each session (Figure 4F). If no priming pellet was delivered, mark the option *Simulate Response*, select the correct active nose-poke (1= left, 2= right), and press Issue.

4. After the end of the session, the number of active and inactive nose pokes is recorded (Figure 4G). The data file is located on the Data folder of the Med-PC Software.

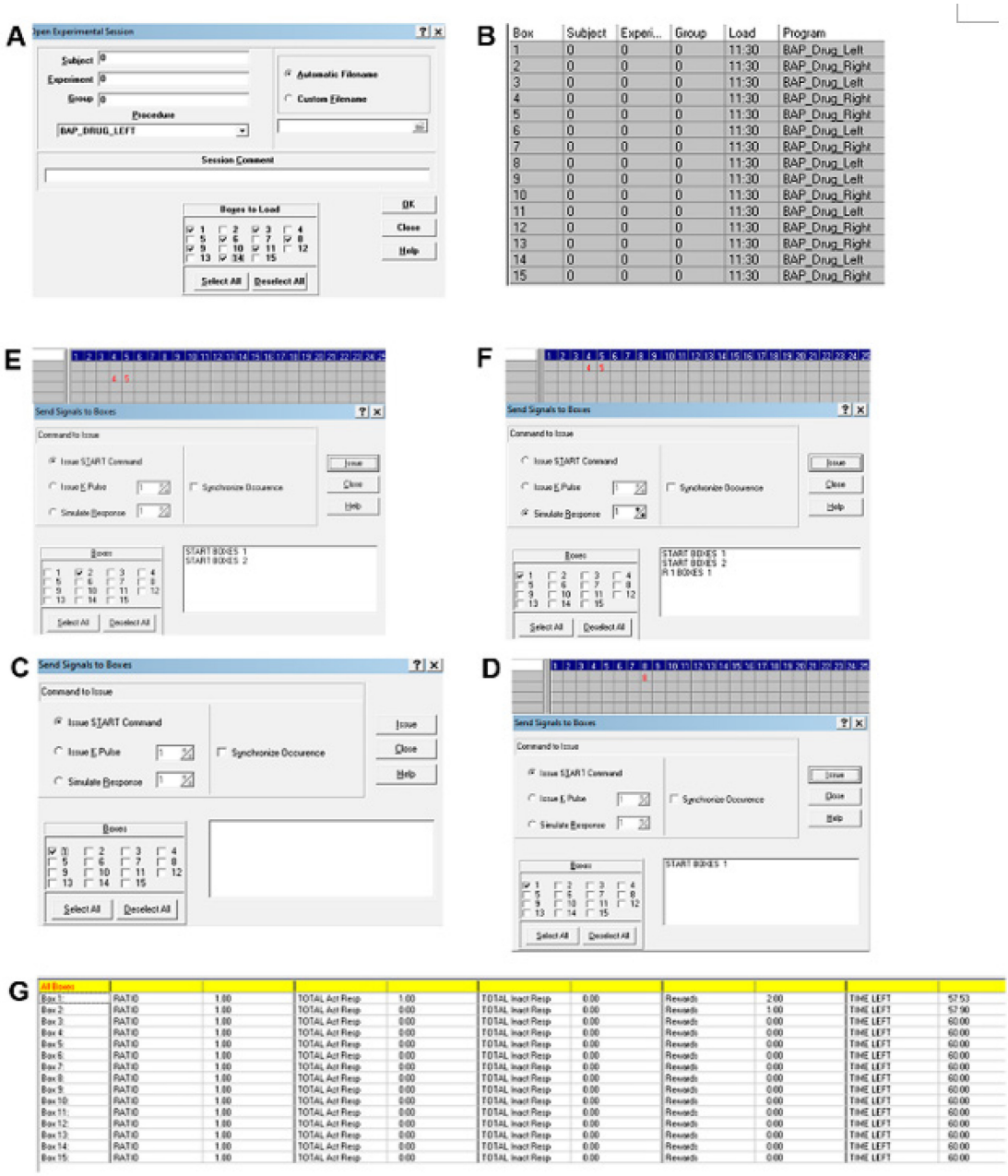


Figure 4. Med-PC IV software.

A) Selection of experimental procedure. B) Information showed in the display of the software; the selected procedure for each box can be observed on the right side, as well as the side of each active nose-poke (included in the name of the procedure). C) Preparation for the start of the procedure. D) Start of the procedure, where the display shows the light-house ON (number 8 marked in red). E) Start of the procedure, where the display shows the cue light ON and the pump ON (numbers 4 and 5 in red, respectively). F) Priming infusion given by the experimenter. G) Display of the data obtained during the session.

F. Drug Self-administration Procedure

1. After completion of food self-administration training, the pain animal model of chronic pain is conducted, and behavioral tests of nociception can be performed to confirm the induction of pain sensitization (Bura *et al*., 2018, Cabañero *et al*., 2020).
2. Once the pain sensitization is induced, the *i.v*. catheter is implanted to allow *i.v*. self-administration.
3. Three days after catheter implantation, mice start the drug self-administration sessions, where the food reinforcer is substituted by drug or vehicle infusions.
4. Self-administration sessions are conducted at the same hour every day and under a fixed ratio of 1 of reinforcement, *i.e*., one nose poke induces the delivery of one *i.v*. infusion. Sessions are conducted during 10–12 consecutive days, until vehicle-treated mice have reached a stable extinction of food-maintained operant behavior.
5. To prevent gas embolism, the pumps are manually adjusted, to remove the remaining air bubbles from the tubes that will bind the syringes loaded with drug or vehicle with the animals. This is done before placing the animals in the operant boxes, by manually rotating the pump knob to expel liquid through the tube until no bubbles are seen.
6. Mice are kindly immobilized as previously described (Section B, point 2), the catheter cap is removed, and the catheter is connected to the infusion pump with the primed tubes.
7. Sessions last 1 h or until 60 active nose-pokes, with active and inactive nose-pokes being recorded after each session.
8. A thiopental test is performed at the end of the experimental procedure to prove the patency of the catheter. Animals with a functional catheter must fall asleep after the infusion of 0.5 mL of thiopental; animals with a negative thiopental test are discarded.

Data analysis

The present model allows measurement of spontaneous pain relief and can detect drug abuse liability. During each operant session, the number of infusions along with active and inactive nose-pokes during the time-in are recorded. Active nose-pokes are the number of nose-pokes on the sensor that induces the delivery of drug or vehicle (Figure 5, filled squares/circles), whereas inactive nose pokes are the number of nose-pokes on the sensor that does not elicit any intravenous self-administration (Figure 5, empty squares/circles). To exclude interferences with food-driven operant behavior, data from the last drug self-administration sessions in which self-administration of vehicle is extinguished can also be analyzed separately. Figure 5A exhibits an example of self-administration data of a drug with pain relieving efficacy. This drug, JWH133, is an agonist of cannabinoid receptor type 2 (CB2), administered in volumes of 23.5 µL at a rate of 0.3 mg/kg/infusion. In figure 5A, it is dissolved in a vehicle containing 5% cremophor and 5% dimethyl sulfoxide. On the left panel of Figure 5A, nerve-injured animals exposed to the drug show an active responding (*Active JWH133* group) significantly different from that of nerve-injured mice exposed to the vehicle without drug (*Active Vehicle* group). On the right panel, drug or vehicle self-administration is extinguished in sham-operated mice (*Active Vehicle* and *Active JWH133* groups). Hence, mice show drug self-administration selectively expressed after nerve-injury, indicative of spontaneous pain relief. Occasionally, pain relieving drugs show abuse liability and can induce drug self-administration in control animals. Figure 5B shows self-administration data of the same JWH133 drug administered at the same dose, but with a vehicle containing 2.5% ethanol and 2.5% cremophor. In this case, the reinforcing properties of ethanol occlude the reinforcing effects of JWH133 in nerve injured mice, and animals receiving JWH133 (*Active JWH133* group) or vehicle (*Active Vehicle* group) show similar operant responding (Figure 5B, left panel), whereas control sham-operated mice do not extinguish the operant behavior (Figure 5B, right panel). This behavior strongly suggests abuse liability of the vehicle, due to the percentage of ethanol.

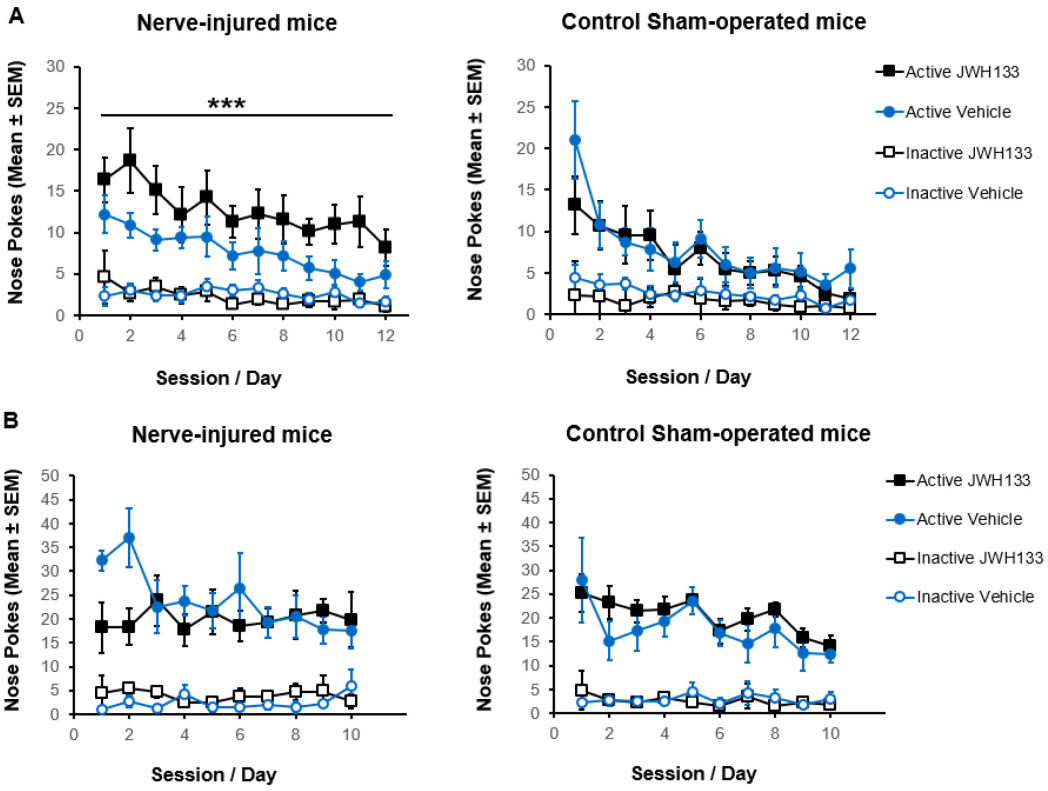


Figure 5. Self-administration of a CB2 agonist with pain relieving efficacy.
A. Self-administration of JWH133 0.3 mg/kg dissolved in a vehicle containing 5% cremophor and 5% dimethyl sulfoxide (adapted from Cabañero *et al*., 2020). B. Self-administration of JWH133 0.3 mg/kg dissolved in a vehicle containing 2.5% ethanol and 2.5% dimethyl sulfoxide. ***p<0.001 Active JWH133 in nerve-injured mice vs. Active Vehicle in nerve-injured mice (A). Nonsignificant differences between Active JWH133 and Active Vehicle in control sham operated mice (A, B). Nonsignificant differences between Active JWH133 and Active Vehicle in nerve-injured mice, associated with the reinforcing properties of the ethanol-containing vehicle (B). Nonsignificant differences between Inactive JWH133 vs. Inactive Vehicle (A, B, nerve-injured and control mice). Data for active and inactive responding in A and B were analyzed with IBM SPSS 19 (SPSS Inc, Chicago, IL) using a linear mixed model with three factors (surgery, day, and treatment) followed by Bonferroni post-hoc tests when appropriate. A diagonal matrix was chosen for the covariance structure of the repeated measures. Data are presented as mean ± SEM.

Notes

1. Mice should be monitored daily for at least 3 days after surgeries. If a mouse removes its skin sutures, anesthetize it with isoflurane (2.5% v/v for induction and maintenance), clean the incision site with saline solution, and close the skin with 6/0 black silk square knots. Afterwards, disinfect with povidone.
2. Mice should be supervised following standardized procedures, as per Morton and Griffiths guidelines on the recognition of pain, distress, and discomfort (Morton and Griffiths, 1985). This adapted protocol considers four variables, and a score is assigned for each variable:
   a. Weight loss:
      0 (normal): There is no weight loss or the animal grows normally.
      1: Weight loss less than 10%.
      2: Weight loss between 10 and 20%. Alteration in the appearance or amount of stool.
      3: Weight loss greater than 20%. The animal does not consume water or food.

b. Coat appearance:
0: Normal.
1: Hair in poor condition.
2: Hair in poor condition and ocular or nasal secretions.
3: Piloerection.

c. Movement/posture/behavior of the animal:
0: Normal.
1: Small changes.
2: Moderate changes.
3: Inactivity, aggressiveness, self-mutilation or vocalizations.

d. Aspect of the incision site:
0: Normal.
1: Slight redness.
2: Local edema.
3: Infection, darkening (signs of necrosis).
The final score is calculated as the sum of the scores obtained for each variable, which determines the application of corrective measures:
0–3: The mouse does not require any corrective measure.
4–7: The mouse requires the application of corrective measures.
≥8: The mouse should be euthanized.
*Euthanasia will be practiced if the score for any of the variables is 3.
Corrective measures include cleaning the wound with disinfectant and applying topical antibiotic, placing the cage on top of a heating pad, and supplying nutrient-enriched hydrating gel or food pellets soaked in water to facilitate feeding.

3. During all the experimental protocol, animals will be handled by the experimenter, allowing them to recognize him/her. This helps to reassure the animals, making them easier to handle, and helping to obtain reliable results. This handling consists of allowing each animal to explore the experimenter's arm for 2 min. This should be done before the start of the experimental protocol, for at least 7 days.

Recipes

1. Heparin 0.0003 mg/mL
0.12 mL of sodium heparin (Heparina Hospira 5%)
20 mL of sterile 0.9% physiological saline
2. Ketamine (7.5 mg/mL) + Medetomidine (0.2 mg/mL)
0.75 mL of ketamine hydrochloride (100 mg/mL Ketamidor)
1 mL of medetomidine hydrochloride (1 mg/mL, Dormitor)
8.25 mL of sterile 0.9 % physiological saline.
3. Atipamezole 1 mg/mL
2 mL of atipamezole hydrochloride (2.5 mg/kg of body weight, Revertor)
8 mL of sterile 0.9% physiological saline
4. Gentamicin 0.3 mg/mL
0.06 mL of gentamicin (40 mg/mL, Genta-Gobens)
10 mL of 0.9% physiological saline
5. Meloxicam 0.5 mg/mL
0.5 mL of meloxicam (40 mg/mL, Metacam)
40 mL of glucose serum (GlucosaVet 5 g/100 mL)

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Competing interests

The authors have no conflicts of interest

Ethics

Animal handling and experiments were in accordance with protocols approved by the Animal Care and Use Committee of the PRBB (Comité Ètic d'Experimentació Animal-Parc de Recerca Biomèdica de Barcelona, CEEA-PRBB; agreement 9569) and the Departament de Territori i Habitatge of Generalitat de Catalunya, and were performed in accordance with the European Communities Council Directive (2010/63/EU).

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