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"A robust and simple catheter connector assembly for long-term self-administration experiments"

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Back mount jugular catheter vascular access port construction for long-term self-administration experiments

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ABSTRACT

Intravenous self-administration in rats is used widely to study the reinforcing effects of drugs and serves as the gold standard for assessing their use and misuse potential. One challenge that researchers often encounter when scaling up experiments is balancing the cost, time investment to construct, and robustness of each implanted catheter. These catheters include multiple components such as surgical meshing and a variety of entry ports designed to facilitate the connection of the rat to a catheter port tethering system. Other considerations include maintaining the catheters free of blockage during the extent of the drug self-administration experiment. These large-scale studies provide ample opportunity for the catheter system to fail. The failure and replacement of commercially purchased catheters leads to ballooning expenses, and the failure of in-lab manufactured catheters requires the manufacture of reserves, also increasing costs, as these handmade products are inherently more variable. We have developed a catheter system that combines a commercially available implantable back-mounted entry connector system with inexpensive medical items such as surgical mesh, sutures, and an air-tight back flow prevention system to bolster the overall success of self-administration experiments.

- Method to bolster commercially available jugular catheter components for long-lasting self-administration experiments.
- Reduces the overall cost per unit of self-administration experiments.
- Easily assembled by laboratory students and staff.

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Name and reference of original method:	Weeks, J. R. [4]. Experimental morphine addiction: method for automatic intravenous injections in unrestrained rats. *Science*, *138*(3537), 143–144.
Resource availability:	22-guage cannula connector, surgical mesh, medical grade catheter tubing, super glue, blunt tip 22-guage needle, small scissors, fishing line.

Introduction

Self-administration is widely accepted as a validated approach to modeling human drug taking behaviors in non-human animals. However, relative to experimenter-delivered protocols for injecting drugs, intravenous self-administration in rats has unique challenges. In a typical self-administration experiment, a rat undergoes surgery in order to implant a permanent indwelling jugular catheter. This catheter creates a delivery system to infuse drug solutions directly into the vascular system. One important challenge is the robustness of the implanted jugular catheter. As a self-administration experiment progresses, there are more opportunities for a catheter system to fail. This catheter failure often leads to the entire rat's data being removed from the experiment and needing to be replaced with another rat. These failures present a significant obstacle for researchers that model a prolonged history of intravenous drug taking.

Selecting the most appropriate type of connector for the rat's jugular catheter to the drug delivery system must factor in the skill of the experimenter connecting the rats to the drug delivery system, as well as the cost of the connector. Several companies have developed different buttons, adaptors, and ports. Each one has distinct hardware for tethering to a leash assembly and drug-dispensing syringe. A significant cost can be accrued through the purchase of individual sterile vascular access buttons and the associated custom-fit tether systems. For this reason, researchers often opt to manufacture their own in-lab ports and buttons out of available and less expensive medical components [3]. While this may be an effective cost-saving strategy, these catheter ports are relatively difficult to construct, must be rigorously tested, and may fail at a higher rate over time than commercial ports due to greater variability typical in lab/hand-made catheters.

As an alternative approach, purchasing some components from a company specializing in intravascular drug administration, combined with in-lab modifications, provides a reasonable compromise between cost and the need for reliable, straightforward catheter construction. Strengthening the junctions or interfaces where different catheter components meet (e.g., catheter tubing and the stainless-steel tube of a connection port within the rat's body) leads to a more robust system capable of withstanding potentially months of testing.

The patency of a catheter system is critical to the success of the experiment. Two sources of catheter occlusion are catheter intraluminal thrombosis and the formation of fibrin around the implanted tip. Intraluminal thrombosis refers to the clotting of blood within the catheter itself. Likewise, fibrin protein formation around the outside and opening of the implanted catheter end leads to blockage and loss of patency. Intraluminal thrombosis occlusion can be reduced by regularly flushing sterile anticoagulant solution through the implanted catheter and by reducing the amount of blood backflow in through the implanted end of the catheter. Fibrin occlusion can also be lessened by flushing a sterile anticoagulant solution, and ensuring the catheter is sterile before implantation. Therefore, adhesives and the components they join must be structurally unaffected by exposure to a gas or liquid sterilant, as the high heat and pressure from autoclave sterilization destroys most catheter materials. In addition, the use of a stainless-steel dummy or plug to seal the entry of the connector when not in use to keep the catheter closed and reduce intraluminal thrombosis is highly recommended.

In this protocol, we provide a step-by-step guide for assembling a system from commercially available catheter ports and other relatively inexpensive medical components that is well suited for large scale self-administration experiments (e.g., 440 rats). This catheter system is readily constructed by students and lab staff. In our hands, routinely 10 catheters are made in approximately an hour.

Method details

Subjects

440 male (~320 g) and female (~220 g) Sprague Dawley rats were implanted with the lab-made catheters described here. These rats were part of various experiments requiring nicotine self-administration, some already published [1]. In the referenced study, rats received a combined passive infusion followed by active infusion (i.e., self-administered) of nicotine at 0.03 mg/kg/~1 s of a ~40 µl infusion in 2-hr daily sessions. Sessions were conducted 7 days a week and the study with patency checks lasted circa 46 days. All procedures were reviewed and approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee.

Preparing components

Table 1 contains a list of suggested purchase locations for the individual components described below. Several items, such as blunt tip needles, surgical suture, and surgical mesh compatible with this catheter system are readily available from several suppliers.

Table 1
List of catheter components. Item name, description and catalog number (if relevant), one suggested retailer, and helpful notes.

Item	Description	Suggested Purchase Point	Notes
Tubing (for backport stopper)	0.024 × 0.064in (0.6 × 1.6 mm) PE/PVC tubing	https://www.instechlabs.com/products/tubing-connectors-pinports/tubing/co-extruded-PE-PVC	*N/A*
Fishing Line (for backport stopper)	0.029 in (0.74 mm) diameter monofilament	https://www.amazon.com/50lb-Omniflex-Monofilament-Fishing-Yards/dp/B00ARJPQOO	*Monofilament of this diameter should work regardless of brand.*
Catheter tubing	MRE 040 from BrainTree Scientific, 0.040″ OD x 0.020 ID (1.02 mm OD x 0.50 mm ID)	https://www.braintreesci.com/catheter-tubing-accessories/tubing/micro-renathane/ SKU: MRE040-S2050	*Each catheter will be 10 cm (female) or 10.5 cm (male) in length*
Prefabricated Connector	22-guage Back mounted connector port, 20 mm height	https://protechinternational.com/products/22-gage-back-mount-pedestal-313-000bm-20-5up?_pos=6&_fid=31a99be3a&_ss=c	*The* 22 G *steel tube entry site and side tube should be* 5 mm *above the pedestal.*
Anchor bead tubing	MRE from BrainTree Scientific, ID 0.050″–0.066″	https://www.braintreesci.com/catheter-tubing-accessories/tubing/micro-renathane/micro-renathane-065-x-030-per-ft	*Just large enough to fit over catheter tubing.*
Detail scissors	Laboratory or craft scissors	https://www.fiskars.com/en-us/crafting-and-sewing/products/scissors-and-shears/renew-detail-scissors-no4-1063073	*4 in (101.6 mm) detail scissors*
Blunt Tip (for flushing)	22 G Blunt tip needle for flushing	https://www.fishersci.com/shop/products/22g-needle-5in-luer-strl-25pk/NC0192755">https://www.fishersci.com/shop/products/22g-needle-5in-luer-strl-25pk/NC0192755	This link is for a manufacturer. This item is common and can be found in many retailers.
Mesh	Pore size of 1 mm is suggested. From this manufacturer, it is part: U-CMY-1010	https://componentsupplycompany.com/product-pages/polyester-screening-mesh.php?gad=1&gclid=CjwKCAjw-eKpBhAbEiwAqFL0mge6BOVXlNsnY8dK1pDWk0bZfWQRTwogsbhj0cPtmhEuRe2qtxFb8BoCn0UQAvD_BwE	*This link is for one possible manufacturer*
Dust Cap Cover	Part C313CAC	https://protechinternational.com/products/dust-cap-cover-crystal-applicator-c313cac?_pos=1&_sid=eea7ee63d&_ss=r	*The cap prevents dislodging of the backport stopper*

Venous catheter

Two options are available for the indwelling portion of the catheter tubing. As one option, prefabricated round tip-end catheters already cut to length are available from a manufacturer. These prefabricated catheters are individually packaged and are already sterilized (SAI Infusion Tech: RJVR-23). The advantage of prefabrication is that it reduces in-house variability in construction quality and decreases overall assembly time. Further, commercial providers state that round tip-end catheters reduce trauma to the vascular endothelium thus increasing the time that a catheter remains patent. The rats used in experiments reported here were implanted with a rounded tip catheter, which can be purchased from the supplier listed in Table 1. A second and more cost-effective option is to construct and assemble the venous catheter in-house, which we also detail in the next section. In addition, the experimenter can fabricate the catheter more flexibly, adjusting its length to account for the rat's body size which can vary by age and biological sex. The catheter lengths reported here were developed in consultation with veterinary staff and postmortem analyses of catheterized rats. Experimenters should work with their institutional veterinarian staff to determine best catheter dimensions when designing self-administration experiments.

Either type of venous catheter can then be adhered and anchored to the side tubing of a prefabricated connector. While others have noted the advantage of using a smaller diameter, more flexible tubing for jugular catheterization [5], in our hands microrenathane (MRE, 0.63 mm inner diameter x 1.02 mm outer diameter) is less likely to collapse when being ligated to the vein during surgery. However, we note that no comparative analysis between catheter material or diameter was done in the rats that we catheterized here, and thus limit our discussion.

Constructing the venous catheter

Use a clean and sharp razor to cut the venous tubing to a desired length. In this example, the MRE 040S tubing is cut to a length of 10.5 cm for male rats and 10 cm for female rats; one end cut to a 45-degree beveled angle. Using a permanent marker, mark the position of the bead anchor on the venous tubing. This bead anchor (seen in Fig. 1) provides a stable suture point at the entrance of the jugular vein to prevent the catheter from slipping out of the vein at the incision point. The length from implanted tip of the catheter to bead anchor (i.e., the indwelling portion of the catheter) should be adjusted based on the size, sex, and strain of the rat. For our adult male and female Sprague Dawley rats, the distance from the catheter tip to the bead anchor is 2.5 cm (males) and 2.3 cm (females); the beveled end and ink marker indicating the location to attach the bead can be seen in Fig. 1a.

Prefabricated 22-guage cannula connector

The prefabricated connector can be purchased from Protech International (Fig. 1b, part number: 313000BM-15–5UP) with a 25 mm mesh disk pre-attached below the side tube. The single mesh disk, designed to allow tissue to grow into its weaving and secure the cannula in place, leaves the side tube exposed directly to the rat's tissue, possibly being an irritant. A second disk is then attached over the side tube, providing two mesh surfaces (above and below the side tube) as areas where rat tissue can grow into the

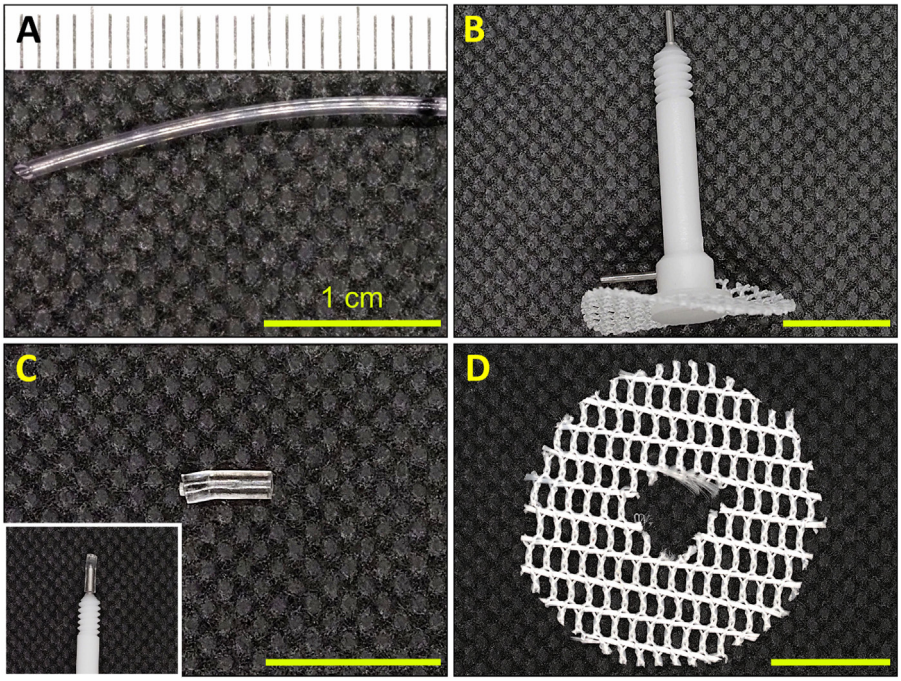

Fig. 1. Individual components for assembly. **(A)** Implanted end of catheter tube, note the beveled end on the left and black mark on the right-hand edge of the image indicating future location of anchor bead. Ruler for scale. **(B)** Commercially available 22-guage cannula connector with steel side tube clearly visible on the left above mesh. **(C)** Backport Stopper, the left side has the fishing line inserted 2 mm. **(D)** Surgical mesh cut in a circle, with an opening that will fit it to the connector pedestal port.

mesh, reducing possible irritation and further securing the entire catheter system. The pedestal height reported here is 20 mm tall. This connector has two open ends at 90-degrees to each other. One end of the connector is implanted into the rat ("the side tube") and will have the catheter tubing attached and anchored. The 20 mm pedestal end protrudes from the rat allowing the experimenter to easily pinch-grip the catheter hub when attaching or removing a rat to or from its infusion tether.

Backport stopper

In the present report, we describe an in-lab made plug (the "backport stopper") that prevents blood backflow. The backport stopper is a critical component that fits over the 22-guage entry of the pedestal end; this stopper creates a tight seal at one end and should be reattached immediately following self-administration or flushing procedures. The attachment of this backport stopper is made even easier by the extra height of the connector. The stopper is made by inserting the fishing line (Zebco® Omniflex Monofilament, 50 lb test, 0.029 in [0.74 mm]) 1 to 2 mm into the PVC tubing (Instech PE/PVC Tubing, BTCOEX-22 [0.61 mm]). Friction will often prevent it from going further. Note that the outer diameter of the fishing line is 0.13 mm larger than that of the tubing and must be so in order to ensure a snug fit. Using a razor, cut the fishing line so that it is flush with the tubing. The piece of fishing line that is now inside one end of the tube will seal that end of the tube. Cut the non-fishing line sealed tubing end to be 2 mm long (Fig. 1c). This completed backport stopper will fit tightly around the 22-guage opening of the catheter connector to prevent any blood backflow (Fig. 1c, inset image). Note that the fishing line itself does not enter the 22-gage steel tubing of the backport connector. We recommend that a threaded dust cap (Protech International, part number C313CAC) be secured to the top of the backport cannula when the rat is not connected to a tether. This cap prevents the rat from an intentional or incidental dislodging of the backport stopper and can be seen in the completed catheter assembly (Fig. 3A).

Mesh disk, anchor bead, and suture

Use small detail scissors to cut a 25-mm diameter circular disk from Dacron® surgical mesh followed by cutting a hole (~10 mm) in the center of the mesh disk. This cut will allow the mesh to fit over the connector pedestal (Fig. 1d). Further, create one "catheter anchor bead" by cutting a ~1 to 2 mm length of Micro-Renathane® "bead tubing" (0.065 inches x 0.030 inches) with a razor. Surgical silk suture (4–0) should be cut into 4 individual 5-cm sections.

Assembling the components

Securing the catheter bead to the indwelling catheter

Position the catheter bead near the 2.5-cm mark. Gently squeeze the tube of cyanoacrylate glue until a drop forms at the end of the applicator. Wipe this drop onto the 2.5-cm mark then carefully slide the anchor bead over the glue with fingers or forceps.

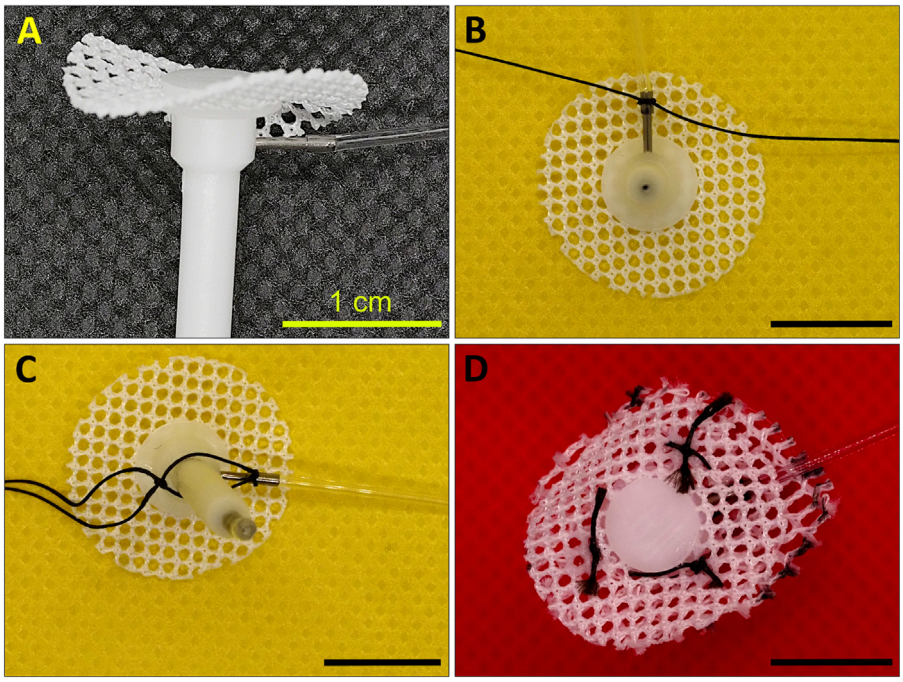

Fig. 2. Assembled components of complete catheter button. **(A)** Steel side tube of the prefabricated connector with catheter tube friction fitted. **(B)** Top view of the connector with overhand square knot securing steel side tube to catheter tube and **(C)** slightly angled view clearly showing the knot around the plastic pedestal of connector. **(D)** Bottom view of connector showing three complete individual suture knots securing the two mesh disks to each other.

Each assembled catheter can then be hung to dry vertically by sticking the catheter to the side of a shelf with a small piece of laboratory labeling tape or adhesive office tape. Other tapes with stronger adhesives will damage catheters. Do not apply any tape to the indwelling portion (i.e., between the beveled end and catheter bead) of the catheter.

Attaching and anchoring the catheter tube to the prefabricated connector

The non-beveled end of the catheter should be slid on to the shorter end of the prefabricated connector (i.e., the side metal tube) using fingers or small forceps (Fig. 2a). Add a small drop of cyanoacrylate glue at the junction of the cannula and catheter to secure the parts together. To anchor the catheter to the side tube of the connector, tie a section of silk suture using an overhand knot (make this knot twice so it holds) on top of both the catheter tubing and steel cannula (Fig. 2b). Wrap both sides of the suture behind the back mount connector plastic and tie an overhand square knot twice to secure; cut off the excess suture and apply a drop of cyanoacrylate glue to all 3 knots (Fig. 2c).

Attaching the mesh disk to the prefabricated connector

Fit the surgical mesh disk over the long end of the connector, sliding the disk down until it rests on the mesh already pre-attached to the connector. Note that both meshes will sandwich the attached and anchored catheter tubing. Attach the mesh disks together with silk suture by making a “U-stitch” through the holes of both mesh disks. A square knot, done twice, is sufficient to secure the two disks to each other (see Fig. 2d). Further secure the knot by carefully applying a drop of cyanoacrylate glue on the knot. Three of these “U-stitch” mesh-locking knots should be tied; the knots from an underneath view of the connector can be seen in Fig. 2d.

Testing and sterilization of constructed catheter connector

Preparing a flushing tip

A side view of the completed catheter system with cap can be seen in Fig. 3a. To prepare the flushing tip, slide a 3.5-cm length of MRE catheter tubing over a blunt tip 22-gage needle until it covers the tip by 1 cm (the individual components can be seen in Fig. 3b). The tubing will be secured to the blunt tip needle with a slip fit. The blunt tip needle with tubing will then be attached to a 1-ml syringe filled with flush solution. The open end of the flushing tip tubing will connect to the 22-gage entrance point of the catheter connector. The flushing tip tubing will provide a tight seal with the entrance point, before, during, and after the flushing solution is pushed through, preventing any backflow while in place.

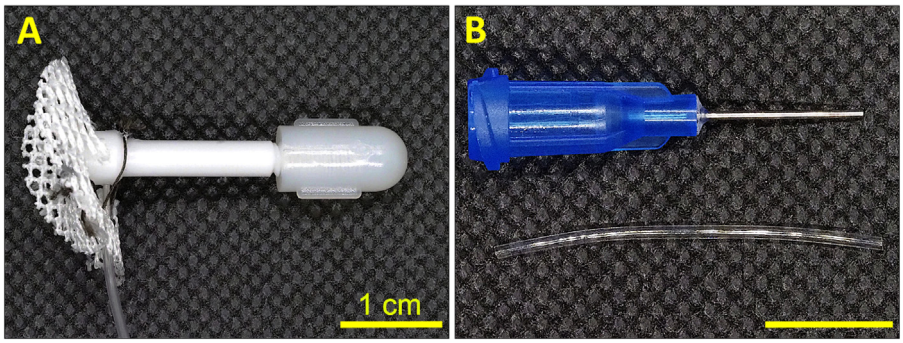

Fig. 3. Final assembly and flushing tool. **(A)** Completed catheter connector with cap (stopper bead underneath cap). **(B)** Blunt-tip 22-guage needle tip (**top)** that can be fitted with a 3 cm length of MRE catheter tubing (**bottom**) for daily flushing.

Quality control for potential leaks

Once the glue is fully dry on the catheter system, attach the flushing tip to a 1-ml syringe filled with distilled water. Connect this flushing tip to the 22-guage catheter connector to form a seal. Firmly depress the plunger, moving water completely though the catheter system. The water should flow unimpeded and without leaking at the junction of the cannula and catheter tubing. If there is a leak, this is often because the catheter tubing is not pushed far enough onto the side metal tube. Push the catheter tubing up further onto the side metal tube and reapply a drop of glue.

Sterilization

The catheter components cannot be heat-and-steam sterilized (i.e., autoclaved) and therefore the experimenter must use alternatives methods to keep the components sterilized. The experimenter should work with their institutional veterinarians to develop a sterilization protocol that does not damage the catheter components. Examples of in-lab manufactured catheter sterilization, using a "cold" (i.e., liquid) sterilant, such as a 2.65% glutaraldehyde solution, can be found elsewhere [3].

Catheter implantation and tethering

Before implantation, the outside and inside of the catheter unit should be rinsed with sterile saline to hydrate the MRE tubing and remove excess ethanol. Guides on jugular catheter implantation and tethering animals have been described in detail elsewhere [2,4]. The catheter system's connectors are designed to easily friction fit to flared tubing inside of Protech International spring tethers (part C313CS/NIT/SPC), which secure via a threaded end.

Maintaining catheter patency

Parameters for determining patency

440 male and female Sprague-Dawley rats were used to determine catheter patency. During recovery from surgery, catheters were flushed with 0.1 ml of sterile saline mixed with heparin (30 U/ml; Midwest Veterinary Supply) for 7 days. Preceding each experimental session, catheters were flushed with 0.1 ml heparin (30 U/ml) in sterile saline and after each experimental session catheters were flushed with 0.1 ml heparin (30 U/ml) + Baytril (enrofloxacin, 5.0 mg/ ml) in sterile saline. These rats were part of several intravenous drug self-administration experiments. Catheter patency was tested by infusing catheters with 0.1 ml of 10 mg/ml xylazine (followed immediately with flush of heparinized sterile saline) and then observing the rats for signs of pronounced ataxia within 30 s. Rats that did not display ataxia within 30-*s* were deemed non-patent even if they displayed ataxia shortly thereafter. Of these rats tested, 387 (88%) were deemed patent 46 days after the catheter implantation surgery.

Conclusions

Here we provide a step-by-step guide to easily adapt a prefabricated catheter connector into a catheter system to be used for long-term rat self-administration studies. The ease of pinch-gripping the tall connector end, removing the back port stopper and connecting the flared self-administration tubing results in less stress on the rat and experimenter. This method generates an inexpensive and robust catheter system for large-scale self-administration experiments that a lab can easily reproduce and maintain.

Ethics statements

All procedures and experiments complied with the ARRIVE guidelines and the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). All procedures and experiments were approved by the Institutional Animal Care and Use Committee at the University at Nebraska-Lincoln.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Mauricio Suarez: Conceptualization, Methodology, Visualization, Investigation, Writing – original draft, Writing – review & editing. **Sergios Charntikov:** Conceptualization, Methodology. **Y. Wendy Huynh:** Conceptualization, Methodology. **Scott T. Barrett:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Rick A. Bevins:** Conceptualization, Writing – review & editing, Supervision, Resources, Funding acquisition, Project administration. **Ken T. Wakabayashi:** Writing – review & editing, Supervision, Resources, Funding acquisition, Project administration.

Data availability

No data was used for the research described in the article.

Acknowledgments

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References

[1] S.T. Barrett, A.T. Flynn, Y.W. Huynh, R.A. Bevins, Appetitive Pavlovian conditioning of the stimulus effects of nicotine enhances later nicotine self-administration, J. Exp. Anal. Behav. 117 (3) (2022) 543–559, doi:10.1002/jeab.740.

[2] J. Feng, Y. Fitz, Y. Li, M. Fernandez, I. Cortes Puch, D. Wang, S. Pazniokas, B. Bucher, X. Cui, S.B. Solomon, Catheterization of the carotid artery and jugular vein to perform hemodynamic measures, infusions and blood sampling in a conscious rat model, J. Visual. Exper. 95 (2015) 1–6, doi:10.3791/51881.

[3] M. Suarez, E.J. Cantrell, K.T. Wakabayashi, C.E. Bass, Assembly of an inexpensive rat jugular catheter button based on a split-septum needleless intravenous system, MethodsX (2023) 102433, doi:10.1016/j.mex.2023.102433.

[4] J.R. Weeks, Experimental morphine addiction: method for automatic intravenous injections in unrestrained rats, Science 138 (3537) (1962) 143–144 New York, N.Y., doi:10.1126/science.138.3537.143.

[5] J. Yang, J.-M.I. Maarek, D.P. Holschneider, In vivo quantitative assessment of catheter patency in rats, Lab. Anim. 39 (3) (2005) 259–268, doi:10.1258/0023677054307033.