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Direct brain excision: An easier method to harvest the pig's brain

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The use of pigs in experimental brain research was advocated more than 30 years ago [9]. However, many factors favour the use of rodents in research including the ease of housing and handling of small laboratory rodents, low cost of both procurement and housing, easy access from veterinary sources and relatively low cost compared to other large mammals. Due to these factors rodents are often the preferred population in pre-clinical research but larger animals, specifically pigs, play an important role in research, particularly in translational work [8].

Many of the prerequisites for conducting neuroscience research are fulfilled for pigs, including the need for standardized laboratory breeds, and an advanced knowledge of the general anatomy and physiology, housing, handling and experimental procedures [8]. However, interbreed differences in neuroanatomy and behavioral reactivity are not fully established for different breeds of pigs [8]. Therefore, it is imperative that well-defined or standardized pig breeds should be employed to ensure increased reproducibility and comparability of research.

The recent advent of imaging technology for studying brain function and structure in vivo has benefited from the relatively large size of the pig brain.

The use of pigs within neuroscience has increased in the past decade to an extent that far exceeds that of other farm large animals, such as sheep [8]. However, basic knowledge about the anatomy, physiology and development of the pig brain is still being collected. A considerable amount has been learned about pig brain anatomy and neurochemistry, but little is known about cortical function [11]. The gyrencephalic pig brain is more similar to the human brain in anatomy, growth and development than are the brains of common small laboratory animals [6,11]. The large size of the pig brain permits the detailed identification of cortical and subcortical structures [11]. Furthermore, the pig is an increasingly popular laboratory animal for transgenic manipulations of neural genes.

Wider use of pigs in research could facilitate extrapolation of preclinical findings to humans, especially in research important areas wherein obvious dissimilarities in brain structure and function render rodents less comparable to humans [4]. Thus, developing an easier and faster method to harvest the pig's brain can be beneficial for a full range of neuroscience experiments, as an alternative to research exclusively in rodents and non-human primates. Included below is a pig brain harvesting methodology derived from our labs dual neurosurgical and preclinical pig lab experience.

The harvesting technique was applied after the end of experiment in human-size pigs (40–60 kg) after the animal care approval. The technique consists of direct excision without decapitation, which it is faster and easier than previous craniotomies methods using longitudinal and coronal craniotomy. Steps: Mark "T-shape" incision line the pig's skull using anatomical references points: the pig's ears and eyes. Approximately, the length should be around 10 cm and the wide 8 cm. The lines are draw using the midline and the ears as reference points. After the skin incision, using a scalp, remove the periosteal membrane to the skull. The coronal suture and the temporal muscle should be exposed. Draw a hexagon picture in the pig's skull using as reference points: superior orbital bones, glabellum, temporal-parietal skull bones division and parietal-occipital line, as demonstrated in the picture behind. Carefully, with handheld circular saw, cut the bone's skull in the hexagonal lines, controlling the force applied in order to prevent brain tissue damage. (Fig. 1).

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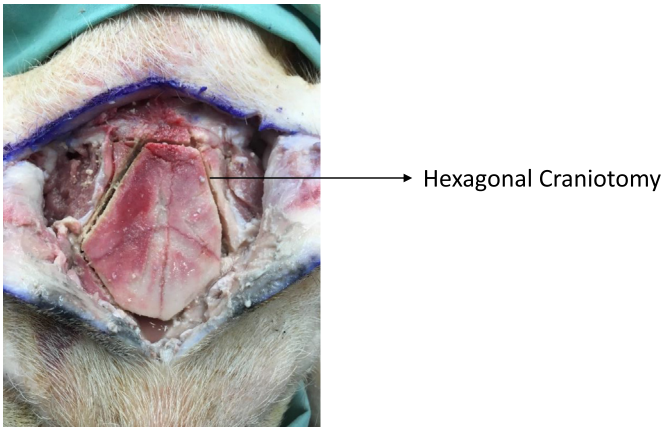

Fig. 1. The craniotomy was made using a circular saw and follows anatomical references to create a hexagonal shape.

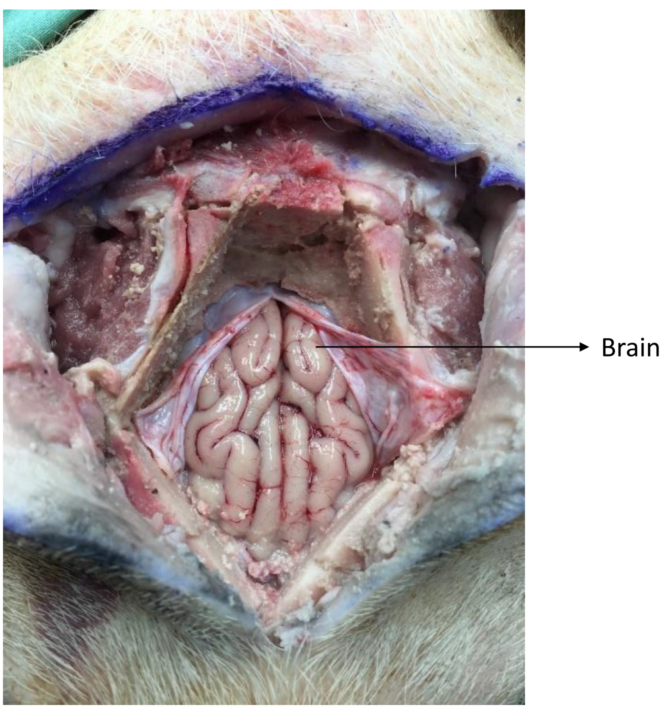

Fig. 2. Duramater was removed to expose the brain.

Remove the skull bone without damaging the dura-mater. Use scissors to cut the remaining meninges and cranial nerve attachments to allow removal of the entire brain. (Fig. 2) Lastly, remove the brain after the dissection of the brain stem and the anterior fossa structures.

The current craniotomy techniques in pigs require decapitation, which consume a large amount of time. The most popular methods are longitudinal and transverse craniotomies [14].

Longitudinal craniotomy removes the head at the atlanto-occipital joint. Then, the tongue and pharynx are removed to expose the hard and soft palate. The mandibular symphysis is split and the head is placed on a non-slip surface such as a rubber mat or onto the ground, using the separated mandible to improve stability. The final part of the procedure is conducting a longitudinal craniotomy to extract the brain [14].

This procedure is unnecessarily time consuming and requires specialized equipment not always available in a pre-clinical lab. Furthermore, a transverse craniotomy has the disadvantage of cutting the brain in transverse slices which it is not appropriate for many studies due to the possible anatomical brain damage [14].However, the technique proposed in this paper has superior advantage in harvesting the cerebrum and the cerebellum, but when comes to the harvesting the brain stem and the caudal cranial nerves there are some anatomical and surgical limitations that turns this technique less appropriate.

The pig brain is comparable to human one in terms of gross anatomy, histology and vascularization with some important differences. The frontal lobe is less developed, and the olfactory system occupies a large portion of the anterior part of the brain. A massive skull protects its brain with size and shape changes during growth making surgical intervention more difficult [11]. The weight of the adult pig brain ranges from 80 to 180 g, and can be comparable to the brain mass of several non-human primate species used for experimental purposes. As such it is it large enough to allow clinical neurosurgical training. [7,10]. Possible training in surgical procedures included craniotomy, dural opening, brain surgery and excision of an artificial tumor created by injection of colored fibrin glue [1]. The model has proved to be an excellent opportunity for young neurosurgeons to train bleeding management and surgical complications. Further aneurysm may be mimicked allow for training several aneurysms clipping techniques, emergency rupture situations and vascular reconstruction procedures [9,12,13]. Borucki, 2003 evaluated the endoscopic anatomy of the cerebellopontine angle through the retro-sigmoid approach in a swine specimen, and found analogy to human's anatomy. Aurich, 2014 studied the cerebellopontine angle exposing and drilling of the internal auditory canal, which is an important step in vestibular schwannoma surgery.

Refining the harvesting of pig brain techniques, with the method described herein, is important to facilitate new research and clinical training involving brain anatomy, histology and physiology. Therefore, we suggest in this paper an easier method to harvest the cerebrum and cerebellum without decapitation.

Conflict of interest

The author and co-authors state that there is not conflict of interest about this article. Additionally, the authors do not have financial support or benefits from the article above.

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