

Video Article

Methods for Intravenous Self Administration in a Mouse Model

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Abstract

Animal models have been developed to study the reinforcing effects of drugs, including the intravenous self-administration (IVSA) paradigm. The advantages of using an IVSA paradigm to study the reinforcing properties of drugs of abuse such as cocaine include the fact that the drug is self-administered instead of experimenter-administered, the schedule of reinforcement can be altered, and accurate measurement of the quantities of drug consumed as well as the timing and pattern of IV injections can be obtained. Furthermore, the intravenous route of administration avoids potential confounds related to first pass metabolism or taste, and produces rapid increases in blood and brain drug levels. As outlined in this video, intravenous self-administration can be obtained without prior food restriction or prior drug training following careful catheter placement during surgery and meticulous daily catheter flushing and maintenance. Experimental procedures outlined in this paper include a description of animal housing and acclimation methods, operant training using sweetened milk solutions, and catheter implantation surgery.

Video Link

The video component of this article can be found at <https://www.jove.com/video/3739/>

Protocol

1. Animal Housing and Acclimatization Procedures

1. Male and female CD-1 mice are housed with same-sex littermates, up to 5 animals per cage, in standard plastic cages containing beta chip bedding and nestlet pads, with wire grid lids to accommodate water bottles and food. Standard mouse chow and water are available ad libitum in the home cages throughout the experiment.
2. Mice are maintained on a reverse light-dark schedule (lights on 22:00 to 10:00).
3. Mice are allowed to acclimatize to the IVSA testing rooms for one week prior to the start of experimental procedures.

2. Operant Training

1. All behavioural procedures including sweetened milk training and intravenous drug self-administration sessions are carried out using operant chambers measuring 15.9 x 14 x 12.7 cm, equipped with 2 ultra-sensitive mouse levers, dipper cups, stimulus lights and microliter syringe pumps (Med Associates Inc., St. Albans, VT, USA). Chambers are interfaced to a computer using Med Associates Smart CR interface and Med-PC software to program the schedule of reinforcement and collect data.
2. Naïve mice are habituated to the operant chambers and trained to lever press using a sweetened milk reward (0.1 ml) presented in a dipper cup. Operant milk training is conducted in 1-hr sessions for 5-7 days. Completion of a fixed ratio (FR) schedule on the active lever resulted in the presentation of the dipper cup and the illumination of a stimulus light. The sweetened milk solution consisted of sucrose (10 mg/ml, ACS Reagent, Sigma-Aldrich Inc. St-Louis MO, USA) added to whole milk (3.25% fat content). Operant training with a sweetened milk solution or a liquid food reinforcer is a common technique used to facilitate operant responding in mice^{1,2}.

3. Preparation of Equipment to be Used during Surgery

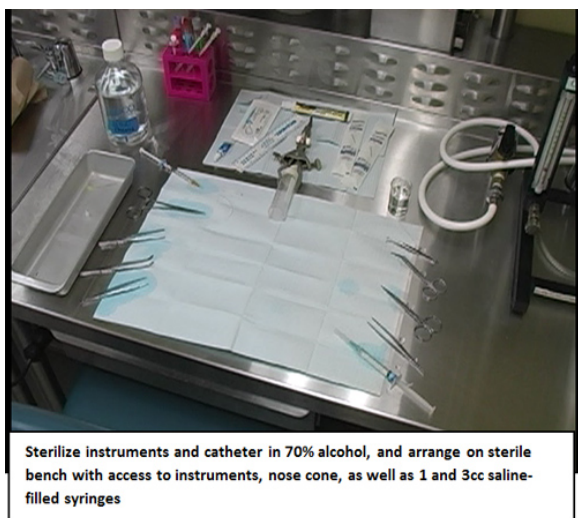
Required equipment - 20, 23 and 26 gauge needles, grinder, 1 cc syringes, tygon tubing, soldering iron, heparin, antibiotics and analgesic.

1. Prepare a needle to guide the insertion of the catheter into the jugular vein by shaving down a 20 gauge needle. The shaft of the needle is shaved down using a grinder to form a channel within the needle to guide the catheter tubing into the vein. The needle channel should be carefully checked for any metal debris that may have been deposited. Any obstructions to the channel should be scraped out using fine tipped forceps.
2. Adapt two 1-cc syringes to be used for flushing and checking the catheter. Prepare two syringes by taking 12 cm pieces of Tygon tubing and attach them at one end to 26 gauge needles affixed to 1 cc syringes, and stretching the other ends of the tubing over 23 gauge needles. One

- syringe should be filled with 0.9% sterile saline and the other with a heparinized ticarcillin solution (33 mg of ticarcillin and 0.3 mg of heparin per 10 ml of sterile saline).
3. Prepare catheter cannula caps. Tygon tubing is stretched over a 23 gauge needle, and cut 1 cm from the bevel. Melt the open end of the tubing to create a thick seal. The tubing should be melted so that the entire cannula cap is long enough to fit over the catheter cannula, with the sealed end fitting snugly over the end of the cannula. Note that if the cap is too long, there is a risk that it will be bent and subsequently pierced during the process of attaching the screw-on catheter covers. Thus careful preparation of the catheter caps is recommended, in order to provide a good fit to the cannula.
 4. Prepare required solutions. All reagents are purchased from Sigma-Aldrich Inc. (St-Louis MO, USA).
 - a. Heparinized Ticarcillin solution for catheter flushing - Dissolve 0.33 g Ticarcillin (disodium salt) and 0.003 g of heparin in 10 ml of sterile saline. 0.03 ml of solution is flushed through the catheter daily. The antibiotic solution is administered to prevent blood clots and infections from developing.
 - b. Amikicin (antibiotic) for subcutaneous injection - A single subcutaneous injection is given following surgery at a dose of 10 mg/kg to prevent post-operative infections.
 - c. Ketoprofen (analgesic) for subcutaneous injection - A single subcutaneous injection is given following surgery at a dose of 5 mg/kg to manage any post-operative pain.

4. Catheter Implantation Surgery

Required equipment and reagents: Isoflurane, sterile saline, alcohol (70%), 1 and 3 cc syringes filled with sterile saline, 1-cc adapted syringes, antibiotic and analgesic solutions prepared in section 3 above, mouse catheters (CamCaths, Cambridgeshire, UK), catheter covers (crystal caps from HRS Scientific, Montreal, Quebec), eye lubricant, 4 cm plastic bar to elevate vein (this can be constructed from a plastic Q-tip), polysporin, sterile swabs and gauze, curved and straight forceps, artery clamps, fine scissors.

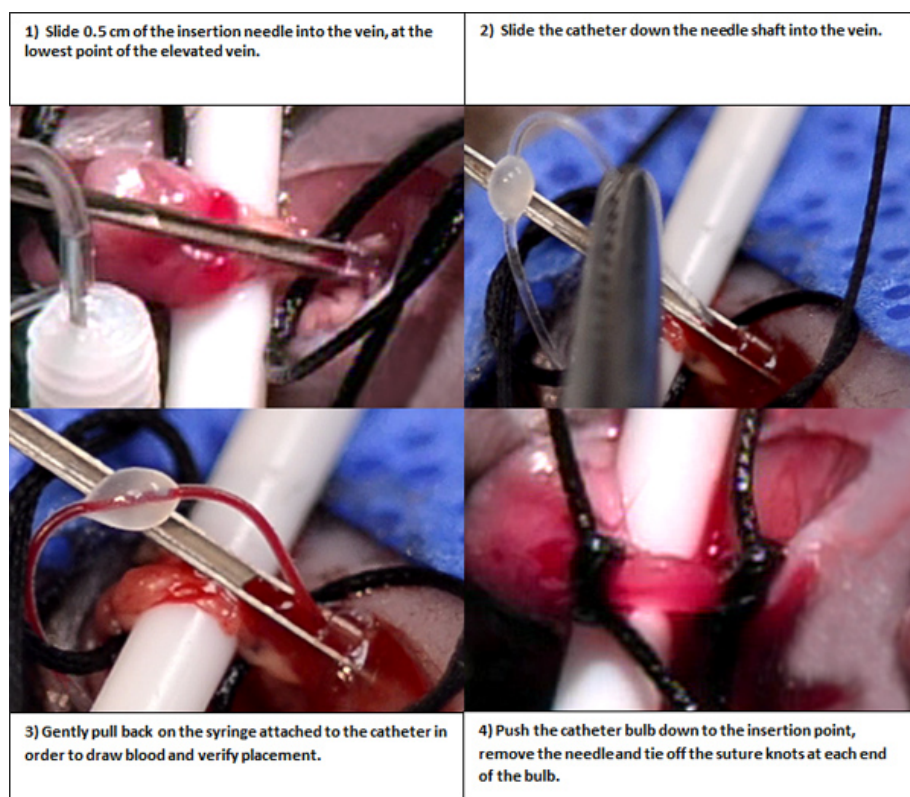


1. Using standard aseptic techniques the surgical bench, the surgical instruments, and the catheters are sterilized prior to surgery. Proper sterilization techniques include steam autoclaving for the surgical instruments and catheters purchased from CamCaths. Glass bead sterilization can also be used on the tips of the surgical instruments. Ethylene oxide sterilization can be employed on more delicate catheters or materials that risk melting. A more detailed description of rodent surgical aseptic techniques can be found in the attached references^{1,2}. Set up of the bench, instruments and nose cone for maintenance anaesthesia is illustrated in the photo.
2. Mice are anaesthetized with isoflurane gas, and maintained under anaesthesia using a breathing tube under a scavenging system. Eye lubricant (Tears Naturale P.M) is applied to both eyes to prevent them from drying out during the procedure. An appropriate surgical margin is shaved on the animal's back and around the neck. Before any incisions are made, the surgical fields are restricted using sterile drapes.
3. In order to prepare the catheter for insertion into the right atrium of the heart, the excess catheter tubing is cut off 1.2 cm from the bulb of the catheter. This is the optimal length established for adult CD-1 mice, approximately 8 weeks of age, 20-25 grams body weight. The length of catheter tubing may need to be adjusted slightly (by trial and error), based on the strain, size and age of the mice. Prior to insertion, the syringe containing sterile saline (section 3.2) is attached to the catheter cannula, and the catheter is flushed and checked for leaks. Keep this syringe attached to the catheter throughout the surgical procedure. It will be used to flush the catheter, and draw back blood in step 4.7.
4. Following sterilization with 70% alcohol, a 2 cm long midscapular incision is made starting midway on the back and ending just below the neck in order to accommodate the base of the catheter. Connective tissue should be forced apart with forceps to make space for the catheter base below the skin.
5. Placing the animal on its back, a second shallow 1 - 2 cm diagonal incision is made from the right clavicle going upwards to the animals jaw, after the area has been swabbed with 70% alcohol. The jugular vein will be found superficially under the skin of the neck. In preparation for insertion of the catheter, tubing from the base of the catheter is pulled through the incision on the back and brought close to the jugular vein by passing the tubing under the skin just over the right shoulder. The end of the catheter tubing is then attached to an artery clamp and placed at the animal's side to keep it in place.
6. The right jugular vein is located by gently moving away superficial connective and adipose tissue from the incision around the animal's neck. Connective tissue around the vein is broken apart using curved forceps and the vein is then elevated using a sterile plastic bar. Loose open

suture knots are made around each end of the vein and the catheter tubing is threaded through the top knot and looped over the suture thread to rest unclamped over the right shoulder.

7. Prior to insertion, wet both the 20 gauge insertion needle and vein with sterile saline to reduce friction. The needle is held parallel to the vein, and inserted gently near the bottom of the elevated vein (**Note:** approximately 0.5 cm of the needle tip needs to enter the vein). Using forceps, slide the catheter tubing down the shaft of the needle into the vein. Resistance would indicate that the tubing is within connective tissue and not within the vein. Push 0.03 cc of saline through the vein to make sure that there are no leaks. Leaks would indicate that the vein may be pierced or that the catheter tubing placement needs adjusting.

Note: To check if the tubing is within the vein, attempt to draw up some blood using the attached saline syringe. If blood cannot be immediately drawn up, the vein or heart wall may be occluding the catheter tip, or the vein has not been pierced; adjust the tubing and try again. Needle reinsertion may be necessary if blood still cannot be drawn up.
8. In order to secure the catheter in place, push the catheter bulb to the insertion point and remove the needle. Tie the bottom knot and then pull the catheter flush against the bar before tying the second knot right above the bulb. Test again to see if blood can be drawn up and loosen the knots slightly if needed. Tuck the catheter tubing under the skin and suture the ventral incision around the animal's neck. Apply Polysporin Heal Fast using a sterile cotton tip applicator, or any other antibiotic ointment preferably containing some analgesic to the closed incision.
9. With the animal on its abdomen, place the catheter base under the skin of the back within the prepared incision. Make sure that the excess tubing is minimally looped and well hidden under the catheter base to minimize the chances of the animal chewing and piercing it. Suture the incision on both sides of the catheter base, and apply Polysporin Heal Fast using a sterile cotton tip applicator.
10. Flush the catheter with 0.03 cc of the heparinized ticarcillin solution using the syringe with the tubing affixed to it (section 3.2). Cap the cannula with the plastic cannula cap and screw on the white catheter cover. In some mice that are fresh from surgery, blood may leak out of the catheter before the cannula is capped. It is important to re-flush the animal and rapidly replace the cannula cap before blood has the chance to flow out. Animals must be flushed on a daily basis to maintain catheter patency.
11. After wiping down the injection area between the animals back legs with 70% ethanol, subcutaneously inject the analgesic ketoprofen at a dose of 5 mg/kg on one side, and the antibiotic amikicin at a dose of 10 mg/kg on the other side.
12. After anaesthesia is discontinued, animals are allowed to recover in a clean cage with easy access to food and water for 5 to 7 days. Mice should be placed in a heated cabinet overnight to prevent post-operative hypothermia.



5. Behavioural testing – Intravenous Self-Administration

1. Prior to behavioural testing catheters are flushed with 0.9% sterile saline. Mice are then placed in the operant chambers and connected to the infusion lines and infusion pumps. Active lever presses result in a 3.2 sec 18 µl drug infusion coupled with the illumination of a stimulus light. Each lever press is followed by an 8 sec time out period during which the stimulus light stays on.
2. Following the operant session, mice catheters are flushed with the heparinized ticarcillin solution before being returned to their home cage.
3. Mice are allowed to self-administer for 3 consecutive 2-hr sessions at each dose. Doses were presented in a random order for each mouse, as shown in the following section.

- Catheter patency is assessed daily by ensuring that both the saline and antibiotic solution can be flushed through the catheter. In addition, a ketamine/midazolam test can be conducted as described in the attached reference⁵. In brief, signs of anesthesia such as immobility within 5 sec of an infusion of 0.02-0.03 ml ketamine (15 mg/ml), or midazolam (0.75 mg/ml) midazolam is evidence of a patent catheter⁵.

Representative Results

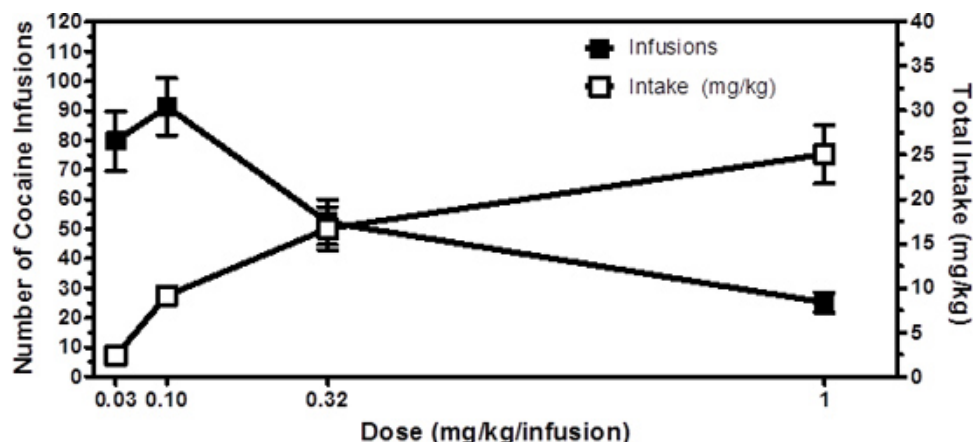


Figure 1. The pattern of responding for intravenous drug self-administration will vary by drug, dose range and mouse strain employed. The figure presented shows cocaine self-administration data following successful catheterization surgeries using the procedure described in the video. The figure shows the mean (\pm SEM) cocaine infusions earned and mean (\pm SEM) cocaine consumption (mg/kg body weight) across a range of 4 cocaine doses presented in a random order on a FR1 schedule of reinforcement. Abscissa: dose of self-administered drug per infusion. Left Ordinate: total number of infusions earned during the 2-hr operant session. Right Ordinate: total cocaine intake in mg/kg during the 2-hr testing session. All 13 catheters remained patent for the duration of the study (4 weeks). A one-way ANOVA conducted on dose revealed that mice were administering cocaine in a dose-dependent fashion [$F(1,12) = 42.8$, $p < 0.05$]. There is an increase in cocaine consumption over the dose-response curve [$F(3,36) = 29.6$, $p < 0.05$] despite a decrease in lever pressing at the higher doses. Each data point represents the average of 3 testing sessions at each cocaine dose (\pm SEM) collected in CD-1 mice ($n=13$ /dose, males and females combined). Comparisons of active (drug-reinforced) vs inactive lever responding across the dose response curve were made using two-way ANOVA to ensure that mice were discriminating between the two levers. For the CD1 mice, the analysis revealed a preference for the active lever [$F(1,12) = 10.255$, $p < 0.05$] over the entire dose-response curve (data not shown here).

Discussion

Animal models of drug abuse are particularly useful in understanding the genetic basis of drug-related behaviours. For instance, mice with different genetic profiles show heritable differences in their sensitivity to cocaine⁶⁻⁸ and help identify potential gene candidates mediating the phenotypic variability observed⁹. The intravenous catheterization procedures described in this paper have been used with considerable success to examine drug IVSA in various strains of mice as well as mice of different genetic backgrounds¹⁰.

The procedures shown in this video highlight important factors to focus on during and following the catheterization surgery in order to obtain reliable intravenous self-administration data. First, the placement of the catheter tubing within the right atrium is vital, in order to prevent catheter failure from clot formation. During surgery it is important to make sure that the catheter end is unimpeded, and not occluded by either the heart or vein tissue. Second, daily catheter flushing is required both before and after operant sessions in order to prevent blockages. Finally, the catheter cannula must be covered constantly with both the cannula caps and crystal covers when the animals are in their home cages, to prevent the entry of debris. Minor blockage of the catheter cannula may be dislodged using a fine 26 gauge needle, however daily flushing with the heparinized antibiotic solution is required, particularly on days when the animals are not tested for IVSA.

To perform animal survival surgery, a good knowledge of aseptic techniques, analgesia and anaesthesia is necessary. While this video does not replace proper surgical training, it may be used as a guide for researchers wishing to acquire the techniques necessary for this paradigm.

Disclosures

No conflicts of interest declared.

Acknowledgements

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