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Acquisition of brains from the African elephant (*Loxodonta africana*): Perfusion-fixation and dissection

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ABSTRACT

The current correspondence describes the *in situ* perfusion-fixation of the brain of the African elephant. Due to both the large size of proboscidean brains and the complex behaviour of these species, the acquisition of good quality material for comparative neuroanatomical analysis from these species is important. Three male African elephants (20–30 years) that were to be culled as part of a larger population management strategy were used. The animals were humanely euthanized and the head removed from the body. Large tubes were inserted into to the carotid arteries and the cranial vasculature flushed with a rapid (20 min) rinse of 100 l of cold saline (4 °C). Following the rinse the head was perfusion-fixed with a slower rinse (40 min) of 100 l of cold (4 °C) 4% paraformaldehyde in 0.1 M phosphate buffer. This procedure resulted in well-fixed neural and other tissue. After perfusion the brains were removed from the skull with the aid of power tools, a procedure taking between 2 and 6 h. The brains were immediately post-fixed in the same solution for 72 h at 4 °C. The brains were subsequently placed in a sucrose solution and finally an antifreeze solution and are stored in a −20 °C freezer. The acquisition of high quality neural material from African elephants that can be used for immunohistochemistry and electron microscopy is of importance in understanding the "hardware" underlying the behaviour of this species. This technique can be used on a variety of large mammals to obtain high quality material for comparative neuroanatomical studies.

1. Introduction

African elephants are the largest extant terrestrial mammals, with adult male body masses ranging between 5500 and 6000 kg (Skinner and Chimimba, 2005). Associated with this large body size is a large brain, with reported brain masses ranging between 4000 and 6000 g (Shoshani et al., 2006). Despite this large brain mass, very little is actually known about the structure, and thus, functional capacities, of the proboscidean brain. A recent review of the neuroanatomical data available for proboscidean brains (Cozzi et al., 2001) reported that only 52 scientific papers have been published that are specifically dedicated to structural aspects of the brain, and that 20 of these were written in the 19th century. Moreover, 46% of these 52 articles were written in French, German, or Italian. Comparatively, there is a wealth of information on the large brains of primates and cetaceans. It was concluded by Cozzi et al. (2001) that the lack of interest in the proboscidean brain is: "...probably due to the feeling that no 'front line' discovery can be derived from these studies..." and a lack of interest in support for such a study from funding agencies. Cozzi et al. (2001) further reason that the wide gap in the amount of knowledge derived for the cetacean and proboscidean brain results from the military interest in the behaviour and physiology of the dolphins and whales, and the need for knowledge of the cetacean brain from countries that previously were whaling countries with a specific need for commercial exploitation.

Behavioural studies of African elephants have demonstrated some exceptional capacities, including ultra-low frequency sound communication (Garstang, 2004), exceptional long-term memory (Markowitz et al., 1975), very complex social structures (Payne, 1998; McComb et al., 2000, 2001), and even basic tool construction and use (Anderson, 2002). For the most part, these behavioural studies do not refer to the structure (and inferred functional capacities) of the brain, as the information required to make this sort of interpolation is just not available. Detailed neuroanatomical studies of the African elephant brain will begin to unlock the neural architecture subserving many of the behaviours recorded, and may in fact provide clues pointing behavioural studies in new directions, leading to a deeper insight and understanding of the African elephant. While many scientists study the behaviour of elephants, both in the wild and in captivity, there is at present no concerted effort directed towards understanding the structure of the brain. Two recent studies have improved our knowledge of elephant brains,

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describing in detail the gross anatomy (Shoshani et al., 2006) and structural anatomy detectable with magnetic resonance imaging (Hakeem et al., 2005). While certain clues relating structure to function have been determined, these papers have difficulty making further inferences due to the conditions of fixation of the tissue, post-mortem immersion fixation between 12 and 24 h after death, and the subsequent inability to apply techniques such as immunohistochemistry or electron microscopy to this tissue.

We set out to obtain perfusion-fixed African elephant brains that can be used for a variety of modern neuroanatomical techniques. The current paper describes the method we used to perfusion fix the brains *in situ* and the treatment of the tissue following perfusion. We successfully obtained three well perfusion-fixed brains from three male African elephants in the age range of 20–30 years. While the method described here may have been used by other researchers, in other animals, previously, we are unaware of this technique being applied to the largest terrestrial mammal, and thus felt our experience is worth relating.

2. Permits and ethical issues

Prior to undertaking this study, permission to sacrifice the animals was granted by the Zimbabwe Parks and Wildlife Management Authority. Ethical permission was obtained from the University of the Witwatersrand Animal Ethics Committee and the animals were treated and used according to the guidelines of this committee, which parallel those set down by the NIH for use of animals in scientific experiments. Permission was granted by the Malilangwe Nature Conservation Trust to obtain African elephants resident on their property near Chiredzi in south-eastern Zimbabwe. Lastly, permission was obtained from the Department of Agriculture, South Africa, to allow the importation of formalin fixed tissue from Zimbabwe into South Africa. All work with the elephants was performed under the direction and supervision of an extensively experienced wildlife veterinarian employed by the Malilangwe Trust.

The elephants selected for use were all solitary males that were old enough to be independent of the maternal herd, but not old enough to be dominant breeders in the region. By selecting animals of this type the potential effect of removing three animals from the population was minimized. In addition, these animals were a subset of animals that were to be culled as part of a larger population management program to be undertaken by the Malilangwe Trust. The bodies of the three animals were subsequently butchered to provide 100 000 meals to the people living in the region. Thus, the

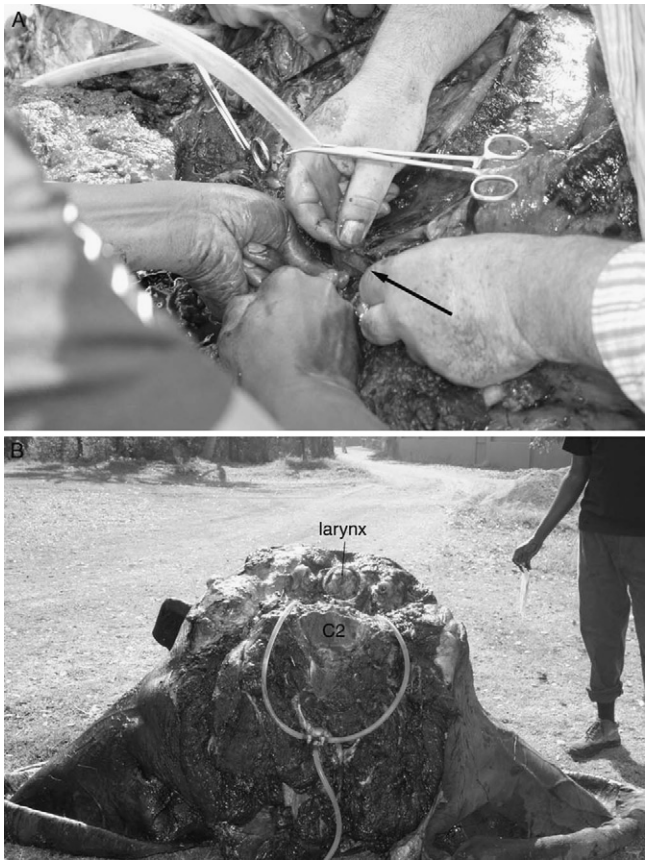

Fig. 1. (A) Insertion of large tubes into the carotid artery of the African elephant (indicated by arrow). Note the size of the carotid artery, being approximately 15 mm in diameter. (B) Position of the perfusion tubes in relation to the larynx and body of the second cervical vertebra (C2).

Fig. 2. Overall depiction of the perfusion set up. The tripod to the right enabled the lifting of the large tank containing the saline rinse or the fixative with a block and tackle. The length of each leg of the tripod was 6 m. A length of flexible plastic tubing was taken from the bottom of the tank to the head of the elephants where this tube was split with each split going to one carotid artery. The height of the tank in this image was that used for the perfusion of the fixative and allowed a slow flow of fixative through the cranial vasculature.

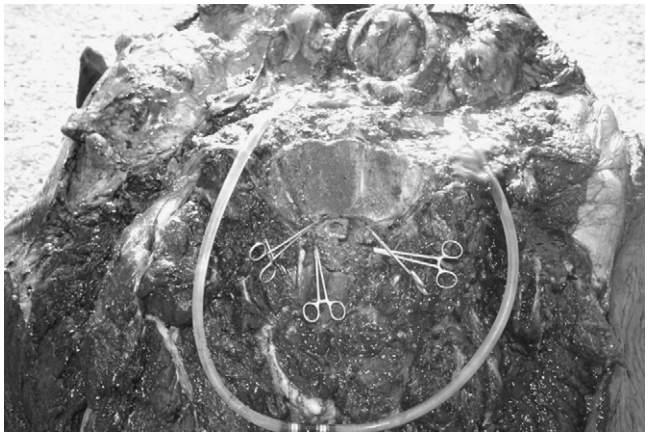

Fig. 3. Image of the cut aspect of the neck showing both the flow of fixative through the cranial vasculature and the artery clamps used to seal off the bilaterally paired vertebral arteries.