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Head-mounted central venous access during optical recordings and manipulations of neural activity in mice

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Abstract

Establishing reliable intravenous catheterization in mice with optical implants allows the combination of neural manipulations and recordings with rapid, time-locked delivery of pharmacological agents. Here, we present a procedure for handmade jugular vein catheters designed for head-mounted intravenous access and provide surgical and post-op guidance for improved survival and patency. A head-mounted vascular access point eliminates the need for a back-mounted button in animals already receiving neural implants, thereby reducing sites of implantation. This protocol, which is readily adoptable by experimenters with previous training and experience in mouse surgery, enables repeated fiber photometry recordings or optogenetic manipulation during drug delivery in adult mice that are awake and behaving, whether head-fixed or freely moving. With practice, an experienced surgeon requires approximately 30 minutes to perform catheterization on each mouse. Altogether, these techniques facilitate the reliable and repeated delivery of pharmacological agents in mouse models while simultaneously recording at high temporal resolution and/or manipulating neural populations.

Introduction

Intravenous (i.v.) catheterization allows precise, time-locked delivery of drugs into the bloodstream, and it has quickly become the gold standard for studying the systemic effects of pharmacological agents[1]. Jugular vein catheterization (JVC) is the primary method

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Author contributions

C.L. designed the protocol, carried out *in vivo* experiments, analysis, and figure preparation, and wrote the manuscript. D.J.F. performed video documentation and assisted with the figure preparation. S.L. supervised experiments, gave conceptual support, provided funding, and assisted with the manuscript preparation.

Competing financial interests

The authors declare that they have no competing financial interests.

Data availability

All data supporting this protocol are available from the lead contact upon reasonable request.

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EDITORIAL SUMMARY: Protocol describing how to implant head-mounted JVC catheters in mice. This procedure facilitates systemic drug administration in a variety of experimental settings, including optical recording and manipulation of neuronal activities and behavioral tests.

for chronic i.v. catheterization in rodents and is well established in rats[2]. However, the technique is more difficult to perform on mice due to their smaller size[3], which may explain the relative sparseness of literature[4] and the increased cost of purchasing mice with pre-implanted jugular vein catheters relative to rats[5].

In mice, intravenous injections are predominantly performed through the lateral tail vein, but this method is typically limited to acute, anesthetized, and/or restrained experimental pardigms[6]. We present a surgical protocol which facilitates i.v. access via a head-mounted port using custom-made implants, combining i.v. drug delivery in awake, behaving, freely moving mice with neural recordings or manipulations.

With this protocol, we delivered nicotine and pharmacological antagonists while performing head-fixed fiber photometry recording or freely-moving behavioral tests and we were able to define nicotine dose-dependent effects on brainstem- midbrain-forebrain circuits at both circuit- and receptor-levels[7]. Furthermore, catheter patency was maintained for 88% of animals at our endpoint of 2 weeks post-catheterization but it could be maintained for longer periods for experiments that require it [4,8]. Here, we provide detailed guidelines for the construction of the head-mounted catheters, the surgical procedure for their insertion, aftercare guidance, and troubleshooting advice to successfully perform repeated optical recordings during i.v. drug administration *in vivo*.

Applications of the method

With an increasing range of transgenic mouse lines[9] and tools for recording or manipulating neural activity in a cell-type and/or circuit-specific manner, mice have become the model organism of choice for many neuroscience studies[10]. However, difficulties in establishing a reliable method to gain intravenous access in mice have limited the potential for leveraging the full range of available techniques. For example, self-administration of drugs has become the gold standard for studying the mechanisms mediating substance abuse[11], but the bulk of these experiments are performed in rat models which have relatively fewer transgenic lines[12]. In mice, systemic drug delivery is typically achieved through intraperitoneal[13] or subcutaneous injections. However, repeated rounds of needle poke can be painful and stressful ultimately obscuring some effects of the drug[13,14]. Using a chronically implanted jugular vein catheter with a head-mounted access point, we were able to perform fiber photometry recordings or neural manipulations concomitantly with drug infusions over multiple days[7]. Due to the small footprint of the vascular access point, this technique can be compatible with a wide range of neural implants, including, but not limited to, optical fibers, cannulas, miniature endoscopes, and/or electrodes. Additionally, we envision that the head-mounted vascular access point may be compatible for serial blood withdrawals in freely moving mice[15,16].

Comparison with other methods

Mouse jugular vascular catheterization has been established by other researchers, predominantly to perform behavioral assays such as i.v. self-administration to model drug addiction. In this case, the vascular access point is generally mounted on the back[4,11,17] even when head-fixed neural recordings are performed[18]. Back-mounted vascular access points

are widely used and appropriate for many applications; however, if animals already receive neural implants, the access point can be head-mounted to limit the total area of implantation on the animal. Here, we describe how to implant head-mounted vascular access ports as a viable alternative to the traditional back-mounted button. Functionally, head-mounted and back-mounted catheters are equivalent for drug delivery, but head-mounted catheters provide advantages in animals with neural implants by adhering the vascular access point to a pre-existing implant location, thereby eliminating the need for a surgical incision on the back. Back-mounted buttons are typically anchored to the skin with a semi-porous disc such as polyester felt or silicone mesh, which may cause skin irritation, inflammation, and/or necrosis[19]. Regardless of the location of the vascular access point, regular flushing of the catheter over the course of the procedure is required to maintain patency which requires animal handling and contact with the vascular access point. In cases where animals will be head-fixed during the experiments, habituation to head-fixation reduces stress during restraint and head-mounted catheters can be flushed with ease. Otherwise, both head-mounted and back-mounted catheters can be flushed with gentle or minor restraint by an experienced handler. If researchers experience difficulty while flushing either head-mounted or back-mounted catheters, they can consider additional handling days, general anesthesia, and/or commercially available restrainers[17–20].

An additional advantage is that the small footprint of the custom catheter could also be compatible with other head mounting approaches, such as head-rings and head-posts. A protocol for a head-mounted vascular access point has previously been published[21] with improved success rate, although the steps implemented to refine the method have not been explicitly stated. We have also found that optimization of this difficult technique based on the individual needs and resources of each researcher and laboratory has considerably improved the success rate in the surgical, post-operative, and maintenance periods.

Expertise needed to implement the protocol

This catheterization protocol (Supplementary Video 2) is readily adoptable by experimenters with previous training and experience in mouse surgery. During the learning phase, the catheterization surgery may take over an hour to complete, but with dedicated practice it can be completed in 25–45 minutes per mouse. While the procedure can be completed by one person with high success rates, a team of two researchers may improve speed and performance especially during critical steps that benefit from an extra pair of hands or by sequentially performing different stages of the surgery based on experience. An experienced surgeon should perform the catheterization, while an assistant surgeon can induce anesthesia, prepare the skin, and perform the final steps of suturing the chest incision and securing the implant with dental cement on the head. While team surgeries do not always decrease the amount of time that an individual animal is under anesthesia, working in pairs can increase the total number of surgeries performed in one day, if animals are shared between surgeons for the relevant steps, with care taken to maintain sterility. Post-operative care (i.e., flushing catheters, Supplementary Video 3) and i.v. drug administration can be performed easily by experimenters with training in animal handling but less experienced in mouse surgery.

Limitations of the protocol

Our protocol was designed for experiments on adult mice, 6–18 weeks old, for up to 2 weeks post- catheterization. We envision that there may be limitations for the application of the method beyond the limits of our study, for example surgical survival rates of juvenile mice or maintenance of the catheter for prolonged experiments. In the past catheter patency has been maintained for months by regularly flushing the catheters with heparinized solution[4,7], similar to what we suggest in our procedure.

Additionally, our animals were single-housed during the 2-week post-catheterization period and provided with additional enrichment. Generally, prolonged social isolation should be avoided as single-housed animals experience stress which can affect their behavior and neural measures[22–24]. Considerations for group-housing animals are discussed in the Experimental Design section.

Overview of the Procedure:

We provide a step-by-step procedure, successfully implemented in our laboratory, along with common troubleshooting solutions. In this protocol, we provide detailed instructions on how to prepare custom-made catheters (Steps 1–17), how to prepare for surgical catheterization (Steps 18–21) and precise guidelines for the surgical procedure (Steps 22–57), including details on how to perform the implantation of a headplate for head-fixation of the animal for neural recordings and catheter flushing, regardless of whether animals will be hand-held or mounted above a running wheel. In addition, we also provide valuable advice on post-operative (Steps 58–61) and peri-experimental (Steps 62–62) care, to improve animal survival and increase success rate. Together, we expect our method to improve success for researchers seeking to establish central venous access in combination with neural implants.

Experimental design

The surgery is most easily performed on adult 6–12 weeks old animals weighing around 18–28g. Application of the procedure in older animals might be challenging due to fatty tissue accumulating around the jugular vein with age, (particularly in males) making the isolation of the jugular vein more difficult; however, we have successfully performed surgery on animals up to 18 weeks. Smaller animals below 18g in weight, (especially younger females), may have smaller jugular veins and increased adverse effects from blood loss if complications arise during the surgery. At the time of jugular vein catheterization, most of our animals had undergone stereotactic surgery for the injection of adeno-associated viruses and implantation of optical fibers and a headplate. When possible, we verified adequate viral expression (such as quality of fiber photometry signal) before selecting an animal to undergo i.v. catheterization to limit the wait time between catheterization and data collection, as catheter patency must be maintained after catheterization. Animals without existing implants received a headplate during the same surgical procedure as jugular catheterization (Step 26). To prevent tampering of the catheter cap by cage-mates, animals were singly housed after catheterization and provided with additional enrichment. To avoid prolonged social isolation, that may alter behavior and neural measures, researchers should consider group-housing animals. In this case, the use of a protective covering over the catheter cap should be included to prevent the catheter cap being punctured or removed by cage mates. Solutions

for threaded catheter caps[7] are available, but these may be bulky and incompatible with head-mounted neural implants.

The combination of a headplate, optical fiber implants, and a head-mounted vascular access point were developed specifically to address our research questions. While we personally did not test this catheterization method with large implants or headplate designs, the technique is likely adaptable to other neural techniques requiring implanting a cranial window, electrodes, or other fixtures. For neural recordings based on *in vivo* two photon microscopy, the angle and the position of the catheter must not interfere with the objective. Under this and other circumstances the construction of the catheter (length) (Supplementary Video 1) and the adhesion process (the angle of the vascular access point) (Supplementary Video 2) can be modified, based on the limitations and specific requirements imposed by each experiment.

Materials

Biological materials: Mice: We applied this procedure to male and female animals between 6–18 weeks of age from the following mouse lines: C57BL/6J mice (Jackson Laboratory, stock number: 000664), DAT::IRES-Cre (Jackson Laboratory, stock number: 006660, strain code: B6.SJL-Slc6a3tm1.1(cre)Bkmn/J), VGLUT2::IRES-Cre (Jackson Laboratory, stock number: 016963, strain code: Slc17a6tm2(cre)Lowl/J), GAD2::IRES-Cre (Jackson Laboratory, stock number: 010802, strain code: Gad2tm2(cre)Zjh/J), and VGAT-IRES-Cre (Jackson Laboratory, stock number:028862, strain code: B6J.129S6(FVB)-Slc32a1 tm2(cre)Lowl /MwarJ). All lines have been crossed onto the C57BL/6J background for at least six generations. Although we applied the procedure to these specific lines, we anticipate the procedure is applicable to any strain. Mice were provided *ad libitum* access to water and chow before and after surgery and they were maintained under a 12 h light cycle. Our experiments were performed during the animal's light cycle, but the light cycle may be reversed for experiments that require it. All procedures complied with the animal care standards set forth by the National Institutes of Health and were approved by University of California, Berkeley's Administrative Panel on Laboratory Animal Care.

CAUTION All procedures must be carried out under the oversight and approval of local and institutional animal care and use programs by experimenters with adequate training.

Reagents—CRITICAL: Reagents and equipment listed are examples; unless otherwise specified, alternatives from other manufacturers can be used.

- Ketamine hydrochloride, 100 mg/ml (KETASET®, Zoetis, cat. no. 54771–2013-1) CAUTION controlled substance. Ketamine is known to have antidepressant properties[25–27] and may interfere with studies involving models of chronic stress and depression. Isoflurane anesthesia may instead be used during the surgical procedure.
- Dexmedetomidine hydrochloride, 0.5 mg/ml (DEXDOMITOR®, Zoetis, cat. no. 54771–2805-1)

- Atipamezole hydrochloride, 5 mg/ml (ANTISEDAN®, Zoetis, cat. no. 54771–6293-1)
- Carprofen, 50 mg/ml (Carprieve® Injection, Norbrook®, cat. no. 55529–131-01)
- Lidocaine hydrochloride Injectable 2% wt/vol (Vedco, cat. no. 50989–417-12)
- Pentobarbital Sodium, 390 mg/ml (FATAL PLUS®, Vortech Pharmaceuticals, LTD., cat. no. 0298–9373-6) CAUTION controlled substance
- Eye ointment (Akorn Artificial Tears, cat. no. 59399–162-35)
- Antibiotic ointment (Neosporin, Original Triple Antibiotic Ointment, cat. no. 0363–0179-34)
- Antiseptic Skin Cleanser (Chlorhexidine Gluconate, CVS, cat. no. 0116–1061-09)
- Ethanol (VWR, cat. no. TX89125–172SFU)
- Bleach (Clorox, Germicidal Bleach, cat. no. 30966)
- CIDEX® OPA (Advanced Sterilization Products, cat. no. 20390)
- Sterile Injectable Saline (0.9% Sodium Chloride Injection, USP, Hospira, cat. no. 0409–4888-02)
- Heparin Sodium Injection (1,000 USP units/mL, Hospira, cat. no. 0409–2720-32)
- C&B-METABOND L-Powder Radiopaque Tooth Shade, Quick Base, & Universal Catalyst (Parkell, cat. no. S396, S398 & S371)
- Dental Cement (Lang Dental, Ortho-Jet Powder 1320& Ortho-Jet Liquid 1304)
- Tissue Adhesive (Vetbond®, 3 ml, 3M, cat. no.1469)
- Paraformaldehyde, 16% w/w aqueous solution (Thermo Fisher Scientific, cat. no. 043368–9M)
- Phosphate Buffed Saline (PBS (1X), Gibco, cat. no.10010002)

Equipment

Catheter and cap construction

- Polyurethane tubing (Instech BTPU-027) CRITICAL The high flexibility of the tubing is critical for limiting damage to the jugular vein after implantation. The inner diameter of the tubing is critical for compatibility with 26g hypodermic syringe needles. Alternative products can be used but compatibility is not guaranteed.
- Silicone Medical Adhesive (Factor II Inc, A-100, LOT #0009460932)
- 26g hypodermic syringe needles (0.5” length, BD cat. no.305111)

- Superglue (Krazy® Glue, All-Purpose Brush-On Formula, 0.18 Oz., Clear, cat. no. 457099)
- 1 ml syringe (BD, cat. no. 309597)
- Soldering iron (Weller, WES51)
- Soldering fan (Aoyue®, Smoke Absorber, 486)
- Razor blade (VWR, cat. no. 55411–055)
- Ruler (FST, cat. no. 30085–30)
- Centrifuge tube holder (Heathrow Scientific, cat. no. HEA29025A)
- Scissors (Office Depot, cat. no. 432212)
- Safety glasses (Pyramex, Flex-Lyte, SBG10510D)
- Sharps container (VWR®, cat. no. 76319–794)
- Scintillation vial (Fisherbrand™ cat. no. FS7450420)

Headplate construction

- CNC machine or CNC machining service provider
- 6061 aluminum sheets, 0.031” thickness

Glass support rod construction

- Bunsen burner (Eisco™ CH0993NG)
- Glass capillary tubes (10 cm, 1.5 mm diameter, Warner Instruments 64–0805)

Surgical procedure

- 10% bleach vol/vol (diluted with water; Clorox, Germicidal Bleach)
- 70% ethanol vol/vol (diluted with water; VWR, cat. no. TX89125–172SFU)
- Temperature controlled heating pad (Harvard Apparatus, cat. no. 50–7212)
- Sterile Injectable Saline (0.9% Sodium Chloride Injection, Hospira, cat. no. 0409– 4888-02)
- Polylined Sterile Field (Fenestrated, Busse, cat. no.697)
- Fur trimmer (Stoelting™ Cordless Trimmer, cat. no. 51472)
- Sharp forceps for piercing jugular (Fine Science Tools by Dumont #5, cat. no. 11251– 10) CRITICAL
- Flat forceps (Superfine dissecting forceps, VWR, cat. no. 82027–402)
- Serrated forceps (FST, cat. no. 11050–10)
- Toothed forceps (Kent Scientific, Curved Iris Forceps, cat. no. INS15917)
- Surgical scissors for cutting skin (Aven, cat. no. 11014)

- Surgical microscissors for cutting the vein (Fine Science Tools, cat. no. 15000–08)
- Hemostat (Kent Scientific, Serrated Curved Hemostat Forceps, cat. no. INS500452)
- Glass bead sterilizer (Keller, cat. no. 250)
- Sutures (AD surgical #S-G618R13-U) Must be flexible, but absorbable or non-absorbable could depend on the intended longevity of animal.
- Cotton Tipped Cleaning Sticks (Puritan® PurSwab®, cat. no. 867-WC)
- 24 well plate (Thermo Scientific, cat. no. 930186)
- Transfer Pipet (Fisherbrand™, cat. no. 13–711-7M)
- Delicate Task Wipers (Kimtech Kimwipes ®, cat. no. 34120)
- Tape (VWR, cat. no. 89097–938)

Aftercare

- Flat forceps (Superfine dissecting forceps, VWR, cat. no. 82027–402)

Reagent Setup

Ketamine/dexmedetomidine cocktail for general anesthesia: To provide each animal with general anesthesia, prepare daily a fresh cocktail of ketamine and dexmedetomidine for a final dose of 60 mg/kg ketamine and 0.6 mg/kg of dexmedetomidine. With the manufacturer solutions of 100 mg/ml ketamine and 0.5 mg/ml dexmedetomidine, the final injection solution should be 2 parts dexmedetomidine and 1 part ketamine. For example, to make enough 60 mg/kg ketamine + 0.5 mg/ml dexmedetomidine cocktail for 4 mice weighing 25 g each, withdraw 0.12 ml dexmedetomidine and mix with 0.06 ml ketamine. Each 25g mouse receives 0.045 ml of the ketamine/dexmedetomidine cocktail via intraperitoneal injection. Ketamine/dexmedetomidine anesthesia provides stable anesthesia for approximately 90 minutes, at which point surgeons should take additional care to monitor changes in heart rate and respiratory rates. Alternatively, surgeons can deliver isoflurane anesthesia, with care to maintain stable anesthesia especially when adjusting the animal between prone and supine positions and to limit chest movement when performing catheterization.

Lidocaine hydrochloride solution: For local anesthesia at the site of skin incisions, prepare a 0.5% (wt/vol) solution of lidocaine hydrochloride by withdrawing 1 ml of the manufacturer solution concentrated 2% (wt/vol) and diluting with 3 ml of sterile injectable saline. The solution should be prepared up to 1 week in advance and stored at 4°C or room temperature. Administer by injecting 0.01 ml of the solution subcutaneously where the incision will be made at the chest and another 0.01 ml subcutaneously where the incision will be made at the head; each animal receives 0.02 ml total regardless of weight. A 20 g mouse receiving 0.02 ml lidocaine is equivalent to a 5 mg/kg dose; the maximum allowable dose is 7 mg/kg.

Atipamezole hydrochloride: Atipamezole hydrochloride at a dose of 6 mg/kg is used as an antagonist to dexmedetomidine, which allows surgeons to effectively control the duration of the anesthesia. Prepare in advance a syringe with the appropriate volume for each animal. From the manufacturer's bottle of 5mg/ml solution, each animal weighing 25g receives an undiluted volume of 0.045 ml of atipamezole hydrochloride via intraperitoneal injection at the end of surgery to counteract the ketamine/dexmedetomidine anesthesia.

Carprofen solution for perioperative analgesia: Up to 1 week in advance, prepare a 1 mg/ml solution of carprofen by withdrawing 0.10 ml of 50 mg/ml carprofen and diluting with 5 ml sterile saline. Store at 4°C or room temperature. Deliver a 5 mg/kg dose via intraperitoneal injection for perioperative analgesia for example a 20g mouse receives 0.10 ml of the 0.1mg/ml carprofen solution.

Heparin solution for catheter patency: Undiluted 1000 USP units/ml heparin solution will be used during catheter construction. For maintenance of catheter patency, prepare a 10% (vol/vol) concentration of heparin solution in sterile injectable saline for a final concentration of 100 units/ml. The solution needs to be prepared fresh for every use.

Paraformaldehyde solution for intracardial perfusion: Prepare fresh daily 4% (vol/vol) paraformaldehyde (PFA) solution in 1X phosphate buffered saline (PBS) by combining 10 ml 16% aqueous PFA with 30 ml 1X PBS in a chemical fume hood.

Equipment Setup

Headplate (custom-made)—The procedure requires headplates designed to allow head-fixation of animals with neural implants. The design (Supplementary Figure 1) provides clearance on the skull for a range of neural implants and the extended screw plates allow manual handling as well as head-fixation. Headplates are cut with a CNC machine from 6061 aluminum sheets that are 0.031" thick.

Glass support rod (custom-made)—To create reusable ~5 cm long support rods for jugular catheter insertion, melt the ends of a glass capillary tube to blunt them following these steps:

1. Light a Bunsen burner and adjust until there is a stable flame.
2. While holding both ends of a 10 cm glass capillary tube with your hands or a pair of rubber tipped forceps, insert the center into the flame and pull the tube apart gently to sever the tube into two pieces.
3. Once the glass has cooled, re-insert each end of each rod to create 2 glass rods with smooth ends, of approximately 4–5 cm in length. Store the rods with the surgical instruments.

Alternatively, substitute single use ~$\frac{1}{8}$ inch diameter sip straws during surgery to support the vein.

Procedure

Preparation of jugular catheters TIMING 45 min for 10 catheters; drying time 24 hours

CRITICAL: Prepare catheters at least 24 hours in advance of surgery. Store the prepared catheters at room temperature (20–22 °C) in a dry and clean environment for up to 4 weeks.

CRITICAL: Always wear gloves and use clean tools to avoid any contamination.

CRITICAL: Refer to Figure 1a and Supplemental Video 1 for the finished products.

1. Clean and prepare your workstation by wiping first with 10% bleach and then with 70% ethanol.

2. With a clean razorblade and ruler, cut 4 cm lengths of polyurethane tubing, one for each catheter. Apply a bead of 2–3 mm in diameter of silicone adhesive around each piece of tubing at a distance of 1 cm from one end. Place the catheter upright in a centrifuge tube holder to dry at room temperature (Figure 1b, Supplementary Video 1 **[0:19–0:40]**). The silicone adhesive cures when exposed to atmospheric moisture; heat does not accelerate cure time and low humidity environments may prolong cure time. Typically, the silicone requires 7–8 minutes for the outermost layer to cure, and 24 hours for complete vulcanization.

   CRITICAL STEP: The size of the bead is important to plug the hole in the jugular vein where the catheter will be inserted, and to anchor the catheter in place following suturing.

3. While wearing safety glasses and working above a sharp disposal container, use scissors to trim away the sharp tip of a 26g syringe needle bevel (Figure 1c, Supplementary Video 1 **[0:40–0:53]**).

   CRITICAL STEP Do not cut the entire bevel but rather leave a wide opening to prevent clots or clogs from blocking the catheter at this junction.

4. Cut the metal needle shaft at the base where it meets the plastic needle hub and discard the plastic. The needle shaft should be approximately 1 cm long (Figure 1d, Supplementary Video 1 **[0:53–1:06]**).

5. Approximately 4 mm from the beveled end, bend the needle shaft using forceps or a hemostat into a ~100–120° angle (Figure 1d, Supplementary Video 1 **[1:06–1:12]**). Examine the metal needle shaft to ensure the opening is not crimped shut from cutting. Use flat forceps (Figure 2a, Supplementary Video 1 **[1:12–1:23]**) to reshape the needle opening into a circle if the opening was made narrow from cutting. To ensure the opening is clear from debris, insert the tip of another sharp 26g needle in the opening and twist to scrape away any blockages (Supplementary Video 1 **[1:23– 1:35]**).

   CAUTION A 90° angle may restrict the inner diameter of the shaft and cause clogging.

6. Insert the beveled end of the needle shaft into the opening of a piece of polyurethane tubing located further from the silicone bead until the tubing extends 1–2 mm past the bend in the needle shaft. Apply superglue at this junction and place the catheter upright in a clean centrifuge tube holder to dry at room temperature for at least 3 minutes (Supplementary Video 1 **[1:37–2:03]**).

7. Prepare a syringe to flush each catheter with ethanol by trimming the bezel of a 26 g hypodermic syringe needle above a sharp disposal container (Figure 1c), as described in Step 3.

8. Attach the needle to a 1 ml syringe and draw 70% ethanol into the syringe. Cut a ~3-inch piece of polyurethane tubing and attach it to the blunted ethanol syringe (Supplementary Video 1 **[2:05–2:17]**).

9. Flush each catheter with ethanol by inserting the metal tip of your custom- made catheter into the tubing attached to the ethanol syringe. Test for leaks in the catheter by using your fingers to gently pinch the end of the catheter shut and apply pressure with the syringe (Supplementary Video 1 **[2:18– 41]**). Discard the catheter if there is a leak.

10. Remove the catheter from the ethanol syringe and let each catheter dry upright in the centrifuge tube holder at room temperature until the ethanol has evaporated.

11. Trim the sharp tip of another 26g needle syringe (as described in Step 3), attach to 1 ml syringe, and draw undiluted 1000 units/ml of heparin solution. Attach a piece of tubing and flush the catheter with heparin. Remove the catheter from the heparin syringe, place it upright in a clean centrifuge tube holder, and let it dry and cure for 24 hours at room temperature before storing in a clean scintillation vial for up to 4 weeks before implantation.

Preparation of catheter caps TIMING 10 min for 20 caps

CRITICAL: Prepare an excess of caps relative to the number of catheters to ensure there are always caps available.

12. With scissors or a razor blade, cut 1 cm segments of polyurethane tubing (Supplementary Video 1 **[2:47–2:52]**).

13. Turn on a soldering iron and fan. Wet a sponge with deionized water to clean the soldering iron tip.

    CAUTION Only operate the soldering iron to melt the tubing in a well-ventilated area, a fume hood or a biosafety cabinet.

14. Hold a piece of tubing with flat forceps and melt one side of the tubing with the soldering iron to seal it (Supplementary Video 1 **[2:54–2:58]**). The final length of the sealed tubing should be about 5 mm.

    CAUTION Never use forceps with serrations (Figure 2b) or teeth (Figure 2c) to handle tubing as it may cause punctures). While sharp flat forceps (Figure 2d)

are suitable for handling tubing, their primary use should be reserved for piercing the jugular vein to maintain sharpness.

15. Clean the tip of the soldering iron as in Step 13 and turn it off when all the caps have been sealed.

16. Prepare a blunted 26g syringe as described in Step 7 and fill it with MilliQ water or 70% ethanol. Test each cap by attaching it to the syringe and applying pressure to test for leaks. The cap should be airtight and prevent inflow of liquid. If liquid enters the cap or escapes through a leak, discard the cap (Supplementary Video 1 **[2:59–3:25]**).

17. Store the caps for later use in a clean and dry environment at room temperature in a plastic box for up to 3 months. For ease of retrieval, caps may be attached to the sticky surface of lab tape in organized rows. (Supplementary Video 1 **[3:26–3:31]**).

Preparation for surgical catheterization TIMING 10–40 min

CRITICAL Don personal protective equipment (PPE), such as gloves, a lab coat or gown, a surgical mask, and protective eyewear.

18. Scrub the surgical area for 5 minutes with 10% bleach and then wipe with 70% ethanol. Sterilize surgical equipment with heat (glass bead sterilizer) or chemical methods (Cidex with 30 min contact time) and rinse with sterile water.

    CRITICAL STEP: Surgical tools and materials could also be autoclaved prior to the surgery, performing chemical or heat sterilization between animals. CAUTION Always adhere to the PPE and sterilization guidelines approved by the animal use programs overseeing your laboratory.

19. Prepare a 1 ml syringe with a blunted 26 g needle tip as described in Step 7, draw ~0.5 ml sterile saline, and attach a ~15 cm piece of polyurethane tubing.

    Take a prepared catheter (Step 11) and catheter cap (Step 17). Connect the metal vascular access point to the tubing attached to the saline syringe and test the catheter for clogging or leakage by flushing it with saline (Supplementary Video 2 **[0:05–0:12]**). It is important to test the catheter again between initial construction and immediately before the surgery in case the catheter became clogged by heparin salt residue after the undiluted heparin solution evaporated. Keep the catheter attached to the modified saline syringe. Spray the catheter, modified syringe, and caps with 70% ethanol and let dry.

20. Turn on the heating pad to provide the animal with heat during the procedure and lamp to provide surgeons with enough light to perform the surgery.

Anesthesia and surgical procedure TIMING 25 min - 1 hour per animal

21. Anesthetize the animal with an intraperitoneal injection of the freshly prepared ketamine/dexmedetomidine cocktail and verify that the animal is deeply anesthetized by pinching the foot to check for a pedal reflex.

! TROUBLESHOOTING

22. If there is no existing neural implant, remove the fur on the skull and on the chest above the jugular vein with an electric fur trimmer. Apply eye ointment to moisten and protect the animal's eyes during the surgical procedure.

23. Provide analgesia with a subcutaneous injection of 5 mg/kg carprofen.

24. Clean the skin on the head with chlorhexidine gluconate then 70% ethanol with sterile cotton-tipped swabs, wiping the skin in a circular motion spiraling outward from the initial contact point.

25. Make a skin incision on the head for the catheter's entry point. Follow option A for animals with an existing neural implant or option B for animals with no pre-existing implant:

- **A.** Incision on animals with an existing neural implant:
  - **i.** Inject 0.01 ml of lidocaine solution subcutaneously where the existing neural implant meets the skin at the corner that is most caudal and on the right side of the animal. The incision for the catheter to exit the skin will be made here.
  - **ii.** Use forceps to create a small opening of ~5mm diameter that will allow the catheter to pass through easily, including the silicone bulb.
- **B.** Incision on animals with no pre-existing neural implant:
  - **i.** Inject 0.01 ml of lidocaine solution subcutaneously at the center of the skin above the skull.
  - **ii.** Use surgical scissors to remove a circular piece of skin 7–10 mm in diameter above the skull, to accommodate the headplate.
  - **iii.** With forceps or cotton- tipped swabs, remove any connective tissue on the surface of the skull and dry it.
  - **iv.** Use a sharp 26 g needle to etch shallow grooves on the skull in a cross-hatched pattern to improve cement adhesion. The headplate will be adhered in step 50.

26. Turn the animal onto its back and clean the neck and chest with chlorhexidine gluconate followed by 70% ethanol as described in Step 26.

27. Inject 0.01 ml of lidocaine solution subcutaneously at the chest above the jugular vein where the incision will be made (Figure 3a).

28. Make a 1 to 2 cm long vertical incision on the chest to expose the tissue above the right jugular vein (Figure 3a, Supplementary Video 2 **[0:13–0:22]**).

29. Using forceps with teeth or serrations (Figures 2b, c), carefully separate the skin, fat, and muscle to expose the jugular vein which pulsates and has a dark red appearance (Supplementary Video 2 **[0:23–0:46]**).

CRITICAL STEP: Adequate clearance of the tissue above the vein will reduce the likelihood of the catheter being inserted into a sheath of connective tissue around the vein (Figure 3b *).*

CRITICAL STEP: Smaller veins will be present in the area, and care should be taken to avoid tearing them. If damage and bleeding from the other veins is a recurrent issue, the smaller veins may be ligated, but we rarely encountered this problem.

30. Insert a pair of superfine forceps (Figure 2a) into the chest opening to the left of the jugular vein (Figure 3a) and exit at the incision made on the head.

31. Grab the end of the catheter with the forceps and pull the catheter through the head incision and out through the opening in the chest (Figure 3a, Supplementary Video 2 **[0:47–1:02]**).

! TROUBLESHOOTING

32. To clear the tissue underneath the jugular vein before inserting the support rod, insert a pair of superfine forceps underneath and perpendicular to the jugular vein and gently puncture the connective tissue. After this, insert the support rod under and perpendicular to the vein by holding the support rod with your fingers in one hand and guiding the rod through the path that was cleared by the forceps (Figure 3a, Supplementary Video 2 **[1:03– 1:34**).

CRITICAL STEP: The support rod allows the surgeon to modulate blood flow through the jugular vein. Restrict the blood flow from the brain to the jugular vein by gently lifting the rod up and away from the chest to increase tension against the vein. If visibility of the jugular vein needs to be increased, the rod can be lowered gently into the chest to reduce contact of the support rod against the vein, thereby increasing blood flow and restoring the vein's dark red appearance (Supplementary Video 2 **[1:35–1:42]**.

CAUTION This step may tear the jugular vein if done too forcefully or with forceps that are too blunt; ensuring adequate clearance of connective tissue (Figure 3b) before this step will help.

! TROUBLESHOOTING

33. Loosely tie two ~5 cm lengths of flexible sutures around the vein, one rostral and one caudal to the support rod (Figure 3a, Supplementary Video 2 **[1:44–2:30]**).

CRITICAL STEP: Do not tighten these knots but rather keep them loose until they are ready to be tied quickly once the catheter has been successfully inserted into the jugular.

CRITICAL STEP: Ensure that the catheter is threaded through the rostral but not the caudal knot.

! TROUBLESHOOTING

34. Before puncturing the jugular vein, ensure that flat forceps are available on both, the left and right side, and that the catheter is filled with saline solution with no air in the tubing (Supplementary Video 2 **[2:30–2:39]**.

35. With the glass rod in your non-dominant hand, pull up gently to reduce blood flow through the jugular vein.

36. Position the support rod ~7 mm rostral to the clavicle, the bone that runs perpendicular to the jugular vein (Figure 3a, Supplementary Video 2 **[2:40–2:46]**).

37. Use your dominant hand to pinch the sharp Dumont #5 forceps (Figure 2d) and pierce the jugular vein ~1 mm caudal to the bend produced by the glass rod (Figure 3a), which provides support and resistance to perform the puncture. Puncture the vein at a 10° angle, almost parallel to the vein.

CRITICAL STEP: A parallel entry reduces the chance of puncturing an exit hole in the vein which will cause excessive bleeding and reduce likelihood of survival.

! TROUBLESHOOTING

38. Verify that the jugular vein has been punctured by loosening the grip on the forceps briefly to allow blood to flow. If there is no bleeding, the vein is unlikely to have been breached. Repeat steps 37 and 38 until successful.

! TROUBLESHOOTING

39. Keep the forceps pinched in the jugular vein to prevent bleeding while releasing the non-dominant hand from the support rod to prepare the catheter (Supplementary Video 2 **[2:47–3:27]**). Optionally, modeling clay may be placed underneath the support rod to maintain upward tension.

! TROUBLESHOOTING

40. With your non-dominant hand, eject saline from the catheter via the syringe that has been connected to the catheter since Step 19 so there is a small bead of saline at the end. Next, pick up the catheter with flat forceps, ensuring that the rostral suture knot does not unravel.

41. Position the catheter immediately next to the hole in the jugular vein. As the dominant hand loosens the grip on the sharp forceps to create an opening in the jugular vein, blood will start to flow. Insert the catheter into the jugular in the space between the tips of your forceps, ensuring that no air has entered the tubing. Once the catheter is ~3–5 mm deep, continue insertion while removing the forceps. Insert the catheter until the silicone bead plugs the hole in the jugular vein (Supplementary Video 2 **[3:28–3:45]**).

! TROUBLESHOOTING

42. While holding the catheter in place with one hand/set of forceps, use the other hand to inject 0.01–0.02 ml of saline via the syringe that has been connected

to the catheter since Step 20, then apply some negative pressure by drawing the syringe plunger 0.02–0.03ml to withdraw blood. If blood is readily withdrawn, the catheter has been successfully implanted and can now be secured in place (Supplementary Video 2 **[3:46–3:56]**). If blood cannot be withdrawn or is followed by an air, reposition the catheter until successful.

! TROUBLESHOOTING

43. Secure the catheter with sutures while keeping the hole in the vein plugged by the silicone bulb, as described in Box 1.

! TROUBLESHOOTING

44. Remove the support rod and clean the area by using cotton-tipped swabs to absorb any excess saline and blood in the chest cavity. Close the chest incision with interrupted sutures (approximately 1–2 mm apart) along the entire length of the opening (Supplementary Video 2 **[5:03–6:03]**).

CAUTION Do not tie the sutures too tightly or apply glue otherwise a scab may form that will cause discomfort.

45. Absorb any blood at the site of the wound with cotton tipped swabs then apply topical antibiotic ointment.

46. Turn the animal over to mount the vascular access point on the skull.

47. Flush the catheter by infusing 0.01–0.02 ml saline, then remove the modified saline syringe and tubing from the metal vascular access point with flat forceps. If the surgery has been successful, saline may appear to pulse in and out of the catheter, due to the heartbeat modulating the saline level in the catheter.

48. Using flat forceps, place a catheter cap onto the metal vascular access point (Supplementary Video 2 **[6:04–6:22]**). Once the cap has been successfully placed and it is airtight, the pulsing of the saline solution should stop. If the cap is leaky, replace the cap with a new one.*! TROUBLESHOOTING*

49. Cement the vascular access point to the headplate. Follow option A, which includes both Metabond and Lang dental cements, for animals without a pre-existing headplate, or option B, which involves only Lang dental cement, for animals with a pre-existing headplate.

CAUTION When mixing cement, avoid applying cement that is very wet. The solvent may dissolve or damage the tubing to create leaks.

CAUTION Monitor the animal’s breathing continuously during the cementing process (Steps 51–54). Excess pressure applied to the skull may obstruct breathing.

CRITICAL STEP: If the vascular access point is not perpendicular to the skull surface and is positioned at a lower angle, the implant may enter the animal field of vision (Figure 3c). *Furthermore, the tubing that attaches to the vascular access*

point is more likely to get caught on the headplate or be within reach of the animal paws.

! TROUBLESHOOTING

a. Securing the vasculature access point in animals without a pre-existing headplate:

i. Mix Metabond cement by combining 4 drops Quick base, 1 drop of Universal Catalyst, and 2 level scoops of L-Powder into a well, then stirring until the consistency is even.

ii. While the cement mixture is a thin liquid, apply a layer of cement on the skull and a layer on the surface of the headplate that will be adhered to the skull. Place the headplate onto the skull.

iii. As the cement thickens to a honey-like consistency, build up the cement in the crevices between the headplate and the skull then use flat forceps to position the catheter tubing on the skull and maintain its position.

iv. Add the remaining Metabond cement while the upright position of the vascular access point is maintained with forceps to allow the cement to cure while ensuring the vascular access point remains perpendicular to the skull's surface (Figure 3c).

v. Build the cement up the metal vascular access point, past the edge of the tubing, to secure it in place. Be careful not to apply too much cement that it would interfere with capping the vascular access point.

vi. Combine Lang Dental Jet Powder and Jet Liquid in a new, clean well in the well plate and mix until the cement thickens into a honey-like consistency. Apply a layer of Lang Dental cement over the Metabond cement, including onto the metal vascular access point.

vii. Hold the catheter in position with forceps until the cement cures.

b. Securing the vasculature access point in animals with a pre-existing headplate:

i. Combine Lang Dental Jet Powder and Jet Liquid in a well plate and mix until the cement thickens to a honey-like consistency and cement the vascular access point to the existing structure in an upright position (Supplementary Video 2 **[6:23–7:12]**).

ii. Build the cement up the metal vascular access point, past the edge of the tubing, to secure it in place. Be careful not to apply too much cement that would otherwise interfere with capping the vascular access point.

iii. Hold the catheter in position with forceps until the cement cures.

50. Clean any incisions made on the head as in Step 46. Apply Vetbond with the manufacturer-provided yellow precision applicator if needed to attach any loose skin to the cement (Supplementary Video 2 **[7:13–7:27]**).

51. Once the surgery has been completed, end the anesthesia with an intraperitoneal injection of atipamezole hydrochloride (Antisedan®).

52. Place the animal in a recovery cage that contains clean bedding on a temperature-controlled heat pad.

53. Once the animal has fully recovered from the anesthesia, prepare them to return to the animal housing room. For animals that will be housed singularly, follow option A. For animals that will be group-housed, follow option B.

A. Preparing for single housing:

i. The recovery cage will serve as the animal's new home cage. Provide wet chow and additional enrichment

B. Preparing for group housing:

i. Provide wet chow in a new cage with clean bedding.

ii. Install a protective covering over the animal's catheter and transfer the animal from the recovery cage to the group-housing cage.

Post-operative care TIMING 5 min per animal

54. At the end of the day after surgery, monitor the animal and allow undisturbed recovery unless there are signs of pain or blood in the catheter (Supplementary Video 3 **[0:05–1:20]**). The heparin coating inside the catheter (Step 11) should adequately thin the blood to allow for uninterrupted recovery from surgery. Excessive heparin delivery may thin the blood excessively and cause blood to spontaneously flow from the catheter whenever the cap is removed, increasing difficulty of catheter flushing.

! TROUBLESHOOTING

55. The day after surgery, repeat Step 55. If there are no signs of blood in the catheter, the animal does not need to be handled.

56. Two days post-catheterization, perform a health check and, after ensuring there is no air in the tubing, flush the catheter with 0.03 ml saline (Supplementary Video 3 **[0:05–1:20**

CAUTION: If any swelling, redness, or purulent discharge is visible, treat the animal for infection according to your animal use protocol.

! TROUBLESHOOTING

57. Repeat Step 56 each day until the catheter no longer needs to be maintained (i.e., the experimental endpoint or euthanasia).

! TROUBLESHOOTING

Peri-experimental care TIMING 5 min per animal

58. Before an experiment, flush the catheter with 0.03 ml saline. If there are any signs of resistance in the catheter, flush with 100 units/ml heparin solution.

! TROUBLESHOOTING

59. After the experiment, repeat the procedure in step 60. Use a new cap if any signs of resistance in the catheter were observed, otherwise the same cap may be used.

Euthanasia and patency verification TIMING 5–25 min per animal

60. Verify catheter patency through i.v. infusion of 0.03 ml 390 mg/ml pentobarbital into the vascular access point. The animal should lose consciousness in less than 3 s. If the animal is still awake after 10 s, inject an additional 0.05 ml pentobarbital intraperitoneally.

61. Perform a secondary method of euthanasia such as intracardial perfusion in a fume hood with freshly prepared 4% paraformaldehyde in 1x PBS or cervical dislocation. Immediately after the animal is intracardially perfused, an autopsy may be performed to confirm the correct placement of the catheter or to identify problems that may have occurred during the procedure, as described in Box 2.

CRITICAL STEP: While we did not check for patency before euthanasia, it is possible to check for catheter patency without euthanizing the animal via infusions of ketamine/midazolam[16] *or brevital.*

Troubleshooting

Troubleshooting advice is listed in Table 1.

Timing

Steps 1–11, preparation of jugular catheters: 45 min for 10 catheters. Steps 12–17, preparation of catheter caps: 10 min for 20 caps.

Steps 18–20, preparation for surgical catheterization: 10–40 min, depending on sterilization method.

Steps 21–53, anesthesia and surgical procedure: 25 min - 1 h per mouse, depending on surgical skill.

Steps 54–57, post-operative care: 5 min per mouse. Steps 58–59, peri-experimental care: 5 min per mouse.

Step 60–61, euthanasia, patency verification, and autopsy: 5–35 min per mouse, depending on the secondary method of euthanasia and whether autopsy is performed.

Anticipated Results

This protocol allows intravenous administration of drugs to freely behaving or head-fixed mice through an indwelling jugular catheter. Drug delivery can also be easily combined with optical imaging, electrophysiology and/or manipulation via neural implants. The protocol has been carefully optimized by thoroughly troubleshooting issues that arose with each cohort of mice and it is now an established and successful procedure in our lab. Animal survival has been greatly improved by piercing the jugular vein with sharp forceps rather than a guide needle, a method that limits blood loss before catheter insertion. We also maintain catheter patency up to 2 weeks post-catheterization, our experimental endpoint. In fact, of the 105 mice (59 males, 46 females) that underwent this surgery, 88% (51 males, 41 females) had patent catheters at the end of the procedure before euthanasia via pentobarbital infusion through the catheter. Moreover, there were no signs of infection (as assessed by looking for redness, inflammation, discharge, pain, and lethargy) in any of our implanted mice, either during the course of the experiments or at the time of euthanasia and autopsy.

JVC in freely behaving mice

This protocol was optimized to investigate the neural circuitry mediating the dose-dependent effects of nicotine. We confirm that i.v. delivery of nicotine causes the same dose-dependent effects on behavior as intraperitoneal (IP) injections of this substance by performing a conditioned place preference (CPP) assay with manual infusions of saline and nicotine (Figure 4a). We performed a 5-day CPP assay where animals freely explored a behavioral box with 3 chambers for 10 minutes on day 1 to establish a baseline preference score for each chamber. On days 2–4 (conditioning days), each animal is confined to one side of the box during the morning session and receives intravenous infusions of saline. During the afternoon session, each mouse is confined to the other chamber and receives intravenous infusions of nicotine. Each animal receives a total of 6 i.v. infusions at 5 minutes intervals during each 30-minute conditioning session. Each infusion contained 1.25 μl of solution per gram of body weight (0.03 ml/infusion for a 25g mouse, 0.18 ml total). Alternative infusion schedules are possible, not exceeding 0.2 ml total[28]. On day 5, animals were once again allowed to freely roam the entire box. By tracking animal locomotion during the pre-test and post-test, preference scores can be calculated by comparing the amount of time spent in the drug-paired chamber after conditioning compared to naïve exploration. Typically, over the course of the CPP assay drugs are administered by IP infusions which involves potentially aversive scruffing and needle-pokes; i.v. infusions through the jugular vein catheter overcome the adverse consequences of handling-related stress and pain and might therefore be advantageous for investigating aversive effects of drugs.

Additionally, i.v. infusions of small doses can be delivered based on chamber entry to perform real-time place preference, which is typically not possible with IP injections. Further, intermittent i.v. infusions may allow a more consistent concentration of drug in the circulatory system compared to a bolus IP or subcutaneous injection. Finally, intravenous access also eliminates potential complications from injections, such as injection directly into an organ or outflow from the puncture site which could affect the rate and amount of drug absorbed.

Head-mounted JVC implants are also compatible with IV self-administration experiments on freely behaving mice in operant chambers (Supplementary Video 3 **[1:21– 1:31]**), which is the gold standard for modeling voluntary drug use in mice. Additionally, i.v. infusions are compatible with experiments aiming at exploring drug effects on oral reward seeking. In this experimental set-up, animals are seeking an orally delivered reward and need to be treated systemically with a drug at a concentration that needs to be maintained constant for the entire duration of the behavioral session.

JVC during neural recordings in head-fixed mice

To understand the effects of low and high dose nicotine infusions on ventral tegmental area (VTA) dopamine (DA) neuron activity, a cohort of DAT- Cre animals were injected in the VTA with Cre-dependent GCaMP6m, a fluorescent calcium indicator[29]. A fiberoptic implant was implanted above the VTA along with a headplate and animals were allowed to recover for 4–8 weeks, to allow for adequate fluorescent signal to develop. After this time, animals underwent jugular vein catheterization and allowed to recover for at least 2 days. Finally, animals were head-fixed on a running wheel in a light- and sound-attenuating chamber and calcium transients were recorded for 10 minutes before and during intravenous infusions of nicotine or saline control (Figure 4b, c). VTA DA calcium transients were recorded for each animal while receiving saline solution, low-dose and high-dose nicotine on separate days (Figure 4d). The head-mounted jugular vein catheter allowed manual intravenous infusions of drugs during neural recordings, without unnecessary animal handling or needle-pokes, which may obscure the immediate effects of drugs. Head-fixed i.v. self-administration is also possible to mimic voluntary drug consumption, with behavioral outcomes similar to those of freely moving animals.

JVC and implanted cannulas for intra-brain and systemic drug delivery during fiber photometry

A head-mounted jugular vein catheter is also compatible with implanted cannulas to observe the effects of intra-brain infusions while systemically delivering drugs during fiber photometry recordings. To do this, we implanted cannulas bilaterally over the laterodorsal tegmentum (LDT) and injected dLight1.2, a fluorescent dopamine sensor[30]. The cannulas are required for the intrabrain delivery of the highly selective alpha7 nicotinic acetylcholine receptor (nAChR) antagonist (ArIB[31]) or saline solution. We also implanted fiberoptic implants targeting the lateral shell of the nucleus accumbens (NAcLat) in one hemisphere and the medial shell of the nucleus accumbens (NAcMed) in the other hemisphere (Figure 4e). Animals are allowed to recover for 4–5 weeks and then a head-mounted jugular vein

catheter is implanted to deliver high-dose nicotine (or saline vehicle). 6 i.v. infusions of nicotine are delivered at regular intervals of 5 minutes, 10 minutes after intra-brain infusions of ArIB, while recording dopamine transients in the NAcMed (Figure 4f). This experiment allowed us to observe the effects of alpha7 nAChR antagonism in the LDT on dopamine release in the NAc in response to manual i.v. infusions of high dose nicotine.

JVC during neural manipulations or recordings in freely behaving mice

To control or record neural activity with population specificity during systemic drug infusion, optogenetics and fiber photometry can be combined with a head- mounted jugular vein catheter. Behavioral experiments such as reward consumption or self-administration in freely moving animals in an operant chamber (Supplementary Video 3 **[1:21–1:31])** can be performed and the infusion tubing can be secured to the fiberoptic cables with tape to provide support. One limitation may be twisting of the infusion tubing and/or fiberoptic cables for long behavioral sessions in open arenas, but rotary joints that allow the simultaneous rotation of fiberoptic cables and fluid swivels are commercially available (Doric HRJ-OL_FC-FC) to prevent this issue. For i.v. self-administration experiments, a syringe pump can be used to automatically infuse a fixed amount of solution in response to animal behavior. Before and after the experiment, it is important to ensure that there is no clogging or resistance in the catheter by manually infusing sterile saline, otherwise animals may not be receiving infusions as expected. Additionally, for experiments that are sensitive to environmental cues that may accompany infusion, a dummy pump that is not connected to the animal may be set up as a control to generate the same environmental stimuli (sounds, vibrations) in the absence of an i.v. infusion.

JVC during *in vivo* electrophysiological recordings

Head-mounted jugular vein catheters can also be compatible with *in vivo* electrophysiological recordings, given the small footprint of the vascular access point. Optical fibers can be implanted to facilitate identification of opto-tagged single units during *in vivo* electrophysiology to record cell-type specific neural activity in response to systemic drug infusion. While optical fibers must be implanted at specific angles and locations to target the brain region(s) of interest, a vascular access point for a head-mounted jugular vein catheter can be placed anywhere on the skull, at any upright angle that accommodates the required neural implants. Similarly, the head-mounted catheter may be compatible with other bulky neural recording techniques and implants, such as prisms, windows, microendoscopy, and more. The catheter can be elongated or shortened during construction to alter the distance between the vascular access point and the silicone bulb to adjust for different placements on the skull (Figure 1a).

Blood sampling

Additionally, researchers may adapt the JVC technique for blood sampling. As described in the procedure, we were able to withdraw blood from the implanted catheters while checking for catheter patency; however, we immediately re-infused the blood without collecting any samples. For researchers interested in collecting blood samples via the jugular vein catheter,

0.25 ml collections have been achieved without apparent adverse effects in mice[32]. Traces of blood can easily clog the catheter thus adequate flushing is required after withdrawing blood and daily heparin infusions may be necessary.

Conclusion

Altogether, this technique provides direct access to the bloodstream in mice with neural implants, expanding the repertoire of experimental methods to combine optical recordings, optogenetic manipulations, electrophysiology, systemic drug infusion and behavioral experiments with intravenous drug administration and/or blood sampling. The small footprint of the catheter improves compatibility with a range of head-mounting methods including, but not limited to, headplates, head-rings, and head-posts. Catheter construction can easily be customized to accommodate limitations that may arise with large implants or recording methods that require sufficient clearance, such as *in vivo* electrophysiology or two photon imaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Box 1:

Securing the catheter with sutures (Timing: 2-5 min)

Procedure (Supplementary Video 2 [3:57–5:03]):

1. Whether the catheter is being held in place with forceps or by the tension generated from pulling the support rod up, use the free hand (fingertips or forceps) to place one end of the caudal suture (Step 33) in the grasp of your hand that is maintaining the catheter in place. CRITICAL STEP: This method will allow you to keep the catheter in place while tightening the knot.
2. Using the free hand, use serrated forceps to pull the free end of the caudal suture to tie the catheter in place.
3. Using both hands, quickly tie the rostral suture with your fingers or serrated forceps, securing the silicone bulb to plug the hole in the jugular vein.
4. Once the sutures have been tied, check for patency by infusing saline and withdrawing blood (Step 42): if blood cannot be withdrawn, the knots have been tied too tightly and must be slightly loosened until the catheter is patent.
5. After the knots have been verified to be appropriately tightened, double knot the sutures.
6. To ensure the silicone bulb stays in place, tie the caudal and rostral sutures together.
7. For added security, tie the suture ends that are diagonal to each other.
8. Trim the excess ends of the sutures so that only ~5 mm extends from the knots.

Box 2.

Transcardial perfusion for autopsy (Timing 5-15 min per animal

Procedure:

CRITICAL: During the perfusion and autopsy, take note of any signs of infection including but not limited to inflammation, redness, and discharge, especially near the chest and head.

1. Transcardial perfusion is performed by inserting a needle into the left ventricle of the heart and making an incision into the right atrium to allow 4% paraformaldehyde solution to flush the circulatory system.
2. To observe whether the catheter entered the right atrium, enlarge the incision in the right atrium. If the terminal end of the catheter is not visible, the catheter may have dislodged, it may have been inserted into the jugular vein too rostrally, or the distance between the silicone bulb and the terminal end of the catheter was too short. Modifications can be made to the puncture site or when constructing the catheters to prevent these problems.

! TROUBLESHOOTING

1. To check whether the catheter maintained its position in the jugular vein at the site of the puncture, make an incision on the chest over the vein.
2. Carefully clear away any tissue obstructing the view of the vein and observe whether the silicone bulb is secured in place by the sutures, and whether the vein remains intact. If the silicone bulb is no longer secured, proper modifications to the procedure can be considered.

! TROUBLESHOOTING

KEY POINTS:

1. This protocol details how to gain head-mounted access to the bloodstream to easily combine recording and/ or manipulation of neuronal activity with the reliable delivery of pharmacological agents.
2. Compared to other drug delivery methods, the intravenous technique explored here eliminates the stress and pain caused by needle pokes. The surgical and post-operative guidance provided in the protocol improves animal survival and catheter patency, increasing results' reliability and reproducibility.

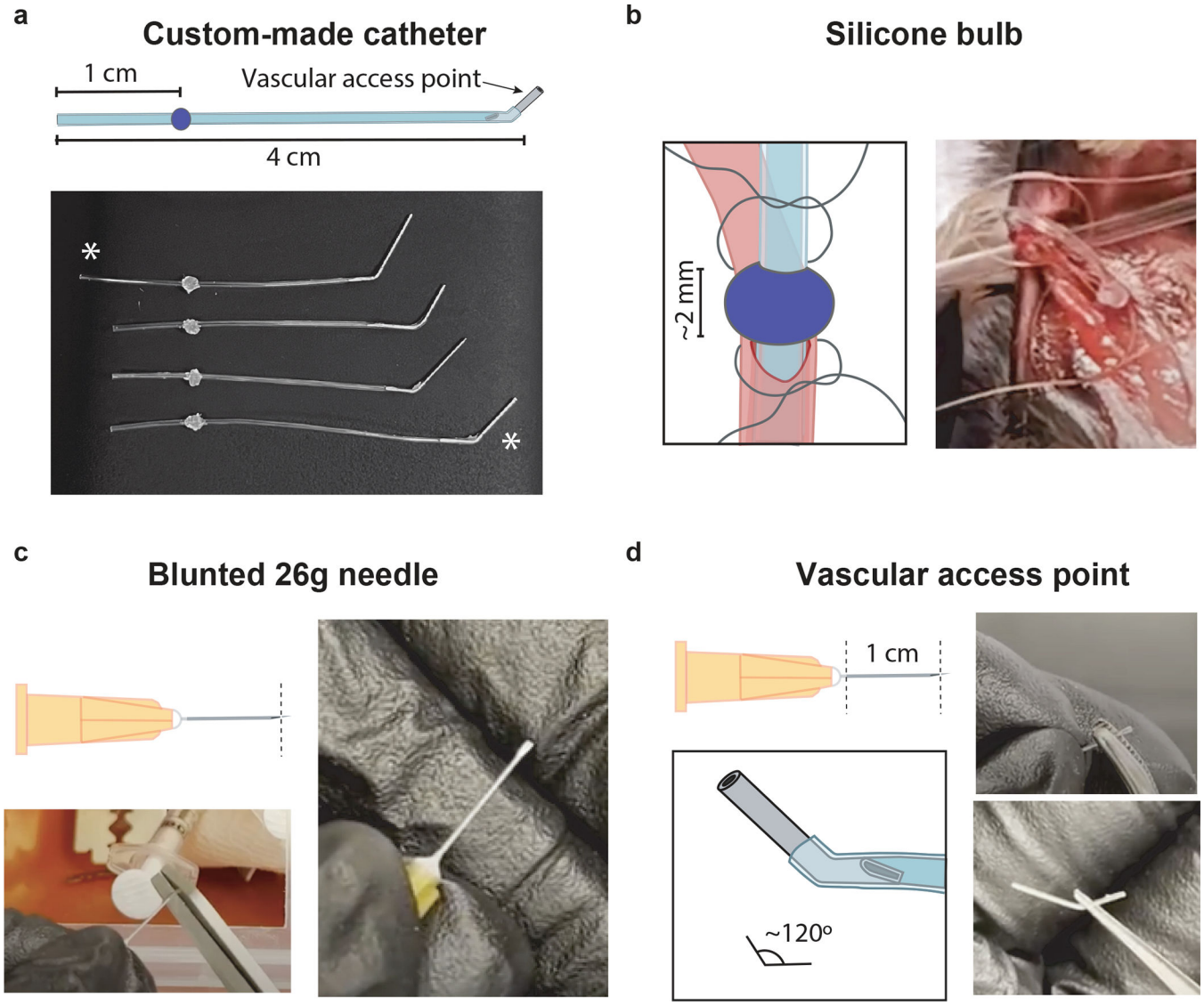

Figure 1. Catheter construction.

(a) Schematic and photograph of finished catheter with 4 cm long tubing and a ~2 mm diameter bead of silicone adhesive (in indigo) applied 1 cm from the end that will be inserted into the jugular vein. A modified 26 g hypodermic needle connected to the end of the tubing serves as the vascular access point that will be cemented to the skull. Asterisks denote catheters with modifications made to the overall tubing length and/or distance between the silicon bulb and vascular access point or terminal end of the catheter to demonstrate adaptability for different neural implants. **(b)** A schematic and photograph of the catheter inserted into the jugular vein where the ~2 mm diameter silicone bead will serve as a plug for the puncture site and anchor for sutures. After successful insertion, sutures tied rostral and caudal to the silicone bulb will secure the bulb in place. **(c)** A schematic and photographs demonstrating how the tip of a 26g needle can be blunted by trimming with scissors. Blunted needles are attached to any syringes that will be attached to the catheter, caps, or infusion tubing for the duration of the experiment and its preparation.

(d) Schematics and photographs for the construction of the metal vascular access point. A modified 26 g hypodermic needle is bent at a ~120-degree angle, with the larger beveled opening inserted into the catheter tubing.

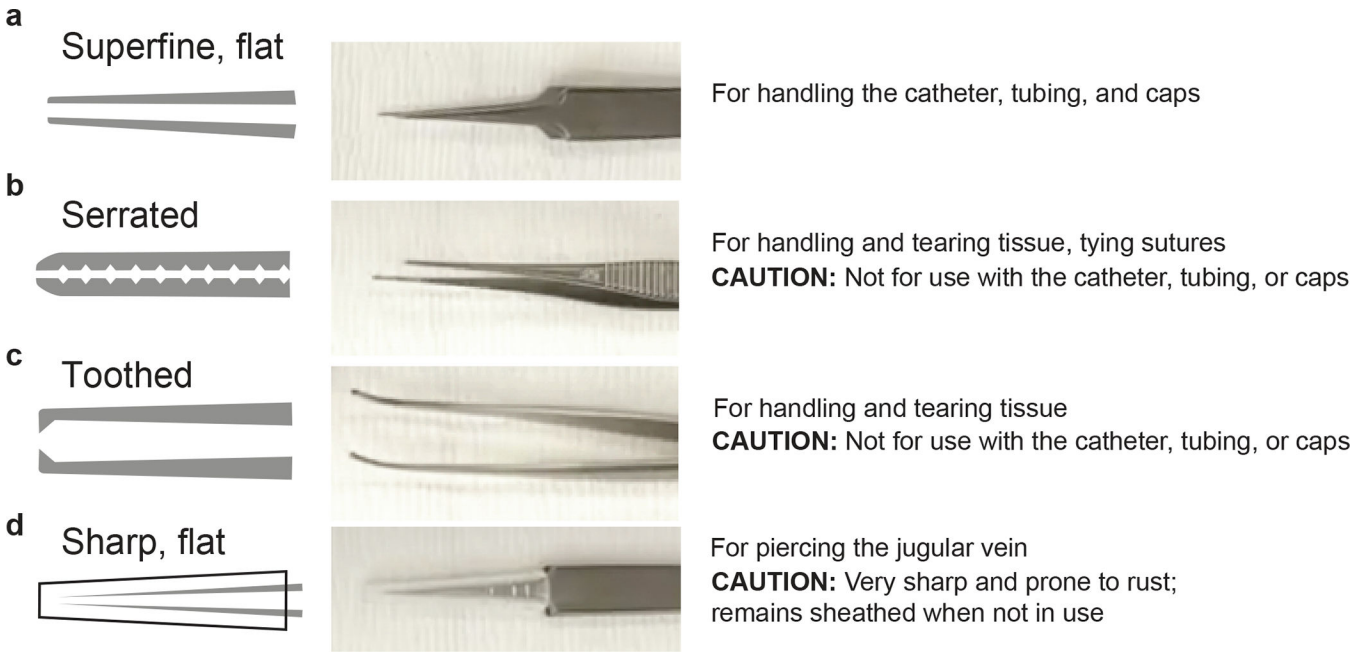

Figure 2. Features of forceps to consider based on the task.

(a) Serrated forceps are useful for handling skin during incisions, tearing adipose and connective tissue, and handling sutures. They should not ever be used to handle polyurethane tubing, as the serrations may cause micro tears that cause leakage. **(b)** Toothed forceps are advantageous for delicately tearing tissue above the jugular vein and handing skin for incisions. They should never be used to handle the catheter, tubing, or caps. **(c)** Superfine flat forceps are ideal for handling polyurethane tubing, fine adjustments to the placement of the sutures and catheter during the surgery, as well as clearing a path underneath the jugular vein to insert a support rod. For best results, use the forceps recommended in our equipment list; some other forceps are unable to securely grip the polyurethane tubing when wet. It may be handy to have two pairs of these forceps available during the surgery and for post-operative catheter maintenance. **(d)** Very sharp flat forceps are primarily used to pierce the jugular vein and should be kept clean and dry when not in use. To facilitate rapid insertion and securing of the catheter, these forceps may also be used to tie sutures and move the catheter tubing.

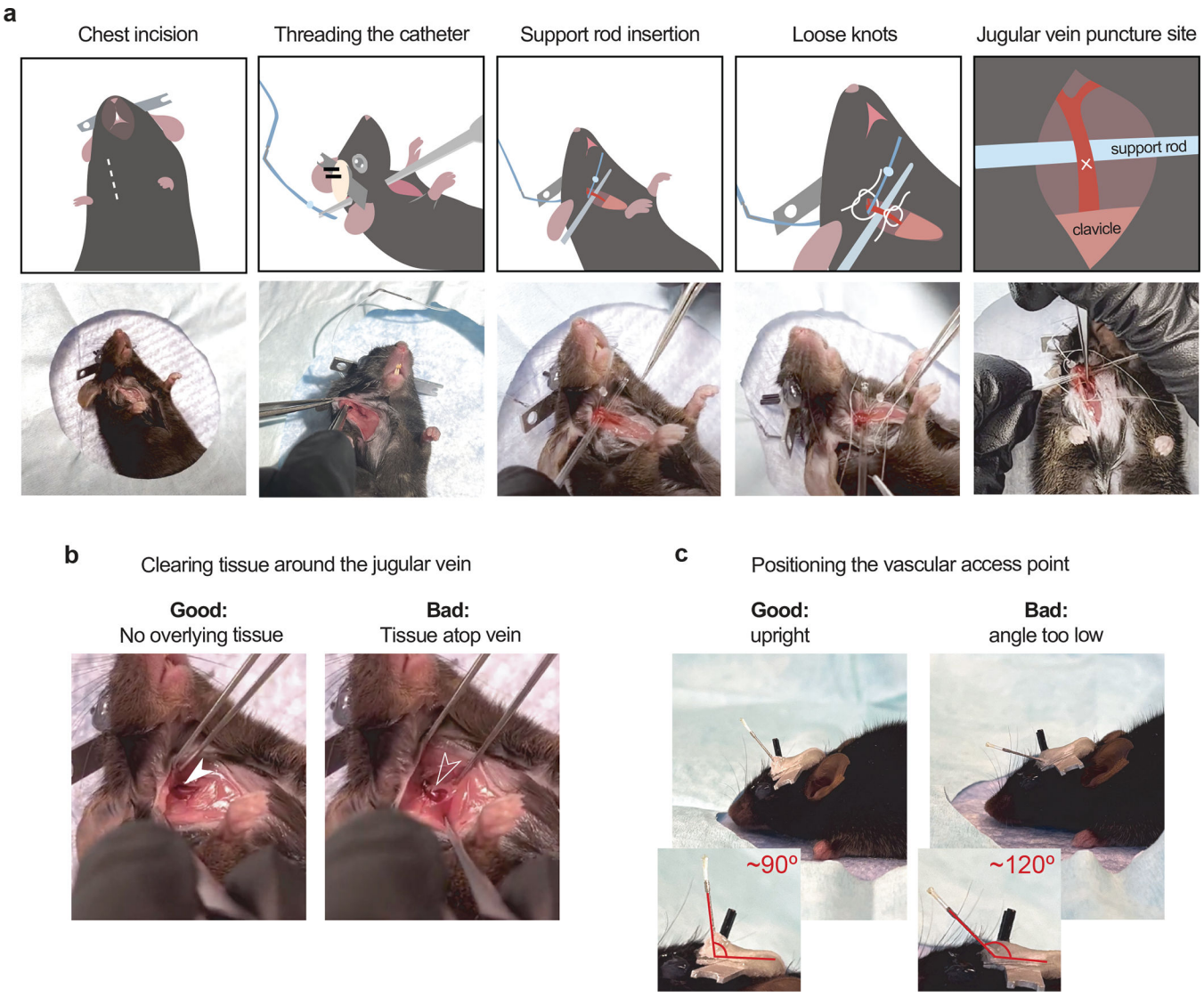

Figure 3. Critical surgical steps.

(a) Schematic (upper panel) and photographs (lower panel) summarizing the critical surgical steps. A chest incision is made (Step 28) to expose the jugular vein. The incision should start ~5–10 mm medial to the base of the right arm and extend 1.5–2 cm rostral to the base of the neck, and approximately 5–10 mm ventromedial to the base of the right ear. The catheter is then pulled by forceps from the chest cavity through the incision made on the skull (Step 25) by inserting the forceps to the left of the jugular vein (Step 30). The catheter is threaded through the shortest path and is situated above the shoulder, beside the neck (Step 31). A glass support rod is inserted underneath the jugular vein (Step 32) to support the jugular vein and modulate blood flow. Upon successful insertion of the catheter, the bulb needs to be secured at the hole made in the jugular vein by inserting two loose knots around the jugular vein rostral and caudal to the support rod (Step 33). The catheter is included in the rostral knot, positioned so that the knot is rostral to the silicone bulb. Finally, the jugular vein is pierced using sharp forceps (Steps 36–37). The goal is to puncture the jugular vein 1 cm from the atrium of the heart, but it is difficult to identify the ideal puncture site due

to the jugular vein can no longer be seen beneath the clavicle. As the support rod is pulled upward, the jugular vein will also stretch. **(b)** Left: Photograph of a jugular vein that has been adequately exposed (Step 29). The vein is unobstructed by any overlying tissue (white arrow), with minimal damage to the surrounding area. Right: Photograph of a jugular vein that remains covered with a small amount of tissue (empty arrow). Removal is performed by inserting forceps in the gap between the tissue and jugular on the lower right and gently lifting the tissue to separate it from the jugular vein. **(c)** Left: Photograph of an animal with an appropriately adhered vascular access point where the metal access point is positioned as perpendicular as possible to the surface of the skull. Right: Photograph of the animal with a vascular access point that is positioned too low and the angle between the surface of the skull and the vascular access point is not perpendicular. The implant hangs over the eye, increasing the likelihood that the mouse may be able to reach the implant with its paws. The implant must be maintained upright until the cement is cured to avoid sagging into this position.

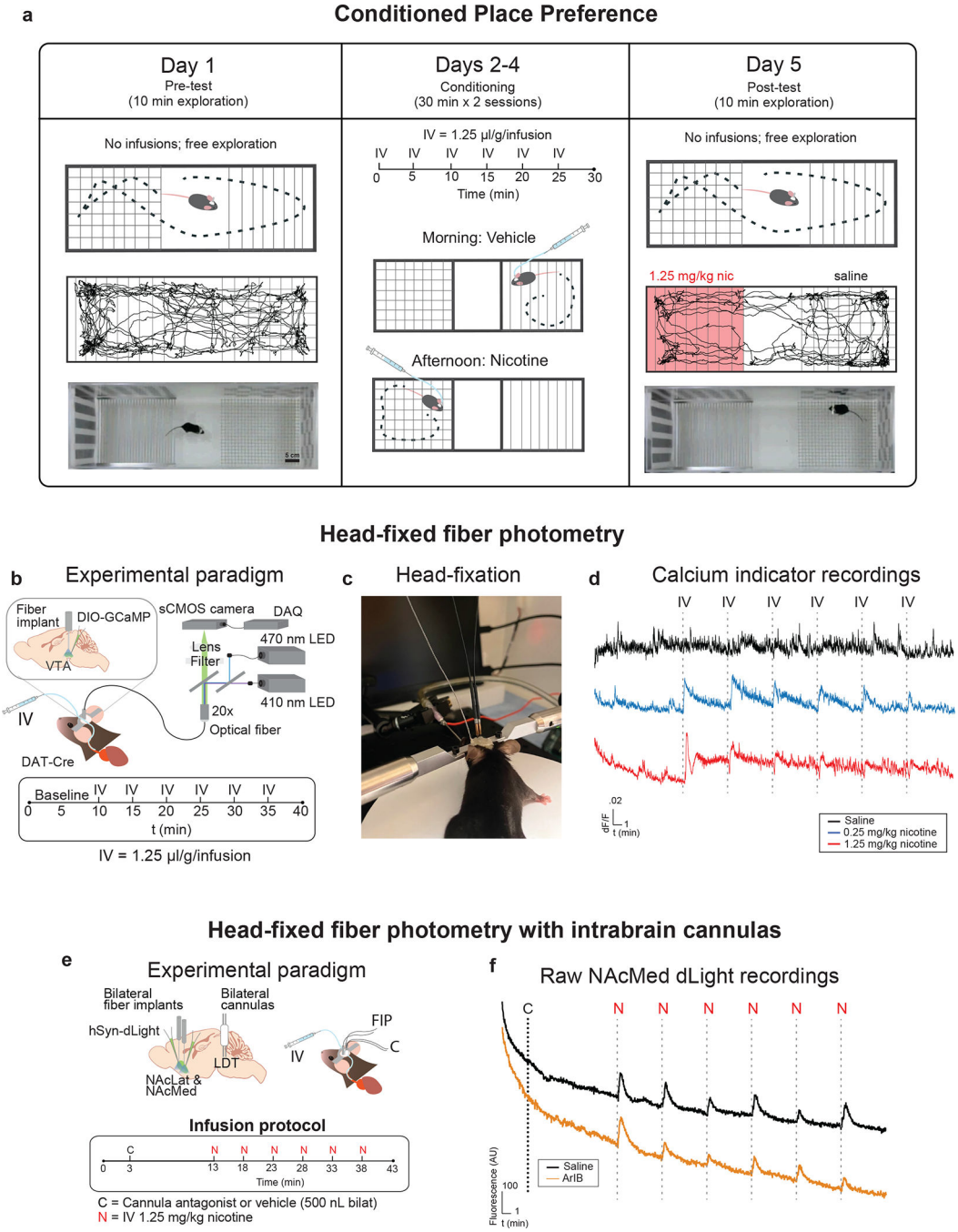

Figure 4. Sample data obtained from intravenous infusions during behavior and neural recordings.

(a) Schematic of a 5-day conditioned place preference (CPP) assay with drug delivery via an indwelling jugular vein catheter. **(b)** Top: Schematic of a DAT-Cre mouse injected in the ventral tegmental area (VTA) with AAV-DIO-GcaMP6m. Animals were implanted with a fiberoptic implant in the ventral tegmental area (VTA) and a head-mounted jugular vein catheter for the combination of fiber photometry during infusions of saline or nicotine. Bottom: Schematic of infusion schedule on head-fixed mice. Fiber photometry recording

starts 10 minutes prior to the first infusion of nicotine or saline control (Baseline). Over a 30-minute period, intravenous (IV) infusions are delivered every 5 minutes for a total of 6 infusions. **(c)** Photograph of an awake animal head-fixed on a running wheel with infusion tubing and fiberoptic cables attached. **(d)** Photometry traces from a single animal while infusions of saline (black), low dose nicotine (blue), or high dose nicotine (red) were delivered. **(e)** Top: Schematic for animals with bilateral injections of hSyn-dLight1.2 and fiberoptic implants targeting the lateral shell of the nucleus accumbens (NAcLat) and medial shell of the nucleus accumbens (NAcMed) on opposite hemispheres of the brain. Cannulas are implanted bilaterally above the laterodorsal tegmentum (LDT) to deliver intrabrain infusions of a nicotinic acetylcholine receptor (nAChR) antagonist (ArIB30) or vehicle in tandem with a head- mounted jugular vein catheter to deliver intravenous infusions of high dose nicotine. Bottom: Infusion protocol for intrabrain cannula infusions (C) occurring 3 minutes after the photometry recording begins, with a 10-minute interval before the first of 6 IV infusions of high dose nicotine (N). **(f)** dLight photometry traces from the NAcMed of an individual animal that received infusions of high dose nicotine with intra-brain pretreatment of the alpha7 nicotinic acetylcholine receptor antagonist ArIB (orange) or saline vehicle (black).

Table 1:

Troubleshooting table

Step	Problem	Possible reason	Solution
21	Mouse is overdosing from anesthesia.	Some mice are more sensitive to IP anesthesia, especially those under 20 g, females, and/or from certain strains.	Inject anesthetic cocktail subcutaneously or use isoflurane gas anesthesia, according to relevant animal use protocols and oversight.
31	Difficulty threading catheter from head to chest	Some forceps do not grip the polyurethane tubing well and slip	Use the recommended forceps in the equipment list.
		Path is not cleared properly	Use forceps to widen and clear the tissue that may be blocking the path for the catheter.
32	Bleeding while inserting support rod under jugular vein	Vein is ruptured when tearing connective tissue surrounding the jugular vein	Allow some connective tissue to remain around the vein if the vein is very fragile (i.e., in smaller animals, particularly females). Use sharper forceps to pierce the connective tissue under the vein with at least 2 mm clearance to avoid making contact with the vein itself.
33	Sutures do not stay in place around the jugular vein	Sutures may be too stiff or thick	Use the recommended sutures in the equipment list, or of equivalent flexibility.
37, 38	Unable to pierce jugular vein	Forceps may be too dull	Use only the specified Dumont #5 forceps. Do not use these forceps for any other purpose than piercing the jugular vein. Do not allow rust to form; clean, dry, and reheat promptly after surgery. Alternatively, use microscissors to cut a hole in the vein instead of piercing a hole with sharp forceps.
		Forceps may be entering sheaths of connective tissue	Remove as much connective tissue above the vein as possible before attempting to pierce the vein. Pierce the vein with adequate force and insert the forceps up to 5 mm inside the vein. If no blood emerges from the puncture site when the forceps tips are widened, try piercing the vein again. A proper puncture will allow the forceps to enter the vein over 5 mm -- this will also widen the puncture hole sufficiently to insert the catheter. If there is physical resistance when attempting to insert the forceps 5 mm into the vein, the forceps are likely in connective tissue and bumping into the clavicle.
38, 39	Excessive bleeding after piercing jugular vein	Blood flow is not restricted enough by the support rod	To slow bleeding, lift the glass support rod up to restrict blood flow. In some cases, the surgery can proceed when bleeding stops.
		Forceps have punctured an extra hole in the jugular vein	Lift the support rod to restrict blood flow until the bleeding stops. Take additional care to ensure that the catheter is secured in place in the jugular vein and does not exit the vein through the additional hole.
			To prevent puncturing the vein twice in future surgeries, pierce the vein with forceps at an angle parallel to the vein. This will guide the forceps into the vein itself rather than piercing an extra hole on the other side of the vein. Alternatively, use microscissors to cut a small hole in the jugular vein instead of piercing a hole with sharp forceps.
41	Difficulty inserting catheter into the jugular vein	The jugular vein may not be pierced	If there is resistance when inserting the catheter into the jugular vein and there is limited bleeding from the puncture site, the forceps may have punctured only the connective tissue around the vein and the catheter is being inserted into the connective tissue. Attempt piercing the vein again using the same puncture site.
		The puncture site in the jugular vein is too small to insert the catheter easily	If blood flows freely from the jugular vein when the forceps are loosened or removed, the jugular vein has been adequately pierced but the puncture site needs to be enlarged to allow insertion of the cathether. Insert the forceps approximately 5mm into the jugular vein to stretch the puncture site and create an opening large enough for catheter entry. Take care not to pierce an additional hole in the jugular by maintaining a near parallel angle during insertion and stopping if met with resistance.
42	Animal dies after flushing catheter	Air bubble in catheter	Ensure that there is no air in the tubing from the saline syringe to the end of the catheter. If necessary, an assistant surgeon may assist with ensuring a bead of saline at the catheter tip as the primary surgeon inserts the catheter into the jugular vein. Alternatively, modeling clay can be placed underneath

Step	Problem	Possible reason	Solution
			the support rod before the jugular is pierced to maintain upward tension as the surgeon prepares the catheter after the jugular vein is punctured.
42	Blood cannot be withdrawn into the catheter	Catheter is stuck in connective tissue sheath	If blood cannot be withdrawn, try repositioning the catheter by sliding it up (but not out of) the jugular vein and reinsert at a more perpendicular angle. The catheter may be in connective tissue, or if the vein was torn or punctured twice, the catheter may have exited the vein. If still unsuccessful, remove the catheter and insert the sharp forceps into the same puncture site to create a path for catheter insertion. As a last resort, another puncture may be made into the jugular vein caudal to the first puncture; the catheter may be trimmed to accommodate this shorter distance between the puncture and the heart. As long as there is no excessive bleeding, the surgeon should make as many attempts as possible to insert the catheter successfully.
		Catheter entered then exited the vein	Attempt to reposition the catheter without exiting the jugular vein by biasing the tubing upwards (and away from the likely exit hole) while inside the vein. If several attempts are unsuccessful, another entry point into the jugular can be created with the sharp forceps that is caudal to the initial punctures, taking care to create only one hole. The catheter may be trimmed to match the length of tubing between the silicone bulb and end of the catheter with the distance from the new entry hole to the atrium of the heart.
43	Difficulty tying sutures while keeping catheter in place	Unable to manage multiple demands with only two hands	An assistant surgeon may tie the sutures while the primary surgeon holds the catheter securely in the jugular vein or modeling clay may be placed to maintain upward tension by the support rod.
48	Blood emerges from catheter while recapping catheter	Blood pressure is too high and/or blood is too dilute	Blood in the catheter causes clogging. If a new cap cannot be placed before blood enters the catheter, it is possible that too much heparin has been given to the animal. Try head-fixing the animal and flush with saline then recap the catheter when the animal is calm. If bleeding persists, flush the catheter again and create a new cap for the catheter by using flat forceps to hold the tubing from the saline flushing syringe, then heat from a soldering iron to melt the tubing and seal it.
49	Animal dies while surgeon is cementing the vascular access point to the skull	The animal airway is obstructed due to excess downward pressure to hold the vascular access point in place	A small pillow made with a folded tissue and tape can be placed under the snout to raise the head or the animal may be head-fixed with a clean apparatus reserved specifically for surgery.
54	The animal is hunched, shaking, and/or displays hypolocomotion	The animal is experiencing pain and/or distress	Provide additional post-operative analgesia approved by your animal use protocol and/or contact veterinarian staff.
	Blood is visible in the catheter tubing	The catheter is not air-tight and blood is able to enter the catheter	Flush the catheter with 0.01–0.03 ml sterile saline and place a new cap on the vascular access point; blood may flow from the heart if the cap is not airtight. Animals can be handled by gripping the headplate or head-fixing them on a running wheel -- scruffing is not recommended for animal handling.
56, 57	Animal's chest incision has formed a scab	Sutures were tied too tightly	In future surgeries, tie the sutures only tight enough to secure the wound without restricting blood flow. If the scab appears to cause any discomfort or pain, consult your veterinarian for next steps.
56, 57, 58	Infusing saline into the catheter requires excess pressure; resistance is encountered when flushing the catheter	The catheter is beginning to clog	Flush the catheter with 100 units/ml heparin solution in increments not exceeding 0.03 ml every 3 minutes until the catheter does not exhibit resistance and recap the catheter with a new cap. Do not inject more than 0.3 ml heparin solution in one session.
56, 57, 58	Catheter is clogged	A clot has formed in the catheter due to suboptimal implantation	Clots are more likely to form in the jugular vein than in the atrium of the heart. Perform an autopsy to identify whether the catheter reaches the heart or terminates in the jugular vein. An autopsy will also reveal whether a clot, in the form of dark, dried blood has clogged the catheter. Try increasing the distance between the silicone bulb and tip of the catheter when making the catheters or pierce the jugular vein slightly more caudal and closer to the heart.
		A clot has formed due to deterioration of the catheter by solvents from cement	While cementing the vascular access point to the skull, the polyurethane tubing or superglue may be partially dissolved and weakened if the cement solutions are too thin. Air may enter the tubing to create clots. Create a thicker cement solution before applying to the skull.
		A clot has formed due to a puncture in the catheter	Only use flat forceps to handle the catheter caps. Serrated forceps may pierce holes into the caps, compromising the air-tight seal.

Step	Problem	Possible reason	Solution
		A clot has formed due to clotting factors in the blood	Catheters can be flushed daily with heparinized solution (instead of just sterile saline).
58	Catheter is not clogged, but drug effects are not observed	Jugular vein atrophy	If no drug effects are observed, and blood cannot be withdrawn from the catheter; the catheter is likely to be disconnected from the bloodstream. Perform an autopsy to identify whether the jugular vein is intact. The jugular vein may have atrophied, which is more likely to occur if multiple holes were pierced, or if the sutures were tied too tightly while securing the catheter. Try tying sutures less tightly around the jugular vein.
		Catheter no longer in place	If multiple holes were pierced in the jugular, the catheter may have exited the vein. Perform an autopsy to identify whether the catheter remained in place. Even if no additional holes were poked, the catheter may be displaced due to jugular vein atrophy. Increasing the distance between the silicone bulb and the end of the catheter may improve the likelihood that the catheter terminates in the heart and not inside the jugular vein which may reduce friction against the vein. Additionally, it is possible that the silicone bulb lost adhesion to the polyurethane tubing. If this is a common issue, consider purchasing catheters with pre-made bulbs, or adding a small amount of superglue to the silicone bulb during catheter construction.
		The puncture site on the jugular vein was not compatible with the length of the catheter	Researchers may need to adjust their jugular vein puncture site or the length between the silicone bulb and the terminal end of the catheter to ensure that the catheter terminates where the jugular vein meets the heart. For catheters constructed with 1 cm between the silicone bulb and the terminal end of the catheter, the ideal puncture site is approximately midway between the branching and the clavicle. Aim to pierce the vein ~5 mm rostral to the clavicle when the jugular vein is slack, and ~7 mm when the support rod is pulled up and the jugular vein is stretched. Pulling the rod upwards temporarily restricts blood flow and facilitates piercing the vein at a near parallel angle.
Box 2, Step 2	Autopsy reveals that the catheter terminates in the wrong position either too distant from the heart or too far into the heart	The sutures may have been tied too loosely, allowing the catheter to dislodge from the vein.	Tie the sutures anchoring the silicone bulb in the jugular vein more securely.
Box 2, Step 4	Autopsy reveals that the catheter is no longer in place in the jugular vein	The jugular vein has atrophied	If the vein looks altered (i.e., it looks stringy around the catheter or it is not visible around the catheter), it may have atrophied or disconnected entirely. Atrophy of the vein might be prevented by tying looser knots when securing the catheter (Step 45, Box 1) or by smoothing the end of the catheter with heat during catheter construction[15].
		The silicone bulb has detached from the catheter	Try a different silicone adhesive when constructing the catheters or purchasing pre-made catheters, if the silicone bulb has detached from the catheter.