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A perfusion protocol for lizards, including a method for brain removal

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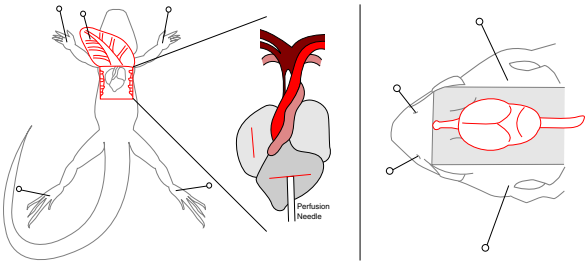
GRAPHICAL ABSTRACT



ABSTRACT

The goal of fixation is to rapidly and uniformly preserve tissue in a life-like state. Perfusion achieves optimal fixation by pumping fixative directly through an animal's circulatory system. Standard perfusion techniques were developed primarily for application in mammals, which are traditional neuroscience research models. Increasingly, other vertebrate groups are also being used in neuroscience. Following mammalian perfusion protocols for non-mammalian vertebrates often results in failed perfusions. Here, I present a modified perfusion protocol suitable for lizards. Though geared towards standard brain perfusion, this protocol is easily modified for the perfusion of other tissues and for various specialized histological techniques.

- The two aortas of the lizard heart, emerging from a single ventricle, mean that care must be taken to place the perfusion needle in the correct aorta, unlike in mammals.
- Only the head and neck perfuse – the visceral organs will not decolour, and the body may not twitch.
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I also include a method for removing a lizard brain, which differs from mammals due to the incomplete and thicker skull of the lizard.

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Method details

Reliable fixation of nervous tissue is a prerequisite for valid histological investigations. Transcardial perfusion utilizes the circulatory system to distribute fixative throughout the organism quickly and efficiently. The animal, traditionally a mammal, is opened up just below the thoracic cavity, which is then entered through the diaphragm [1]. The heart is exposed, incisions are made into the right atrium and left ventricle, and a specialized, blunt-tipped needle (perfusion needle) is inserted into the left ventricle or the aorta. Fixative is then perfused throughout the animal. This has the added advantage of removing all blood, which may obscure histological features.

Recently, there have been significant advances in our understanding of the neurobiology of squamate reptiles (lizards and snakes), with particular emphasis on lizards [2–4]. Although perfusion is a commonly used method of fixation in lizard neuroscience, there is no published methodology for perfusing lizards. Lizards differ from mammals in anatomical and physiological ways that affect the perfusion method. Here, I have taken a standard mammalian perfusion protocol and modified it for lizards. This procedure has been tested on fifteen species of agamid and scincid lizards ranging between 3 g and 300 g. All materials necessary for this procedure are listed in Supplementary Materials 1.

1. Prepare buffered solutions

1.1 Prepare prefix perfusate, fixative perfusate and storage buffer (example solutions are listed in Supplementary Materials 2).

1.2 Prior to perfusion, warm buffers to room temperature. For mammal perfusions, solutions are often warmed to 37 °C (mammalian body temperature), however lizards are ectoterms and as such room temperature is their body temperature.

2. Prepare apparatus

Two types of devices are commonly used to perfuse liquids into the circulatory system: those depending on gravity to propel solutions, and those that use a pump system. I use a pump system, which is generally sufficient for protocols such as Nissl staining and immunohistochemistry. However for some purposes, such as electron microscopy, a gravity system may be more appropriate. Furthermore, gravity systems are useful in the field, as they do not require electricity.

2.1 Run distilled water through the perfusion tubing and perfusion needle to rinse.

2.2 Draw prefix perfusate into the perfusion tubing. 2 mL/5 g of lizard is sufficient to flush the blood from the circulatory system. Take care to avoid any bubbles forming in the tubing.

2.3 For a small lizard, the amount of prefix needed is so small that it is possible to store the entire amount in the perfusion tubing. In this case, measure out the required amount of prefix, draw it into the tubing, and mark off the appropriate place on the tubing with a permanent marker. Then continue to fill the tubing with fixative until there is no air left in the tubing. For larger lizards and when using a gravity system the switch from prefix to fixative has to occur during the perfusion, as with mammals. Always take care not to leave any air bubbles in the tubes.

2.4 Affix the perfusion needle to outlet end of the tubing.

2.5 Turn on the perfusion pump and adjust the flow rate until there is a weak but even flow of liquid out the end of the perfusion needle (no dripping). Flow rate is heavily dependant on the gauge of the needle and the model of perfusion pump. To find the correct pressure, start with a pressure at which liquid drips from the needle. Increase pressure slowly until the stream flows steadily. I use pressures