

New Approaches to Identifying and Measuring Pain

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Abstract

The search for objective measures of pain is ongoing. In human pain research, self-reporting is considered to be the gold standard against which prospective measures are compared. Because self-reporting is not possible in non-linguistic species, studies that utilise standardised noxious stimuli are often used to evaluate prospective measures of pain. Two approaches to the quantification of animal pain have emerged in recent years that seem to offer significant advances over previous techniques.

Quantified analysis of the electroencephalographic response to noxious stimulation has been used in many species of mammal. This technique has the advantage that it is carried out under general anaesthesia and so has the potential to give insight into pain without actually subjecting animals to pain.

In conscious animals, ethological quantification of behaviour is able to identify pain-related behaviours with much greater precision and assurance than older methods of behavioural analysis.

This paper will discuss both techniques with examples taken from the work of the Massey University Comparative Analgesia Group (Johnson et al. 2005; M^cCracken et al. 2006). Used together, behavioural and EEG analysis have the potential to provide much insight into pain in animals, both in mechanistic physiological and applied animal welfare research.

Introduction

Research into pain and its relief in animals has utilised a wide range of techniques with the aim of finding objective measures that can quantify the experience of pain suffered by the animal. The objective quantification of pain is complicated by our understanding that pain is inherently subjective in nature and

the experience of medical pain researchers that objective measures do not always correlate well with a patient's subjective perception of their pain. In recent years, two research measures, spectral analysis of the electroencephalogram (EEG) and ethological analysis of behaviour, have proved to be extremely useful in this area and have contributed significantly to our understanding of animal pain and the techniques that we use in its alleviation. These two methods have very different applications and limitations, but used in conjunction with each other they can give a very complete picture of the pain felt by groups of animals and the impact of different analgesic regimes upon that pain. This presentation will focus on these two methodologies. After a brief description of the usefulness, advantages and limitations of each technique, an example will be given of the way in which these two techniques can be combined to give an overall picture of an animal's experience of pain.

Power Spectral Analysis of the EEG

The EEG has long been known to reflect changes in CNS function. In particular, it gives a direct indication of the activity of the cerebral cortex that contains the bipolar cells believed to be its primary generators (Silva 2004). Historically, the cerebral cortex was not believed to be an important structure in pain perception, but functional imaging techniques developed in the last decade have demonstrated that certain cortical structures, in particular the anterior cingulate gyrus play an important role. These developments have greatly increased the importance of EEG analysis in pain related research. Studies in human volunteers and pain patients have demonstrated that EEG variables correlate well with subjective evaluation of pain. These findings indicate the value of EEG analysis as an indicator of the degree pain perceived by an animal rather than the magnitude of the noxious stimulus presented to that animal.

Power spectral analysis gives an indication of the frequencies that form the component parts of the EEG using the Fast Fourier Transformation developed by Cooley and Tukey (1965). This mathematical algorithm contains no indication of how the components of the EEG change over time, but the analysis of successive short periods of EEG can demonstrate changes over time. The resulting changes over time are often illustrated as a compressed spectral array (Fig 1). This gives an excellent visual indication of EEG changes, but is not easily amenable to quantification and statistical evaluation of these changes.

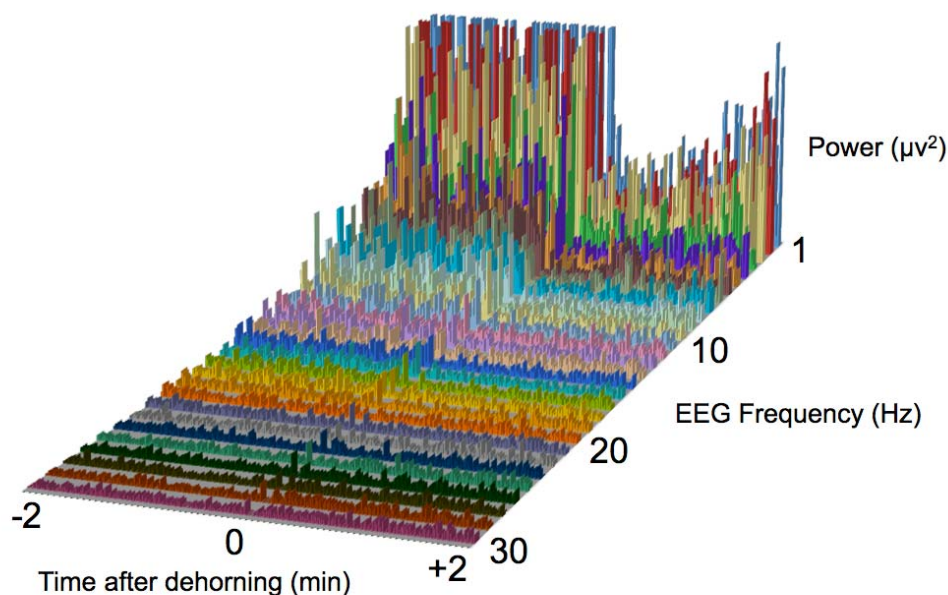


Figure 1: Compressed spectral array of EEG during scoop dehorning. This represents the EEG response to dehorning (at time 0) in an anaesthetised heifer. An immediate reduction in low frequency power and increase in high frequency power lasting for approximately two minutes a can be seen. Data from Gibson et al. (2007).

In order to facilitate formal quantitative analysis, a variety of descriptive variables have been derived from the power spectrum. The most frequently used of these are the median frequency, 95% spectral edge frequency and total EEG power.

The median frequency is the statistical median of the power spectrum. As a marker of central location, it gives an overall picture of both the high and low frequency components of the EEG. It appears to be a stable and robust measure, but its response to changes across the frequency range of the EEG make it less specific than other measures. The 95% spectral edge is the 95th percentile of the power spectrum. As such it is a very sensitive measure of the activity of the higher frequencies of the EEG. Total EEG power is the area under the power spectrum curve. Since the power spectrum contains much more power at lower frequencies than higher frequencies, total power is preferentially sensitive to changes in these lower frequencies. For a more detailed discussion of the derivation and uses of these variables, see Murrell and Johnson (2006).

Power spectral analysis of the EEG has been used in many studies as an indicator of noxious stimulation in animals. Studies have been undertaken in man, horses, sheep, pigs, rats, red deer, wallabies, cattle and dogs (Chen et al. 1989; Murrell et al. 2003; Johnson et al. 2005; Haga and Ranheim 2005; Murrell et al. 2006; Johnson et al. 2005b; Diesch et al, 2005; Gibson et al. 2007). In general, the response of the EEG to a noxious stimulus manifests as a decrease in low frequency activity and an increase in high frequency activity. This results in increases in 95% spectral edge and median frequency and a concurrent decrease in total EEG power. The relative responses of these three variables

appear to differ with different noxious stimuli. In particular, somatic noxious stimulation is associated with changes in spectral edge and total power and visceral noxious stimulation with median frequency.

Electroencephalographic changes appear to be similar in awake and anaesthetised animals, but practical recording of the EEG during noxious stimulation in animals is only practical under general anaesthesia. Although anaesthesia blunts the responses to noxious stimulation, we have developed the technique of 'minimal anaesthesia' (Murrell and Johnson 2006) that enables investigations into pain perception to be carried out in lightly anaesthetised animals. Although the experimental subjects are anaesthetised and so cannot experience pain, their cerebral cortex responds to noxious stimulation in the same way as when the animal is conscious and so inferences can be made about the relative magnitude of responses to noxious stimuli. The need for general anaesthesia is an obvious limitation of this technique. It does however have several advantages over other methodologies used in pain research. Many studies into animal pain give equivocal results because of the absence of a negative control. In conscious animals the inclusion of a control group subjected to the noxious stimulus without any analgesia raises obvious ethical concerns. The minimal anaesthesia model allows for a control group to be included with no welfare cost as the animals in each group can be given analgesia utilising routine clinical techniques following data collection and before they recover from anaesthesia. Indeed, it is often possible to carry out pain research with a negative welfare cost in the sense that animals involved in the study receive better pain relief than those undergoing similar procedures under normal farming conditions. The sensitivity of the EEG response to noxious stimulation means that statistically significant results can be demonstrated whilst using fewer animals than with other methodologies. Taken together these advantages represent a significant reduction and refinement in experimental methodology and allow pain research to be undertaken without inflicting pain.

Behavioural analysis

Pain related behaviour has been used as a tool in pain research for many years. Traditional methods have relied on the experimenter making some kind of subjective assessment of the degree of pain and recording this using either a descriptive or numerical scoring system. These techniques all suffer from the common flaw that the previous experience and bias of the operator constitute a very large source of variability in the data. For example, it has been shown that different groups of people score a patient's pain differently depending on how well they empathise with the patient. The patient's partner will attribute the most severe pain score followed by other relatives, nurses, anaesthetists and surgeons in descending order (Hodgkins et al. 1985; Klopfenstein et al. 2001).

An ethological approach to pain related behaviour provides a powerful means to look at individual behaviours without the need for the observer to make judgements about their significance. Behaviour is analysed in a group of animals

before and after a noxious stimulus such as surgery and again after the noxious stimulus with the provision of analgesia. The incidence or prevalence of individual behaviours is analysed in order to identify behaviours that discriminate between the three conditions. Any behaviour that increases its incidence after noxious stimulation, but decreases with analgesia may be a good candidate for a pain related behaviour. Once a specific behaviour has been identified, it can be used to quantify the effect of different analgesic strategies by analysis of the degree to which they mitigate changes in the expression of the behaviour.

This approach to behavioural analysis has been successfully applied in species such as the rat (Flecknell and Roughan 2004) and Horse (Price et al. 2003), in which pain related behaviour has been traditionally disappointing. Whilst this technique has been shown to be a very powerful tool, it is extremely labour intensive, analysis of a single hour of video often taking several hours to complete. Other disadvantages of this technique include the great specificity of pain related behaviours, meaning care must be taken not to generalise when applying the results of these studies in the clinical arena.

The advantages and limitations of EEG analysis and behavioural analysis mean that neither technique can give a complete picture of animal pain in all circumstances. However, the very different nature of the two techniques makes them complimentary and together they can give a very complete picture of situations in which animals are subjected to noxious stimuli. Table 1 contains a brief comparison of the advantages and disadvantages of EEG analysis and behavioural analysis as research tools in the area of animal pain.

Table 1: Advantages and disadvantages of EEG and behavioural analysis in the study of pain in animals.

| EEG Analysis | Behavioural Analysis |
|---|---|
| Animals must be anaesthetised | Animals must be conscious |
| Statistical differences with small numbers | Larger numbers required for statistical differences |
| Mathematical concepts complex | No complex mathematical concepts |
| Rapid analysis of data | Data analysis laborious |
| Suited to rapidly applied stimuli | Suited to more prolonged perception of pain |
| Consistent responses in wide variety of mammalian species | Behaviour specific to species and even type |
| Differentiation of visceral and somatic pain | Behaviour specific to noxious stimuli |
| Pain research without causing pain to research animals | Research animals must be suffer pain in order to measure pain-related behaviour |

Castration in lambs – an example of the two techniques in action

International animal welfare guidelines concerning castration vary in detail, but agree that castration without local analgesia should be carried out as early as is practicable. Lambs express pain related behaviour following castration and these behaviours are qualitatively different in lambs of different ages (Thornton and Waterman-Pearson 2002). It is difficult to draw conclusions based solely on behaviour about changes in the welfare impact of castration as the animals get older.

In two studies carried out over two successive lambing seasons, we investigated the effect of castration under minimal anaesthesia on the EEG of lambs of differing ages. The first study (Johnson et al. 2005a) compared the EEG response of two groups of lambs aged $12 \pm X$ and $29 \pm X$ days respectively. This study demonstrated that the younger lambs had a greater median frequency response to castration compared to their older counterparts (Fig 2). In the second study (Johnson et al. 2004), we investigated the EEG response to castration in lambs between 3 hours and 44 days of age. The most striking change in response revealed by this data was an increase from almost no response to castration in the youngest animals to an adult-like response by one week of age (Fig 3). The lack of cerebral response in the very young suggested that castration at this age would be associated with a reduced welfare impact since the animals had no perception of castration as noxious.

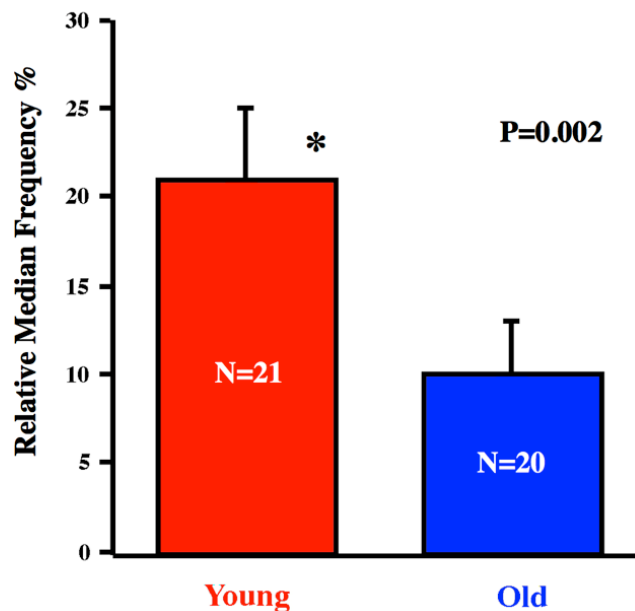


Figure 2: Relative response in median frequency following castration in lambs of differing ages. Young lambs 12 ± 2 days old: Old lambs 29 ± 1 days old. Data from Johnson et al. (2005a).

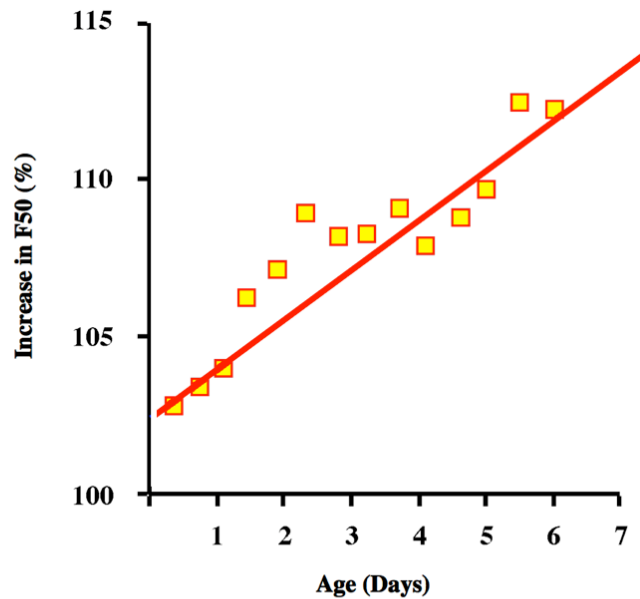


Figure 3: Relative response in median frequency following castration in lambs During first week of life. Each data point represents rolling mean of 10 animals. Data from Johnson et al. (2004).

The welfare impact of pain has two facets: contemporaneous perception of a noxious stimulus as pain and subsequent changes in the nervous system leading to the development of hyperalgesia which can persist for months or years (Taddio et al. 1997, Grunnau 2000). In order to build a complete picture of the welfare implications of castration, we felt it necessary to explore any hyperalgesia that may result from castration at different ages. In a further study (McCracken et al. 2006), we castrated two groups of lambs from the same flock at either one or ten days of age. These lambs were then docked at one month of age and their behaviour recorded for 15 minutes following docking in order to identify any differences in pain related behaviour between the two groups. This study demonstrated that the lambs castrated at one day of age had increased expression of some behaviours compared to their counterparts castrated at ten days of age. In particular, there were increases in the expression of abnormal posture and restlessness. These data suggest that despite the absence of EEG responses to the noxious stimulus of castration in very young lambs, they demonstrate hyperalgesia to tail docking at one month compared to their counterparts castrated at ten days of age.

Taken together, these three studies indicate that the perception of pain is greater in lambs castrated at one week of age or more compared to those castrated whilst very young, however the very young lambs demonstrate increased hyperalgesia compared to those castrated when older. The mechanism of this hyperalgesia is not clear, it may be due to the absence of descending cortical inhibition of nociceptive pathways in those animals where there is no cerebrocortical response to castration. The results of these studies have led to the reformulation of our original question. Instead of asking when is the best time

to castrate lambs without analgesia, we are now questioning if it is ever acceptable to carry out castration without the provision of effective analgesia.

Conclusions

This paper has given a brief overview of two of the most useful techniques currently available for the study of animal pain. The example of our investigations of castration in lambs demonstrates how these techniques complement each other and can be used together to build a picture of the overall experience of pain in animals subjected to specific painful husbandry procedures. Whilst these techniques document the effects of noxious stimuli, they do not comment on the acceptability of the use of such techniques. Decisions as to the acceptability or otherwise of farming practices can only be made by society as a whole through the mechanism of public policy formulation. The provision of objective information as to the impact of these practices on animal welfare will hopefully inform this process and lead to the development of improved animal welfare policy.

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