

INDWELLING JUGULAR VEIN CATHETERIZATION IN THE UNRESTRAINED CONSCIOUS RATS

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ABSTRACT

Blood is removed from the animals for a variety of scientific purposes. This process may well be unnecessarily stressful for an animal, simply because of the handling, the type of anaesthetic used or the discomfort associated with a particular technique. The physiological changes associated with increased stress may even invalidate the results. Cannulation is considered when repeated samples of blood are required so as to avoid multiple needle entries at any one site. This enables us to design complex experiments in which time course information or evaluation of repeated treatments is necessary. It in turn reduces the number of animals necessary to acquire such information and thus facilitates the compliance with the animal use. In this paper we have described a simple microsurgical technique to collect blood samples from the right atrium through a catheter (cannula) implanted into the right external jugular vein of the adult rats. Blood is easily sampled while the rats reside in their home cages.

Keywords: Microsurgery, cannulation, repeated blood sampling, jugular vein.

INTRODUCTION

Whenever animals are used in laboratories, minimizing any pain and distress they suffer should be an important objective as achieving the experimental results. The refinement of procedures to make them more humane should now be an integral part of all scientific research. This is important both from humanitarian concerns and in order to satisfy the requirements of the animals Scientific Procedure Act (1986). In recent years, more attention has been focused on the need to recognize and control adverse effects of scientific procedures on animals. There is a great deal of scope for improving current laboratory practice for the benefit of animal welfare. Such techniques in turn no doubt improve the quality of scientific research since suffering and distress in animals can result in physiological changes which are likely to add another variable to experimental results. The physiological changes associated with increased stress may even invalidate results (Ajika *et al.*, 1972; O' Neill and Kaufmann, 1990; Sarlis, 1991). Comparison of normal blood obtained through chronic indwelling cannulae in unrestrained animals with blood obtained by more conventional methods has shown significant differences, for example in the levels of prolactin, cortisol, corticosterone, glucose, catecholamines as well as in counts for red and white cells and platelets and packed cell volume. Since stress may cause physiological reactions which may affect the research therefore due attention should always be given to the method of blood sampling being employed. To reduce stress and release of stress related hormones, it is desirable to refine

experimental procedures, avoiding the stress caused by handling, restraint and sampling Royo *et al.* (2004). This article is an attempt to simplify the technique of indwelling jugular vein cannulation with simple drawn illustrations so that the technique can be used easily by more and more researchers conducting animal studies.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (200-230 gm in weight).

Surgical Items

Stainless steel surgical tray with lid, Angular forceps 4.25 inches with 1.2 mm wide serrated tips, Half curved forceps 4 inches with 0.8 mm wide serrated tips, Curved hemostat 3.5 inches Hartman Mosquito type, Iris scissors 4.5 inches, Operating scissors 5.5 inches, Vessel dilator forceps 4.5 inches Dumont type atraumatic with oblique points, and Spring scissors 3.5 inches.

Tubings

Silastic tube Dow corning medical grade I.D. 0.025 inches, Polyethylene tubing PE-50, 12-15 cms in length clay Adams brand, and Plastic tygon tube 1 to 1.5 inches in length.

Anaesthetic

Sodium pentobarbital (Nembutol) 40 mg/kg body weight Intraperitoneally (I/P).

Solutions

Saline: 0.9% sterile normal saline (sodium chloride) solution, Heparinized saline (10 units/ml sterile) Shum *et*

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al. (2001). It is prepared by mixing 1.0 ml of heparin sodium solution (injectable, 1000 USP units/ml) in 100 ml of normal saline, Toluene, 70% ethanol, and Betadine surgical scrub.

Other Items

Drapes, towels, gloves, face mask, surgical cap and gown, Cotton swabs, sponges 2 x 2 inches gauze pads, ruler, 1.0ml syringes, injection needles 23 G, 25G, precision needles, centrifuge tubes, electric shaver, three way cannula, adhesive tape, heating pad, fiberoptic cold light source with gooseneck guides, rat cage with fresh bedding, infrared lamp, weighing balance, and operating table.

Suture Thread

Ethicon brand black braided 3-0 silk for cannula, and catgut/ethilon 3-0 suture for skin.

Catheter Assembly and Implantation

1. The catheter is a length of polyethylene (PE-50) tubing ending in a segment of silastic tubing.
2. A 12-15 inches long piece of clay Adams brand PE-50 tubing (i.D. 0.023 in x O.D 0.038 in, wall 0.008 in) and a 3.5-4.0 cm long piece of Dow corning medical grade silastic tubing (I.D. 0.025 in x O.D. 0.047 in wall 0.011 in) are used to prepare the catheter.
3. One end of silastic tubing is soaked in toluene solution. A 2-3 mm high toluene column will be drawn into it by capillary action.
4. Silastic tube will become soft in 10-15 secs.
5. Now take the PE-50 tubing and advance it into the silastic tubing for a distance of 1-1.25 cm.
6. Silastic tubing is then cut at a length of 2.9-3.2 cm measured from the center of the overlap.
7. The PE-50 tubing end of catheter is filled with 70% ethanol through a connector.
8. Then it is left immersed overnight in the ethanol.
9. Before use the ethanol is drained out of the catheter and it is filled with heparinized saline using 1.0. ml syringe.
10. Outer side of the catheter is wiped free of ethanol with sterilized gauze pad.

Preparation of Surgery

(a) Preparation of surgical kit and other items

All the instruments needed for surgery, cotton plugs, drapes, gauze pieces, surgical gown are sterilized by autoclaving for 1 hour at 350°F, silk 3.0 about 20-25 cm is kept soaked in 70% ethanol overnight.

Before use excess ethanol is squeezed out and thread is dried up. Other items mentioned under materials are kept ready in the surgical table.

(b) Preparation of Animal

Animal's weight is determined. The rat is anaesthetized by giving sodium pentobarbital (Nembutol) 40 mg/kg body weight I/P. Depth of anaesthesia is check by absence of eye blink, tail pinch, foot withdrawal on pinching. Skin over the right shoulder (ventrally) and at the back of neck shaved with electric shaver. Rat is then placed on the blue sheet pad. Adhesive tape is applied on all 4 arms in extended position and tail to secure the animal in position. Area over the skin of right shoulder joint is thoroughly cleaned with betadine surgical scrub as right jugular vein is to be catheterized. Light source is turned on and sterilized drape is placed over the animal thereby exposing only the area where surgery is to be done.

Microsurgical Technique

In the rat the external jugular vein is a major superficial vein of the neck located rostral, to the clavicle (collar bone). It is easily visualized through an incision on the shoulder close to the base of the neck (Remie, 2000).

Steps of Technique

1. Shave the skin a little lateral to the right sternoclavicular joint on the ventral aspect of neck and about half inch on the back of neck using an electric shaver.
2. Lift up the skin rostral to right clavicle with an angular forceps.
3. Make an incision 2 cm long in skin with operating scissors and separate the flaps. Jugular vein can be seen right there under a thin tissue cover.
4. Give a small cut in this tissue with iris scissors and widen it carefully.
5. Separate the underlying vein from the fascia and clear out about 1.5- 2 cm long portion of the vein.
6. Pass the curved forceps beneath the vein and open up its end to expose the vein more prominently.
7. Pass two sterile 3.0 silk sutures beneath the vein at the proximal end and distal end. Tie the proximal one tight but keep the distal one with a loose knot.
8. Stretch up the exposed portion of vein by holding the proximal suture with tissue holding (mosquito) forceps by placing it on the shoulder to stretch the vein gently.
9. Make a clear cut v-shaped hole in the vein using sharp corneal spiral scissors.
10. Introduce the tip of vessel dilator forceps in the v-shaped hole and stretch the vein slowly and carefully.
11. Slide pass the silastic cannula thru this hole about an inch into the vein so that it is close to the heart that is until the center of the silastic-PE tubing overlap is at the incision.
12. The knot should be tied over this center to secure the cannula.
13. Withdraw the dilator forceps slowly as the cannula is advanced.

14. Wash the cannula with heparinized saline (10U/ml of saline) 200-250 μ L before insertion during surgery and on day 2 after insertion.
15. Confirm that the cannula is in the vein. Pull out with insulin syringe. If blood is there then it is deep in the vein.
16. If resistance is encountered, reposition the cannula till blood can be easily withdrawn.
17. Tie the distal knot tight over the cannula but taking care that it should not be tight enough to occlude the lumen of cannula
18. Double tie the proximal suture around the cannula to fix it firmly.
19. Snip off the extra suture thread.
20. Close the skin in layers using 3.0 ethilon catgut by intermittent sutures.
21. Now turn around the animal so that its dorsum is facing up.
22. Make an incision half inch on the back of neck previously shaven and scrubbed at the site of interscapular region.
23. Pass an artery (tissue) holding forceps thru the incision subcutaneously and pull out the jugular cannula through it on to the back of neck.
24. Lower the end of the cannula and again check for smooth flow of blood.
25. Fill up the cannula with heparinized saline.
26. Close the cannula with a lid and burry up this lid beneath the skin.
27. Pass a precision needle through the lid and connect the other end of the needle with 2.5 to 3.0 cm of long plastic tygon tube. This tube will remain standing at right angle over the neck of the animal and will secure the cannula from being chewed by the animal.
28. Close up the skin incision similar to the ventral side.
29. Alternatively one can use the skin tether buttons which are secured through peripheral holes to the surrounding muscle tissues beneath the skin & through the central hole which has tunnel already attached to it, cannula can be moved out & kept closed with a lock or precision needle.
30. Animal should be given a single dose of analgesic (Buprenorphine) 0.03 mg/kg intraperitoneally after surgery .
31. Experiment should be done 3-4 days after the surgery.
32. Take out the saline from the cannula.
33. Flush the cannula again with heparinized saline.
34. Take out the blood and again fill the cannula with heparinized saline.

Post operative care

Place the animal in separate cage. Maintain the temperature of the animal with infra-red lamp.

When ambulatory shift the animal to the animal facility. Check the animal daily.

DISCUSSION

From 1952 through 1983, 9 arterial and 12 venous procedures were described for multiple blood sampling in unrestrained rats. The large number of different procedures for chronic blood sampling suggests a lack of reliability and reproducibility in the techniques Giner (1987). In fact it would not have been possible to evaluate temporal pattern of hypothalamic pituitary adrenal (HPA) axis responses to stressors in conscious rats without the use of repeated blood sampling technique (Plotsky, 1992; Engelmann *et al.*, 1996; Thrivikraman, 1997; Thrivikraman, 1999; Arborelius, 2000; Ladd, 2000; Hout, 2001). Vascular access techniques have been employed to collect blood samples since 1960's Cocchetto and Bjornsson (1983), Lestage (1985) in many neuroendocrinological (Fagin, 1983; Yoburn, 1984; Pich 1993; Bohus, 1998; Lightman *et al.* 2000), pharmacological studies Rawlings (1994), Booze (1997), Rivier (1999) and Chan and Sawchenko (1994) have reported that the presence or absence of jugular catheter did not affect the basal expression of the immediate early gene product Fos-protein in the brain. No overt effects are reported in humans implanted with vascular catheters to monitor circadian patterns of plasma cortisol and other constituents (Portaluppi *et al.*, 1990). In the rat the presence of jugular catheter does not compromise the effects of immune challenges Lee (2000). Chronically implanted catheter allows remote blood sampling from conscious undisturbed rodents. This is especially important when measuring blood levels of hormones, cytokines or other endogenous substances whose production and/or release into blood is affected by stress on the animal. Maintaining patent catheters that cause minimal pathology in the animals require careful attention to the following elements:

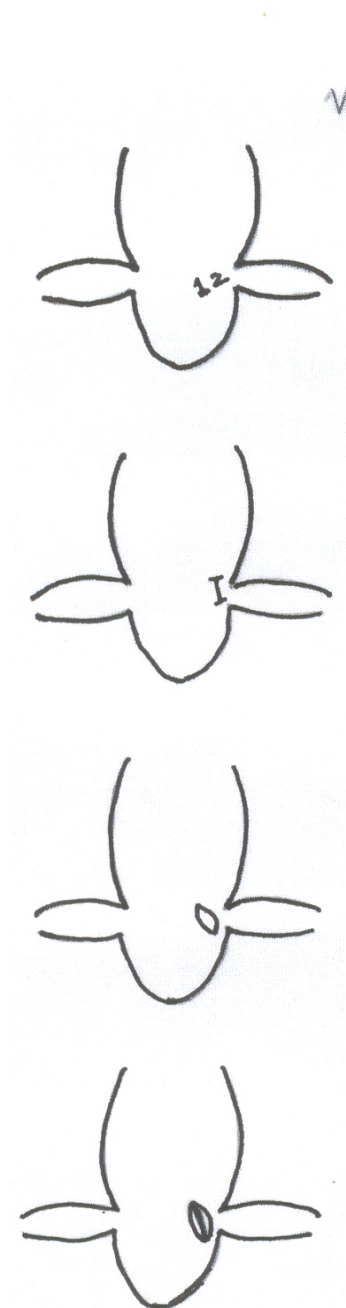
- (a) Aseptic surgical technique.
- (b) Proper catheter material.
- (c) Optimal catheter tip placement.
- (d) Routine and careful catheter flushing technique.

It is possible to maintain patent catheters and healthy animals for routine blood sampling for weeks to months Fink (2007). Vachon and Moreau (2001) have reported that stress was slightly less in the cannulated (jugular vein) rats than the rats that underwent repeated anaesthesia. The profound effects of anaesthesia and surgical stress on a number of physiologic functions are well established Cox and Bagshaw (1980) and Carruba (1981). Thus to get meaningful results one has to use conscious, nonstressed, freely moving animals but this may be difficult, especially in small laboratory animals such as the rat. Experimental procedures often involve repeated withdrawal of blood samples, continuous monitoring of blood pressure and heart rate, short term intravenous infusions and other maneuvers. There have

been a number of attempts to overcome these difficulties, Weeks and Jones (1960), Fink (1981), but experiments involving vascular catheterization in conscious rats are still not common place. Chronic implantation of vascular catheters therefore is a more physiologic approach. However, chronic rat preparations are not necessarily devoid of difficulties for instance cannulation of a common carotid artery Popovic and Popovic (1960) may interfere with blood pressure regulation or implantation of catheters into the femoral artery may lead to paralysis and

gangrene of the corresponding hind leg in some strains of rats. None of the surgical procedures in humans or animals is absolutely free of complications. Vascular access in small laboratory animals if performed carefully and aseptically is a very good tool for the researchers to carry out not only the time course studies but also in determining the cytokine and hormonal levels using less number of animals at a time. We believe that other laboratories will adopt this simple method for studying truly conscious, unrestrained animals.

VENTRAL ASPECT OF RAT

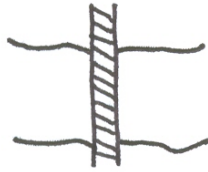


1. Base of neck
2. Right Clavicle

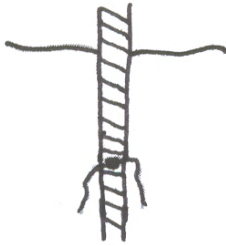
Site of incision for right jugular vein about 2 cm long.

Retracted flaps of incision.

Exposed right jugular vein.



Loose silk thread beneath the rostral & caudal part of exposed portion of right jugular vein.



Tied caudal end of silk thread to be held by the mosquito forceps.



V shaped nick in the anterior wall of right jugular vein.



Tip of vessel dilator in the V shaped nick.



Introduction of cannula assembly into the hole over the vessel dilator.



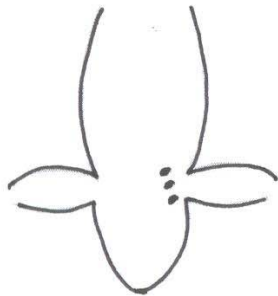
Cannula assembly into the right jugular vein with junction of silastic tube with PE-50 tube at the level of nick in the wall of vessel.



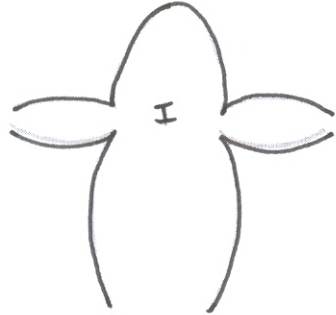
Securing of cannula with rostral silk thread with the vessel wall at the junction of silastic tube with PE-50 tube.



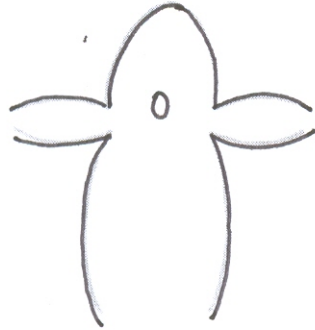
Extra silk thread snipped off.



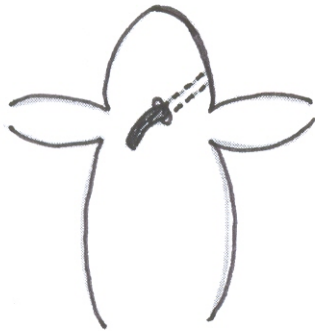
Skin closed with intermittent catgut sutures.

DORSAL ASPECT OF RAT

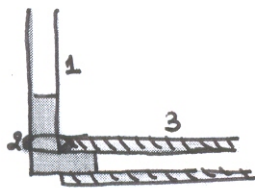
Incision site at the back of neck 1.5 cm.



Retracted flaps of incision.



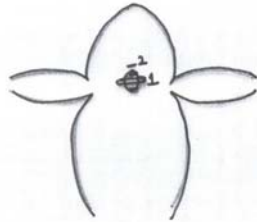
Subcutaneous tunneling of cannula and exit through hole at the dorsum of neck.



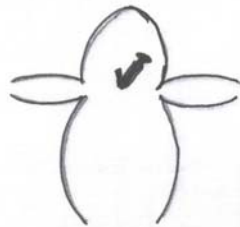
1. Precision needle
2. Centrifuge tube cap
3. Tygon tube



Precision needle bent at 90° and passed through cap of centrifuge tube. At 'a' it will fit into PE-50 tube and at 'b' it will fit into tygon tube.



1. Cap buried beneath skin.
2. Skin closed with intermittent catgut sutures.



Cannula with overlying tygon tube is standing at the back of animal's neck with insect pin closing it.

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