A Method for Chronic Intravenous Infusion of Fluids in Unrestrained Rats

by

R. PICKENS and J. DOUGHERTY
A METHOD FOR CHRONIC INTRAVENOUS INFUSION OF FLUIDS IN UNRESTRAINED RATS\textsuperscript{1}

Roy Pickens and John Dougherty
Psychiatry Research Unit
University of Minnesota

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I. Introduction

The purpose of this note is to describe a device which is used in our laboratory for chronic intravenous injection of drugs in unrestrained rats. The device is essentially a tube with one end inserted in a vein near the animal's heart and the other end connected to a remote syringe or infusion pump. Drugs can be injected through the tube directly into the circulatory system. The device also has design features which allow the animal freedom to move and turn about in the cage.

The major components of the device consist of (1) venous catheter, (2) harness, (3) leash and swivel unit, and (4) connecting tubing. The catheter is a small silicone rubber tube that enters the animal's precava via the posterior facial vein and passes subcutaneously to an exit on the dorsal neck. The harness, which is implanted under the skin of the back, provides a point of attachment for the catheter to a needle-tubing leash that passes out a hole in top center of the animal cage. The upper end of the leash contains a leak-proof swivel-joint that is connected by rubber and vinyl tubing to an injection pump. This paper presents details for construction and assembly of all components of the device, as well as a description of the surgical procedure for implantation of catheter and harness.

One use for the device is in studying self-administration of drugs by animals. In these studies the device is connected to an infusion pump and the animal is placed in an operant conditioning chamber. Responding by the animal is programmed to operate the infusion pump, which delivers a specified volume of drug solution through the catheter tubing and into the animal's bloodstream.

The device is based in large part on features of similar devices originally described by J.D. Davis (1966) and J.R. Weeks (1962), and on features developed by Roy Pickens at the University of Mississippi working in collaboration with W.F. Crowder. It is a revised version of the method reported by Pickens (1967).

Many of the improvements in construction are attributable to the work of Earl Olson and Steve Arhelger of the Psychiatry Research Unit Shop. The surgical procedure partly reflects the contributions of Laird Miller, Dave Muchow, Bob Yokel, Dr. Wayne Harris, and other students and staff of the Psychiatry Research Unit.
II. Construction of Intravenous Cannula

Materials:

1. Silastic silicone rubber tubing (Dow Corning):
   a. one 15-cm. length .012 inch I.O. x .025 inch O.D.
   b. one 25-cm. length .025 inch I.D. x .047 inch O.D.

2. Silastic 382 Medical-Grade Elastomer, with Catalyst M (Dow Corning). One 1-lb bottle for general use. Ask for two extra vials of Catalyst M for each bottle of elastomer.

3. Adhesive/Sealant #RTV-102 (General Electric). One for general use.

4. Syringe (6 or 12 ml) with blunted 20 or 22 ga. needle, and one 25 ga. needle.

5. Surgical silk (#3-0 and #5-0), one spool each for general use.

6. Ivory Snow soap, one box for general use. General laboratory supplies, including scissors, adhesive tape, shallow stainless steel or ceramic dish, spoon, beaker or cup.

7. Toluene.

Assembly of Cannula:

1. Wash the outside of both sizes of rubber tubing with hot soapy (Ivory Snow) water. Also flush the inside of both rubber tubes with hot soapy water using syringe with appropriate size needle attached to tubing. Attach syringe to one end of larger tubing. Immerse free end of larger tubing about 10 cm into toluene for about 30 seconds. Then, draw some hot, soapy water into the enlarged lumen and insert the smaller tubing into the larger tubing as far as possible, but no more than 10-12 cm. Twisting the smaller tubing and keeping both tubes well soaped will facilitate entry. Flush with tap water after tubing has resumed normal size and then aspirate the water from the tubing.
2. Tie a length of #5-0 silk snugly around the larger tubing about 2 mm. from the junction of the two tubes. Taking care not to constrict the lumen of the smaller tubing. Use a square knot and cut off the ends of the silk. Tie additional knots at 2 cm. intervals along the larger tubing. The final knot should be about 1 mm. before the point where the smaller tubing terminates inside the larger tubing (see Figure 1). Flush with tap water and check for leaks and/or blocks.

Coating of Cannula

Coating cannula with elastomer prevents ballooning of cannula during infusion. Coating also serves to protect the cannula from destruction by the rat's claws.

1. Dry cannula. Cut several strips of adhesive tape about 1 inch long.

2. Pour about 2 tablespoons of elastomer into a shallow dish. Add 2-3 drops of Catalyst M with an eye dropper and mix thoroughly.

3. Coat the larger-tubing part of the cannula with elastomer by drawing and twirling the larger tubing through the elastomer, picking up an even coat about 1 mm. thick. Also coat the junction of the large and small tubes with elastomer (Figure 2). Immediately wipe off any elastomer adhering to exposed part of smaller tubing. Hang up cannula using the adhesive tape.

4. Immediately cut two lengths of 3-0 silk, each about 20 cm. long. Tie one of the lengths through the elastomer to the larger tubing about 2.5 cm. above the tubing junction. Do not cut the ends of the silk. Tie the other length of silk 2 mm. above the tubing junction. See Figures 3a and 3b for details.

5. Allow cannula to cure just until it can be touched without adhering to one's finger. Lay cannula on clean flat surface and bend end to form a half circle as illustrated in Figure 4. Use adhesive tape strips to hold formed cannula in place.

6. Allow cannula to harden until it retains this shape (about 1/2 hour). Trim off any excess of elastomer and touch-up any exposed spots with RTV-102.
7. Next day, wash cannula in detergent. Cure cannula until catalyst odor can no longer be detected. (Catalyst M is irritating to body tissues. Using incompletely cured cannulas may promote the formation of subcutaneous edematous sacs and granulation tissue with possible total encapsulation of cannula.)

III. Construction of Subcutaneous Harness

Materials (for either disposable or reusable harnesses):

1. Disposable type: 2.5 cm. x 1.75 cm. curved plate cut from Nalgene conventional polyethylene one-liter bottle (#32-1000).

![Fig. 5](image)

2. Reuseable type: 1.2 cm. x 2.0 cm., .020 inches thick stainless steel plate. Drill two 3/32-inch holes as illustrated.

![Fig. 6](image)

3. Harrison's Teflon Mesh (C. P. Bard, Inc.): One 3.0 cm. x 2.5 cm. piece (Catalog #3050).

4. Nylon binding head screws, size 2-56 x 1/2-inch, and nylon hex nuts, size 2-56 (Product Components, Inc.). Two screws and six nuts for each unit.
Harness Assembly

Disposable:
1. Cut out polyethylene plate and round all sharp edges with sharp knife.
2. Punch two small holes in polyethylene plate to align with holes in external back harness (see Figure 7 for dimensions), allowing for spread of screws due to curvature of plate.

Disposable and reusable:
3. Cut out teflon mesh.
4. Push screws through Teflon mesh then through holes on concave side of steel or polyethylene plate. Attach hex nuts and tighten.

IV. Construction of Leash-Swivel Unit (Type I and Type II)

Type I

Materials:
1. Needle tubing, 21 ga. One 4-inch length.
3. Needle tubing, 21 ga. One 12-inch length (NOTE: The actual length of this item will depend on cage dimensions. The length given is for a cage 8" high by 9" long by 8-1/2" wide. Longer, higher, and/or wider cages will require a longer length).
4. Needle tubing, 18 ga. One 11-inch length (see NOTE above).
5. Teflon tubing (Becton-Dickinson Co.). Two 1/8-inch lengths of .027 inch I.D. x .051 inch O.D. tubing (#6458).

6. Screws, machine, brass, #4-40 x 1/4". Two screws.

7. Nuts, brass, #4-40. Two nuts.

8. Spring, expansion type, medium flexibility, 3/8" diameter, 1/2" long. One spring.


11. Teflon rod, 1/4" diameter. One 1-inch length.

12. RTV adhesive-sealant, RTV #102 (General Electric Co.). One tube.

Procedure for Assembly

1. Smooth and round edges of all needle tubing using a fine file.

2. Slip one 1/8-inch length of 18 ga. needle tubing over one end of the 4-inch length of 21 ga. needle tubing. Solder needle tubing together using as little solder as possible. Clean the joint.

3. Slip the remaining 1/8-inch length of 18 ga. needle tubing over one end of the 12-inch length of 21 ga. needle tubing. Solder together with a minimum of solder. Clean the joint.

4. Slip the 1/8-inch lengths of Teflon tubing behind the 18 ga. needle tubing pieces, as shown in Figure 8.

Figure 8. Preparation of ends of 21 ga. needle tubing
5. Place the 3/8-inch diameter Teflon rod in a lathe. Drill a 9/16-inch deep hole in the center of one end of the Teflon rod using a #8 drill bit. Drill a hole through the remainder of the rod using a #66 drill bit. Thread the wall of the #8-size hole using a 1/4-20 tap. Clean the piece of all Teflon particles. (see Figure 9).

6. Place the 1/4-inch diameter Teflon rod in a lathe. Drill a hole 3/8-inch deep in the center of one end of the rod using a #45 drill bit. Drill a hole through remainder of the rod using a #66 drill bit. Put 1/2-inch of threads over the end containing the #45-diameter hole using 1/4-20 die. Clean the piece. (see Figure 10).

![Fig. 9 Preparation of 3/8-inch Teflon piece](image)

![Fig. 10 Preparation of 1/4-inch Teflon piece](image)

7. Insert the 4-inch length of 21 ga. needle tubing into the 1/4-inch diameter Teflon rod. and the 12-inch length of 21 ga. needle tubing into the 3/8-inch Teflon rod. The ends of the 21 ga. needle tubing containing the short length of 18 ga. needle tubing and the Teflon tubing should be pulled into the #45 holes of the Teflon rods.
8. Coat the threads of the Teflon rods with RTV and screw the rods together. Wipe off the excess RTV. (see Figure 11).
9. Slide 11-inch length of 18 ga. needle tubing over the 12-inch length of 21 ga. needle tubing. Push the 18 ga. tubing up to touching the 3/8-inch diameter Teflon rod (swivel). Bend the excess 21 ga. tubing at opposite end 90 degrees. At 18 ga. and 21 ga. needle tubing junction solder the head of a #4-40 brass screw. Let the solder overlap the screw head, 18 ga. tubing, and 21 ga. tubing (see Figure 12).

Fig. 12 Construction of leash

10. Attach the spring to the machine screw with a #4-40 nut.

11. Cut and drill the stainless-steel piece as shown in Figure 13.

12. Attach the stainless-steel piece to spring with the #4-40 screw and nut. (see Figure 14).
Fig. 13 Construction of stainless steel back piece

Fig. 14 Details of connection of leash to stainless-steel back piece
13. Curve 4-inch length of 21 ga. needle tubing (on top of swivel) to form 90 degree bend. (see Figure 15).

Fig. 15 Details of top of swivel

14. Figure 16 shows completed leash-swivel unit.

**Type II**

**Materials:**

1. Needle tubing, 21 ga. One 13-inch length. (Actual length depends upon dimensions of experimental chamber. Length quoted is for a 9 x 8.5 x 8-inch high rat chamber).

2. Needle tubing, 18 ga. One, 11.5-inch length. (Again, actual length depends upon dimensions of experimental chamber. Length quoted is for a 9 x 8.5 x 8-inch high rat chamber).


4. Nylon screw, #8-32 x 1/2-inch or longer.

Fig. 16 Completed swivel-leash unit

7. Steel spring, 1/8-inch diameter. One 3/4-inch length.

8. High chemical resistance stainless steel plating, 1-inch x 1/4-inch x .021 inch thick.

9. Drill bits: #42 and #69.

10. Two steel fuse clips.

11. Solid wire solder and muriatic acid.

12. Linen-filled bakelite sheet, 1/32-inch thick.

13. Nylon nuts and bolts, #3/32 x 1/2-inch (Product Components).


Assembly:

1. Cut the head from the nylon screw. Use a #42 drill bit to make a 1/4-inch long cylindrical hole in center of screw. Use a #69 drill bit to make a hole that continues through the remaining 1/4-inch of screw. (see Figure 17).

2. Use a #69 drill bit to make a center hole in the .093-inch diameter stainless steel cylinder. Cut the cylinder to a 3/16-inch length. Slip the cylinder over the 21 ga. needle tubing until it is flush with the end. Use acid to etch the steel before soldering and then solder. Be sure that the solder does not plug the needle tubing. Slip two 'O' rings onto the needle tubing.
1. Insert the needle tubing through the large-diameter hole end of the nylon screw.

Fig. 18

2. Slip the 18 ga. needle tubing over the 21 ga. needle tubing, and etch and solder. Leave 1/2-inch of 21 ga. tubing exposed at the screw end and 1 inch exposed at other end.

Fig. 19

3. Bend the free inch of 21 ga. tubing 80°. Slip spring over tubing and etch and solder.

4. Cut a 1-inch x 1/4-inch piece of steel plate and smooth edges and drill two 3/32-inch diameter holes 5/8-inch apart. Etch and solder the free end of the spring onto center of plate. Bend the plate slightly to correspond to the curvature of the rat's back. (see Figure 20, next page).

5. Smooth the outside hub of a 21 ga. stainless Luer-Lock needle and tap the inside of the needle barrel with a #10-32 tap (Figure 21). Flatten the nylon screw as shown in Figure 22 and screw the threaded part of the screw into the needle barrel.

Fig. 20

Fig. 21

Fig. 22
Fig. 20  Swivel-leash unit

- NYLON SCREW
- SOLDER
- 18 ga.
- SPRING
- EXTERNAL PLATE
- TOP VIEW
8. Solder the fuse clip to the outside of the smoothed 21 ga. needle barrel. Cut 5-8 strips of linen-filled bakelite (1 x 6 cm.). Drill 3/32-inch holes, 1/4-inch from each end and also in the middle. Loosely attach the bakelite strips together end to end with nylon screws and nuts, forming a chain.

9. Leaving the female Leur-Lock intact, solder the other 21 ga. needle to the fuse holder and attach the bakelite chain. Cut an appropriate length of Teflon tubing and slide it over the needles. Loosely tie the Teflon tubing to the bakelite chain with loops of surgical silk. Figure 23 shows the completed system.

Fig. 23
10. Pump connections: Fit the female Leur-Lock of the 21 ga. needle into a male Leur-Lock adapter anchored over the center of the top of the experimental chamber. The use of a rotating adapter (#625, Becton Dickinson) provides an additional swivel, but this is not essential. Attach the blunted 21 ga. needle to the other end of the male adapter and connect to an infusion pump with Teflon tubing.

11. Pumps: Teflon tubing can be connected to syringe pumps without further modifications. Piston pumps (Milton-Roy Co., Instrument Mimirpump #196-31) provide the best results. These pumps require an adapter and a back-pressure valve. The accuracy is good over a wide range of adjustable output rates.

V. Surgical Procedure for Implanting Cannula and Harness

Materials and Instruments:

1. Cannula (see Section II).
2. Subcutaneous harness (see Section III).
3. Leash & Swivel unit (see Section IV).
4. Rat, at least 90 days old.
5. (a) Methoxyflurane anesthetic (Metophane, Pitman-Moore Laboratories) or (b) Pentobarbital sodium solution (Nembutal, Abbott Laboratories).
6. Atropine sulfate in saline or water, 25 mg/ml.
7. Heparin sodium, 1,000 units/ml (Upjohn Company).
8. (a) Penicillin, Benzathine penicillin G (Bicillin) 300,000 units/ml (Wyeth Laboratories) or (b) Tetracycline, water soluble (Polyotic, American Cyanamid).
10. Nitrofurazone powder .2%, (Furacin, Eaton Laboratories).
11. Sterile saline for injection, 500 ml bottle.
12. Syringe, 1-ml. Two disposable or one glass, with 25 ga. needles.


15. Scalpel #3 or #7, with blade #15 or #10. One each.

16. Halstead mosquito forceps, 5-in. One curved and one straight. (May substitute one small Derf needle holder for the straight mosquito.)

17. Iris scissors, 4-1/2 inch.

18. Tissue forceps, serrated tips. One broad and one fine.

19. Suture needles:
   (a) Cutting edge, 1/2 circle, small (Anchor 1831-4)
   (b) Taper point, 1/2 circle, small (Anchor 1943-22)

20. Trocar, 17 cm., 8 ga. needle tubing, with 2 cm. sharpened bevel on one end.

21. (a) Chromic gut, 5-0, with taper point needle. (Ethicon U-202H)
   (b) Plain gut, 4-0, (Ethicon M-301H) or 5-0 surgical silk.

22. Stainless steel pans, 2 each, approximately 9 x 5 x 2 in. deep.

23. Rubber tubing, 3/8-inch diameter. Two 3 ft. lengths; one for anesthesia administration and one for artificial respiration.

24. Small animal electric clippers, with size 40 head.

25. Towels (at least two), non-sterile gauze sponges, surgery lamp, table, chair.

26. Teflon mesh squares, 5 x 5 mm., 2 each.

Surgical Procedure:

1. Inject 10-15 mg/kg atropine sulfate, i.p., 10 min. before anesthetizing animal.

2. Soak surgical instruments, cannula, harness, etc., in 500 ml. tincture of Zephiran in a steel pan for 5 minutes. Rinse instruments in a pan containing 500 ml. saline (non-sterile) and place on a towel to dry.
3. Cut off the tip of a needle cover of a 6 ml. disposable syringe case, and insert the cut-off needle cover into one end of 3-ft. rubber tube. Attach the other end of the tube to an air jet. Place approximately one-half of a gauze sponge inside one syringe case and saturate it with methoxyflurane. Hold the rat's nose near open end of syringe case and allow a slow flow of air to push the anesthetic gas out.

![Fig. 24](https://example.com/image1.png)

4. After rat is anesthetized (does not respond to loud noises), clip hair from one side of the ventral neck, from mandible to sternum, midline to shoulder. Do not cut off the vibissae. Clip the hair from a 4 cm. wide band from mid-back to behind the ears. Re-anesthetize the rat as necessary.

5. Transfer the methoxyflurane-soaked sponge to a 6 ml. syringe case with intact needle cover. Use this for further anesthesia.


7. Wet the skin on back and make a 2-3 cm. transverse incision about 1.5 cm. (a finger width) behind scapulae. Use curved forceps to separate a channel between skin and muscle to a point on the dorsal midline 3 cm. anterior to the incision. Insert trocar into channel and, using bevel as a "bed", make a small (.5 cm.) incision and push trocar through. Remove the trocar, cover incisions with gauze sponges and turn the rat on its back.

![Fig. 25](https://example.com/image2.png)
8. Wet the neck skin and make a 1-2 cm. incision at an angle to the midline. See Figure 26a. Use curved mosquito forceps to bluntly dissect subcutaneous tissue and fascia in a downward direction.

(a) Posterior facial vein cannulation: Dissect down between submaxillary and diffuse parotid salivary glands. See Figures 26b-d. Free the p. facial vein from membrane by opening forceps points on top of and parallel to the vein (Figure 26e). Insert the trocar under vein and lift. Strip all other tissue from vein wall with the fine tissue forceps (Figure 26f). Remove the trocar and separate the submaxillary gland from underlying muscle to provide a site for cannula anchorage. Separate the submax. gland from overlying fascia to provide space for the loop of the cannula.

or (b) Jugular vein cannulation: Bluntly dissect tissue .5 to 1.0 cm. below submaxillary and parotid glands. (Figure 26g next page). Other procedures the same as above.

9. Starting from the posterior end of the neck incision, push the trocar, with bevel down, subcutaneously down the ventral midline about 4-6 cm., around behind the foreleg, and then anteriorly to the back of the neck, exiting through the .5 cm. incision. Insert the large tubing end of cannula into back of trocar and push the cannula in as far as possible. Pull the trocar from the beveled end while pushing the trocar and cannula from behind. Take the cannula out of the trocar when the trocar pulls free, leaving cannula in place under skin. Adjust cannula so that the loop faces laterally and slightly downward. See Figure 27.
FIGURE 26

Incision; Dissection; Isolation of Vein.
10. Attach the saline and heparin filled syringe to the cannula with a blunted 20 ga. needle. Fill cannula with saline. Estimate distance between vein and heart, trim small tubing accordingly (should be approx 1.75 in.). An unbeveled tip is preferred, but pointed end may be used if difficulty is encountered when attempting to insert cannula into the vein.

11. Lift the vein with the trocar and insert 25 ga. needle, bevel down and tip toward heart, into the lumen of the vein. Remove and insert a 23 ga. needle into the hole in the vein. Grasp the small tubing 2 mm. from the tip with the broad tissue forceps. Lift the needle and insert the small tubing into the vein behind the bevel of the needle (see Figure 28a and b, next page). Thread cannula into vein until coating prevents further entry. One should be able to infuse saline and withdraw blood freely. If column of blood in cannula pulsates, cannula tip is in right atrium of heart. Cannula should be withdrawn until pulsations are no longer seen.

12. Remove trocar carefully. Using the taper-point needle, suture one anterior silk tie through muscle below the posterior facial vein, being careful not to penetrate anterior facial vein which courses in this area. Slip a small 5 x 5 cm. Teflon mesh square down the silk tie to the cannula. This mesh helps to anchor the cannula in the neck. Before making square knot with other anterior silk tie, insure that the cannula goes straight into the vein without kinking. Tie the knot, cut the ends, and insert the loop of the cannula between the fascia and submaxillary gland. Anchor the posterior silk ties to adjacent muscle on sternum, also including a small Teflon mesh square.

13. Check patency of cannula by infusing a small amount of saline into vein.

14. Close fascia over cannula using 5-0 chromic gut with interrupted stitches. Subcutaneous tissue overlying fascia may also be approximated. Close skin with 4-0 plain gut or 5-0 silk and cutting edge needle. Clean wound and apply nitrofurazone. Check patency of cannula by infusing a small amount of saline into the rat.
15. Insert subcutaneous harness into back incision. Insure that it is in the center of the back; then close skin with 4-0 plain gut or 5-0 silk and cutting edge needle, leaving 1/2-inch screws protruding from wound. Apply nitrofurazone. Screw on one hex nut on each screw to about 1 mm. from wound surface. See Figure 29, next page.

16. Inject .25 ml. (75,000u.) penicillin i.m. in hind leg or put soluble tetracycline into drinking water. Do not administer both.

17. Attach the rat to leash-swivel unit by fitting external harness plate onto protruding screws and secure with hex nuts. Fill the leash-swivel unit with saline and attach cannula to leash. Secure the cannula with silk ties and coat junction with RTV. Attach leash to infusion pump containing saline and heparin (one drop heparin from 25 ga. needle to 20 ml. saline). See Figure 30, next page.

18. Postoperative care: Schedule hourly infusions of saline (.2-.5 ml.) to keep cannula open. Allow access to 25% dextrose in drinking water (with or without tetracycline) but give no dry food for 12 hours. (Because of the atropine, the rat cannot salivate and may choke on the dry food). Before beginning experimental manipulations it is best to wait until rat is eating, drinking, and defecating normally and has regained pre-operation body weight (2-5 days).
VI. Addresses of Suppliers

Becton Dickinson Company
Rutherford, New Jersey

Medical-Grade Elastomer:
Dow Corning Corporation
Medical Products Division
Midland, Michigan

Silastic Tubing:
Dow Corning Corporation
4825 N. Scott Street
Schiller Park, Illinois 60176

General Electric Company
Silicone Products Division
1285 Boston Avenue
Bridgeport, Connecticut

Milton-Roy Company
711 W. Devon Avenue
Park Ridge, Illinois 60068

Minnesota Rubber Company
3630 Wooddale Avenue
Minneapolis, Minnesota 55416

Product Components Corporation
15 Washington Avenue
Hastings-on-Hudson, New York
VII. References

