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Methods

Retro-orbital Blood Sample Collection in Rats-a Video Article

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ABSTRACT

Rats are the most commonly used laboratory animals for wide range of scientific research. Collection of biological fluids (such as blood, gastric fluid, breast milk and urine) is one of the important step in experiments. In many experiments, blood samples are collected for analysis of biochemical parameters and genetic profile. Blood samples are commonly collected through the tail vein, retro-orbital sinus and cardiac puncture. Retro-orbital sinus is commonly employed for collecting large volume of blood (200 µL to 1 mL) and it is employed in terminal procedures too. The main objective of this paper is to discuss the general principles of blood sample collection and the method used for orbital sinus blood sample collection.

Key words: Blood, Biological fluids, Orbital sinus, Rats, Retro-orbital.

BACKGROUND

Rats are the most commonly used laboratory animals in research due to their frequent reproduction, genetic purity and similarities to human biological system.[1] In biological research, rats are the preferred species for studies on aging, neoplasia, toxicity, gnotobiology, dental caries, metabolic disorders, behaviour, inflammatory disorders, pharmacokinetics, pharmacodynamics, embryology and genetics. The estimated number of rats used in the USA is nearly 4–5 million annually, and it is approximately 3 lakhs/ year in Canada. Normal adult rats weighing 200–400 g have 20–40 mL of blood i.e., total blood volume of rats is 9–10% of its total body weight. The maximum volume from a single blood sample collection is 10% of the rat's total blood volume i.e., approximately 2–3 mL, and exsanguination volume of blood is around 8–12 mL.[2]

Anatomy of rat eyes

The rat's eye has the same basic structure and function as all mammalian eyes, including the human eye (Figure 1a and 1b). In rat's eyes, light passes through the cornea and pupil. The pupil size of the rat is highly variable, and diameter of the same changes very rapidly from 2 mm to 0.5 mm in half a second. Later, light passes through the lens and strikes the retina. Rats are unable to change their lenses' shape because they have poorly developed ciliary muscles, and it acts as a filter by filtering 50% of ultraviolet A light and allows the remaining 50% of ultraviolet A and all visible lights. Once light hits the retina, it is detected by photoreceptors. Rats have rods and two types of cones which detects green and blue colour. Rats have poor visual acuity (20 times worse than humans). A normal pigmented rat has about 20/600 vision (1 cpd). The albino rat has vision of 20/1200 (0.5 cpd).[3-5]

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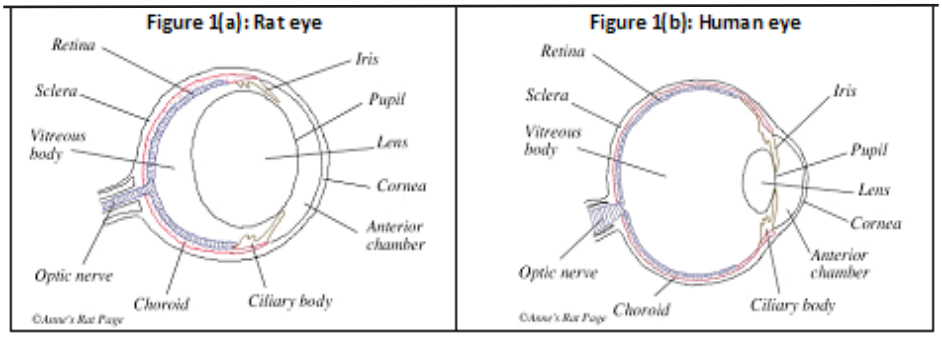
Circulatory system in rats

Rats and humans share an almost identical circulatory system. Blood is carried to lungs for oxygenation and back to heart by pulmonary circulation. Other parts of the body are supplied with blood from the left ventricle by systemic circulation. The estimated total blood volume is 55-70 ml/kg for a normal adult and lower (-15%) in obese and older animals.[6]

General principles of blood collection in rats

- Blood sample collection in rodents is a stressful procedure because of handling, restraint and use of anaesthesia. During any method of sample collection the animal stress levels should be kept to a minimum. The person colleting the blood should have appropriate training, knowledge of rodents' anatomy, physiology and management of critical situations.
- The blood sample collection method which is used for the experiment should be described in the Institution Animal Ethics Committee approved protocol or the used method should be recommended by national guidelines on animal experimentation.
- Frequency of sampling in rodents is very important. Once in two weeks is ideal for sample collection in rodents.If the study requires multiple sampling, samples can be collected using micro needles (capillary sampling through dorsal pedal vein) or higher animals such as lagomorphs (rabbits, hares) can be used.
- The researcher should decide the nature of the sampling before initiating sampling itself. The sample can be collected either by



[Figs.1(a) and 1(b) adopted from http://www.ratbehavior.org/Eyes.htmwith permission]

terminal or non-terminal methods. If the researcher uses non-terminal procedure, he/she has to make ready replacement sterile fluids to manage the medical emergencies, if any. To avoid animal suffering from hypovolemia when the volume of blood collected is more than the 30% of the circulatory blood volume, Lactated Ringer's solution (LRS) recommended as the best fluid replacement by National Institutes of Health (NIH) should be administered.

- In general, total volume of blood in mammals is about 7% of total body weight and any bleeding should not exceed 1.5% of total blood volume.If blood sample is collected in two-week intervals, 10% of animal total circulatory blood volume may be collected at one time. If blood sample is collected in four-week intervals or less frequent, 15% of the animal total circulatory blood volume may be collected using any of the safe, recommended methods.[7]

Adverse effects of blood sample collections

- Stress, haemorrhage, bruising, thrombosis, infection at the site of needle entry, phlebitis, scarring, and nerve damage are some of the potential adverse effects which should be avoided.
- Venipuncture may cause 'bruising', so proper monitoring is required.
- Failure in aseptic procedures may cause thrombosis and phlebitis.[8]

1 to 2% of the adverse effects include hematoma, corneal ulceration, keratitis, pannus formation, rupture of the globe, damage of the optic nerve and other intraorbital structures and necrotic dacryoadenitis of the Harderian gland.

Signs of hypovolaemia

- Anaemia
- Fast and thready pulse
- Pale dry mucous membranes
- Restlessness and anxiety
- Cold skin
- Hyperventilation
- Sub-normal body temperature[8]
- Temporary autonomic dysfunction (Orthostatic hypotension)
- Loss of >40% total blood volume may cause lethargy and coma.[9]

Management of hypovolaemia

- Warm (30-39°C), normal buffered saline can be administered to prevent hypervolemia.

PROTOCOL FOR ORBITAL SINUS BLOOD SAMPLE COLLECTION

- Requirements include the rat species (Wistar/Sprague Dawley), anesthetic agent(s) (diethyl ether, isoflurane, and halothane), induction chambers, cotton, capillary tube and blood sample collection tubes.
- This method is used with recovery in experimental circumstances and is also called periorbital, posterior-orbital, retro-orbital and orbital venous plexus bleeding. It is performed as a terminal procedure to collect large volumes of blood sample. Usually, short-time general anesthesia is preferable while adapting this technique.[10,11]
- The animal is anesthetized with a general anesthetic agent/ gaseous anesthetic agent such as rapid-onset, short acting (halothane/ isoflurane) or slow onset, long-acting (ether, methoxyflurane).[12]
- Ensure the level of anesthesia by checking loss of righting reflex. If animal shows any righting reflex it indicates that the animal is not in complete anesthesia.
- To avoid post-experimental pain to the animal, small amount of topical ophthalmic anesthetic agent may be applied on the eye before the initiating the procedure.
- The rat is restrained, neck gently scruffed with thumb and forefinger of the non-dominant hand and the skin around the eye is pulled taut.
- A capillary (2-2.5 cm) is inserted into the medial canthus of the eye (30 degree angle to the nose) using dominant hand.
- Slight thumb pressure is enough to puncture the tissue and enter the sinus/plexus.
- Once the sinus/ plexus is pricked/punctured, blood will flow through the capillary tube.
- After collecting required volume of blood from the plexus, the capillary tube is gently removed and wiped with sterile cotton to avoid further bleeding.
- Bleeding can be stopped by gently applying little finger pressure for approximately 30 seconds. Thirty minutes after blood collection, the animal is checked for postoperative and periorbital lesions. Figure 2(a) and 2(b) representing sequence of blood sample from rat orbital sinus using both right and left predominant hands.

Caution

- Repeated blood sampling, sequential sampling are not recommended in order to prevent histological changes and discomfort.
- This technique or procedure requires skill, honed by intensive training.
- Even a minor mistake will cause permanent damage to the eyes (Figure 3).
- Minimum two-weeks interval should be allowed between two bleedings.
- Adverse effects reported from retro-orbital sampling method is around 1 to 2% which includes hematoma, corneal ulceration, keratitis, pannus formation, rupture of the globe, damage of the optic nerve and other intraorbital structures and necrotic dacryoadenitis of the harderian gland.

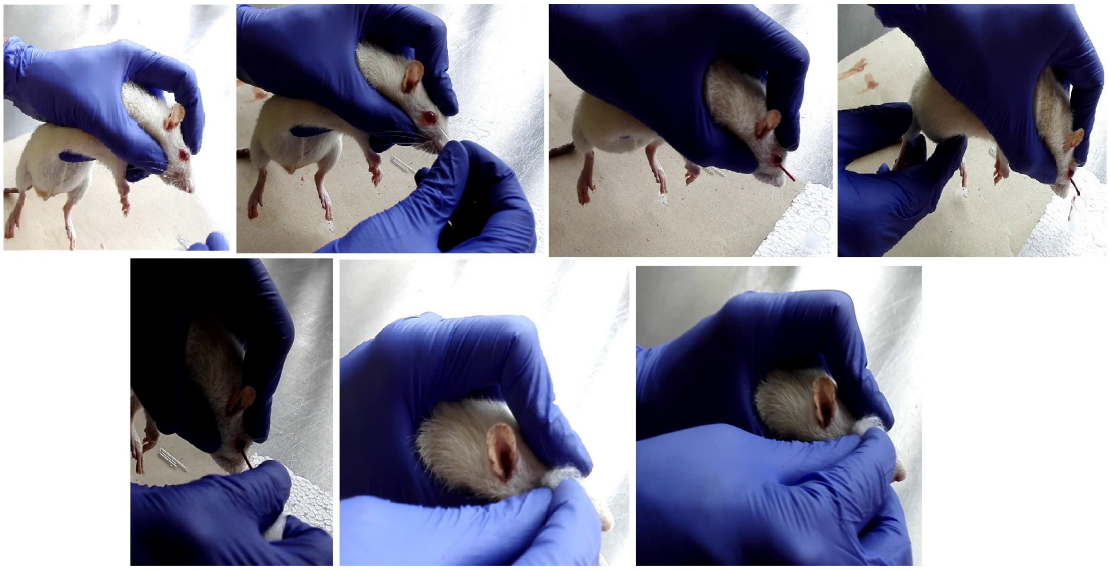


Figure 2a: Blood sample collection from rat orbital sinus (using right predominant hand)

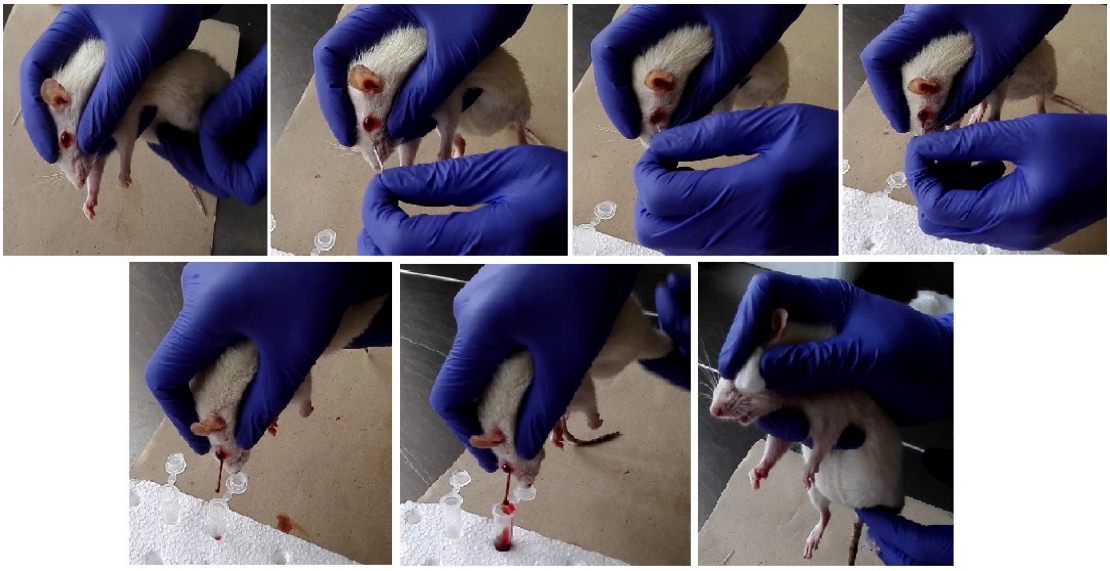


Figure 2b: Blood sample collection from rat orbital sinus (using left predominant hand)

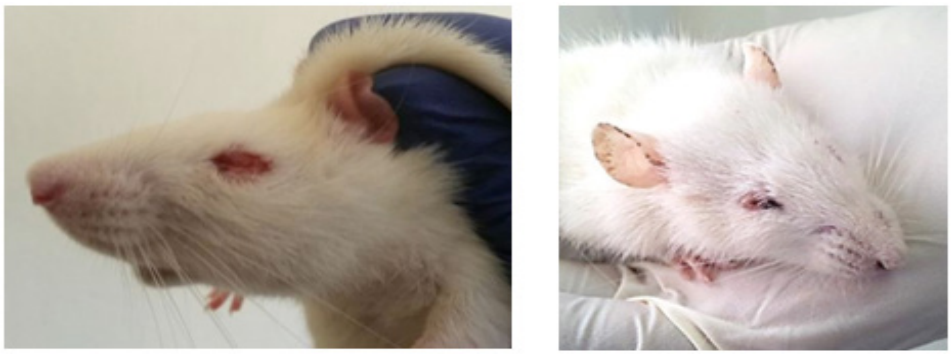


Figure 3: Abnormalities caused by wrong way of blood collection

Video 1: Blood sample collection from rat orbital sinus (using right predominant hand)
Video 2: Blood sample collection from rat orbital sinus (using left predominant hand)

CONCLUSION

Blood sample collection in any pre-clinical and/or clinical experiment is an essential step for assessing the normative functions and pathological alterations. Even a minor error while sampling can cause untreatable adverse events. Hence, the person carrying out the procedure must undergo appropriate training. The first few samples should be collected and handled with/under the supervision of the subject expert or supervisor to avoid and manage the unexpected events, if they do occur.

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REFERENCES

1. Why Are Rats Used in Animal Testing? Available in http://www.wisegeek.org/why-are-rats-used-in-animal-testing.htm. [Last accessed on 27/04/2015].
2. Harkness JE, Turner PV, VandeWoude S, Wheler CL. Harkness and Wagner's Biology and Medicine of Rabbits and Rodents. 5th edition, Wiley-Blackwell; 2013. pp. 95 – 109. [Book source: Google Books]. Available in http://books.google.co.in/s?hl=en&lr=&id=SFSXqsWvsMQC&oi=fnd&pg=PR11&dq=why+rats+are+used+for+testing&ots=pczCjCoWq2&sig=lD4Wj7QByn3fQce11joFkCQAlRM#v=onepage&q=why%20rats%20are%20used%20for%20testing&f=false[Last accessed on 27/04/2015].
3. The Rat's Eyes. Available in http://www.ratbehavior.org/Eyes.htm [Last accessed on 29/03/2015].
4. Majo F, Rochat A, Nicolas M, Jaoudé GA, Barrandon Y. Oligopotent stem cells are distributed throughout the mammalian ocular surface. Nature 2008; 456(7219): 250-4.
5. Representative Eye Images. Available inhttp://www.med.wayne.edu/anatomy/berkowitz/high_resolution_of_a_newbo.htm [Last accessed on 26/04/2015].
6. Rat-Circulatory System. Available in http://www.biologycorner.com/worksheets/rat_circulatory.html [Last accessed on 30/03/2015].
7. Animal Care & Use Program. Available in http://vetmed.duhs.duke.edu/GuidelinesforBloodCollection.html [Last accessed on 13/04/2015].
8. General principles. Available inhttps://www.nc3rs.org.uk/general-principles[Last accessed on 13/04/2015].
9. Marchand S, Dahyot C, Lamarche I, Plan E, Mimoz O, Couet W. Lack of effect of experimental hypovolemia on imipenem muscle distribution in rats assessed by micro dialysis. Antimicrob Agents Chemother. 2005; 49(12): 4974-9.
10. Rat: Retro-orbital (non-surgical/terminal). Available in https://www.nc3rs.org.uk/rat-retro-orbital-non-surgicalterminal [Last accessed on 26-04-2015].
11. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2010; 1(2): 87-93.
12. Use of ether for animal anesthesia at Johns Hopkins University. Available in http://web.jhu.edu/animalcare/policies/ether.html [Last accessed on 26/04/2015].