AATEX **14**, Special Issue, 97-100
Proc. 6th World Congress on Alternatives & Animal Use in the Life Sciences
August 21-25, 2007, Tokyo, Japan

Innovative refinements to anaesthesia techniques can deliver pain research without pain

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Abstract
Research into analgesia has traditionally not been possible without subjecting animals to pain. The practice of inflicting pain in some animals in order to relieve pain in others leads to an obvious ethical dilemma. Over the last 15 years we have developed and refined a novel approach to anaesthesia that allows the cerebral cortex of an anaesthetised animal to respond to noxious stimuli in a similar manner to that of a conscious animal experiencing pain. Under these conditions, changes in specific electroencephalographic variables seen in response to noxious stimulation and their attenuation by different methods of analgesia have allowed various analgesic strategies to be directly compared with each other. Our approach has enabled analgesia research to be undertaken for the first time without subjecting animals to pain. We have studied pain and analgesia in this way in cattle, deer, sheep, horses, rats, dogs and wallabies. This paper will outline our new approach to analgesia research and discuss the advantages of this novel technique over more traditional approaches. We will draw on examples of applied analgesia research from several species of mammals in which our techniques have been applied.

Keywords: pain, analgesia, anaesthesia, refinement

Introduction

Pain has been described as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP, 1979). The dual sensory and experiential aspects of pain make it the most subjective of the sensory modalities. It has been suggested that the degree of sensory stimulation is a less important factor in the degree of perceived pain than the prior state of the central nervous system that receives the stimulation (Vossen et al. 2006). This subjectivism has made pain difficult to quantify objectively and this has lead to the widespread use of a variety of subjective pain scales in human patients. Although subjective pain scoring has proved to be a very powerful tool, its use is limited to those patients that are able to describe their pain. Non-communicative patients such as very young children, adults with various forms of cognitive and communicative impairment and animals are not suitable candidates for these methods. The need to assess pain in these groups has fuelled a continuing search for objective measures that correlate well with subjective pain scores.

Objective measures of pain and nociception can be broadly divided into four categories:

Autonomic responses
Endocrine stress responses
Behavioural responses
Neurophysiological responses

This paper will discuss the latter category and in particular the analysis of electroencephalographic responses to noxious stimulation during controlled general anaesthesia, the so-called minimal anaesthesia model (Murrell and Johnson 2006).

The relationship between pain and the EEG

Prior to the mid 1990s, the perception of pain was thought to be a function of the limbic structure (Lico et al. 1974). The development of functional magnetic resonance imaging allowed the areas of the brain involved in the processing of pain to be firmly identified. Cerebral structures, particularly the insula cortex and anterior cingulate gyrus were found to be specifically responsive to pain in human volunteers (Craig et al. 1996). The discovery of the inherent role

of the cerebral cortex in turn lead to renewed interest in electroencephalographic analysis as a means of measuring pain and nociception.

Principles of EEG analysis

Electroencephalograms are often analysed using the Fast Fourier Transform (FFT). The following is a very brief explanation of this methodology. Any signal or waveform whose statistical descriptors (mean frequency, relative frequency components etc.) do not change over time is said to be stationary. Signal analysis theory states that any stationary signal can be considered to be the sum of an infinite number of sine waves of different frequencies and strengths. Fast Fourier Transformation transposes a signal in the time domain into the frequency domain, that is it converts a conventional signal into a power spectrum, a histographic representation of the original signal (Fig 1). For a more detailed explanation of FFT analysis, see Young (2001).

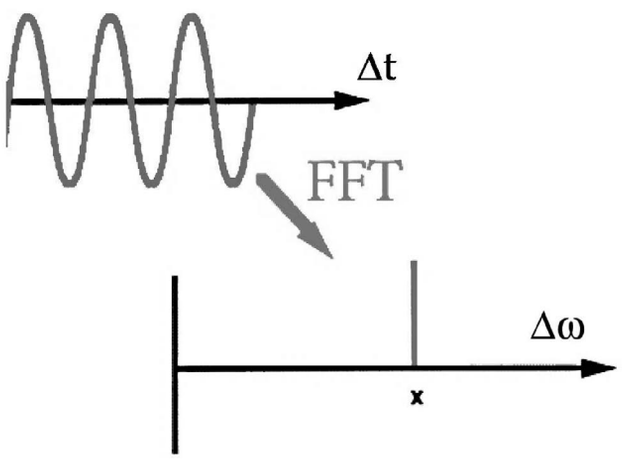

Fig. 1. Graphical representation of Fast Fourier Transformation (FFT) of a time domain signal (Δt) to a frequency domain signal (Δω). The point x in the frequency domain represents the frequency of the signal.

Fast Fourier transformation of one short epoch of EEG (typically one second), gives an indication of the frequencies present at that time. The power spectra of consecutive epochs can be displayed adjacent to each other to give an indication of how the frequency components change over time. This is a compressed spectral array (CSA). Typical CSAs are illustrated in Fig 2. A CSA gives a good visual representation of EEG changes, but in order to perform statistical analysis, it is necessary to derive mathematical descriptors from this waveform. The most frequently used descriptors are: median frequency (F50: the statistical median), which gives a general view of the CSA; 95% spectral edge (F95: the 95th percentile), which responds to changes in high frequencies; total EEG power (ptot: the area under the curve), which responds to the lower frequencies. For more details on these variables, see Murrell and Johnson (2006).

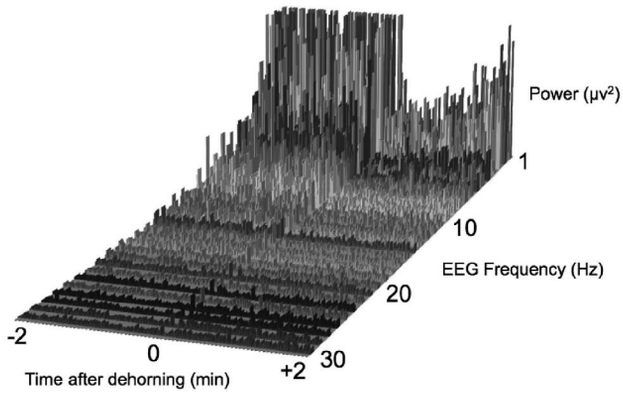

Fig. 2. Compressed spectral array of EEG during scoop dehorning. This represents the EEG response to dehorning (at time 0) in an anaesthetised heifer. An immediate reduction in low frequency power and increase in high frequency power lasting for approximately two minutes can be seen.
Data from Gibson et al. (2007).

The minimal anaesthesia model

The minimal anaesthesia model takes advantage of the finding that under carefully controlled conditions of general anaesthesia, noxious stimulation can result in EEG changes (Murrell et al, 2003) that are similar to those seen in conscious animals (Ong et al. 1997). In conscious human volunteers, these changes have been shown to correlate well with subjective perception of pain (Chen et al. 1989). This means that we can compare the pain perception resulting from different noxious stimuli in animals that are anaesthetised. By definition they cannot feel pain as they are anaesthetised, but the EEG changes give us an indication of the degree of pain that they would have perceived were they consciously aware. This gives us, for the first time, a method of investigating pain in animals that does not require us to subject experimental animals to pain. Even if animals form part of a negative control group and receive no analgesia in addition to general anaesthesia, they are not conscious throughout the study and can be given appropriate analgesia before they recover from the general anaesthetic.

To date the minimal anaesthesia model has been used to investigate pain in 8 species of mammal: horses (Murrell et al. 2003); sheep (Johnson et al. 2005a); red deer (Johnson et al. 2005b); cattle (Gibson et al. 2007); pigs (Haga et al. 2005); rats (Murrell et al. 2007); wallabies (Diesch et al. 2008); dogs (data in preparation). Two examples of the practical applications of this model will be discussed below:

Scoop dehorning in calves
Velvet antler removal in red deer

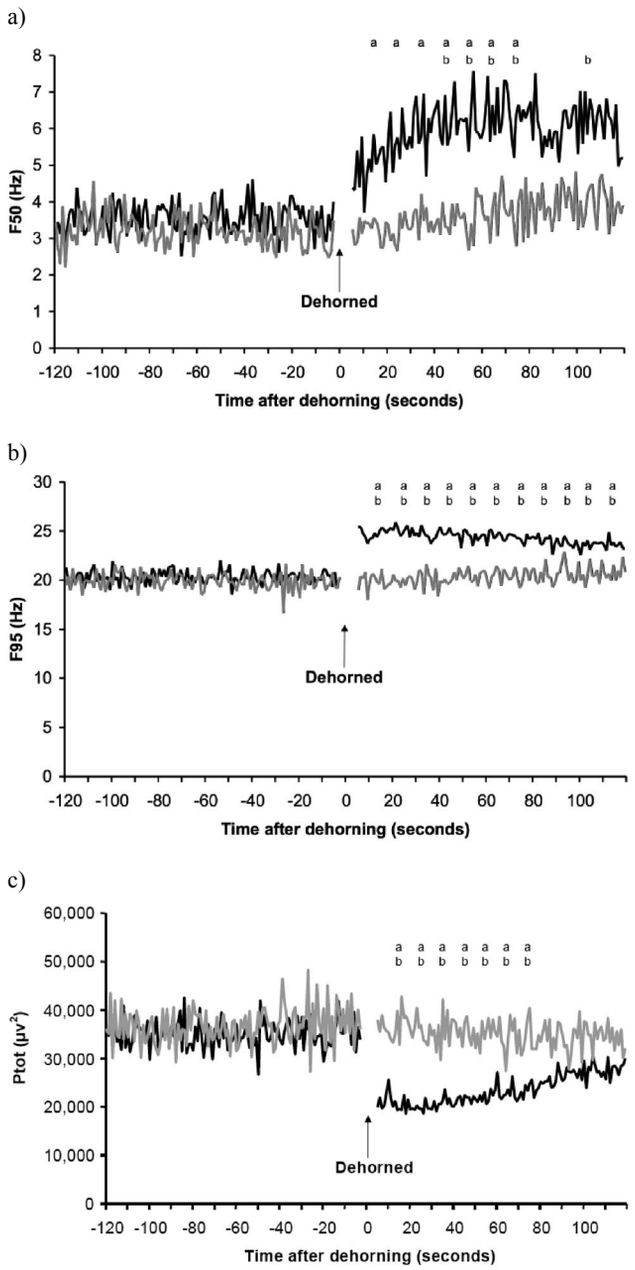

Fig. 3. Changes in: a) median frequency (F50); b) 95% spectral edge frequency (F95); c) total EEG power (Ptot) following scoop dehorning at time 0 in anaesthetised cattle. Data from Gibson et al. (2007) reproduced with permission.

Scoop dehorning in calves (Gibson et al. 2007)

Changes in F50, F95 and ptot in the two minutes following scoop dehorning in cattle are illustrated in Fig 3. Dehorning resulted in an immediate increase in F95 and decrease in ptot and a more gradual increase in F50. The addition of a local anaesthetic ring block prevented these EEG responses demonstrating the effectiveness of local anaesthetic ring block as an analgesic technique for this procedure. The experiment validated the use of the minimal anaesthetic technique in cattle and has formed the basis for further studies (data currently under analysis).

Velvet antler removal in red deer (Johnson et al. 2005b)

This study compared the use of local anaesthetic ring block or antler pedicle compression to no analgesia for the surgical removal of velvet antler in red deer. Antler pedicle compression was proposed as a method of field analgesia for velvet harvesting. This study demonstrated that antler pedicle compression was not as analgesic as local anaesthetic ring block and in addition that the application of the compressive band was itself a significant noxious stimulus. As a result of these and other studies, the National Animal Welfare Advisory Committee declined to recommend the approval of antler pedicle compression in New Zealand and it has not been adopted for use in the field. This is an example of how results generated using the minimal anaesthesia model have been used to influence animal welfare policy at a national level in New Zealand.

Conclusions

In conclusion, the minimal anaesthesia model offers significant advantages over other methodologies available to pain researchers. All animals are anaesthetised throughout the period of data collection. This means that a control group with no additional analgesia can be included into studies against which to compare the effects of proposed techniques of analgesia. Together with the very tight degree of control afforded by the conditions of general anaesthesia, this increases the statistical power of research studies and allows significant effects to be identified using fewer animals than would be possible with other experimental techniques. Experimental animals can be given analgesia using appropriate clinical techniques after the completion of data collection, but before they recover from general anaesthesia. This represents significant reduction in the numbers of animals used and refinement in terms of the welfare impact to those animals in an area of research where replacement is not currently a realistic option.

The ability to give better analgesia to experimental animals than they would be expected to receive under routine animal husbandry conditions means that for the first time, pain research can be carried out whilst simultaneously improving the welfare of the animals involved in the studies.

Acknowledgements

I am indebted to all past and present members of Massey University Comparative Analgesia Group "Team Ouch" for their enthusiasm and dedication to our studies. Research studies discussed in this paper were funded in part or in whole by: Ministry of Agriculture and Forestry (NZ); Department of the Environment, Food and Rural Affairs (UK); Velvet Antler Removal New Zealand.

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