Electroencephalographic responses of anaesthetised pigs to intraperitoneal injection of sodium pentobarbital

NJ Kells*, NJ Beausoleil†, MA Sutherland‡ and CB Johnson†

† Animal Welfare Science and Bioethics Centre, School of Veterinary Science, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand
‡ AgResearch Ltd, Ruakura Research Centre, Hamilton 3240, New Zealand
* Contact for correspondence and requests for reprints: N.J.Kells@massey.ac.nz

Abstract

Small laboratory animals are commonly euthanased via intraperitoneal (IP) injection of sodium pentobarbital. However, there is concern that animals may experience pain prior to loss of consciousness with this delivery route. The present study investigated electroencephalographic (EEG) nociceptive responses of anaesthetised pigs to IP sodium pentobarbital injection using an established minimal anaesthesia model. Thirty commercial white line entire male pigs aged 10–15 days were minimally anaesthetised with halothane in oxygen. Following 10 min of baseline EEG data collection, pigs had their tails docked using side-cutters and, after a further 5-min interval, were euthanased via IP injection of sodium pentobarbital (250 mg kg⁻¹). The summary variables median frequency (F50), 95% spectral edge frequency (F95) and total power (P_TOT) were derived from the EEG data. For each variable in each pig, means were calculated for the following 60-s periods: immediately prior to tail-docking (baseline 1); immediately prior to pentobarbital injection (at least 4 min after docking; baseline 2); and for two consecutive 60-s periods immediately following pentobarbital injection (P₁ and P₂). Statistical analyses revealed no differences between the two baseline periods, indicating that transient EEG changes induced by tail-docking had resolved prior to pentobarbital injection. IP pentobarbital injection induced a significant increase in F50 and decrease in P_TOT of the EEG during P₁. This response is characteristic of acute nociception, indicating that conscious pigs likely perceive IP sodium pentobarbital as painful in the period prior to loss of consciousness.

Keywords: animal welfare, EEG, euthanasia, nociception, pentobarbital, pig

Introduction

Intraperitoneal (IP) injection of sodium pentobarbital is frequently employed for anaesthesia and euthanasia of laboratory rodents (Svendsen et al 2007). Whilst intravenous delivery is preferred as it achieves more rapid distribution of the agent and has lower potential to cause tissue irritation (Wolfensohn & Lloyd 2003; Svendsen et al 2007), IP delivery is most common in small animals, such as rodents, due to its relative ease of administration. However, there is concern that pentobarbital preparations may cause pain or irritation to the parietal and visceral peritoneum and associated tissues when delivered IP, particularly at concentrations required for euthanasia (Ambrose 1998; Wolfensohn & Lloyd 2003). Such irritation is thought to arise due to the high pH of euthanasia solutions, which have been reported as ranging from pH 10–12.5 (Wadhams 1997; Ambrose 1998).

Previous studies have investigated pain and/or distress associated with IP pentobarbital injection in rodents through measurements of behaviour, plasma stress hormone concentration and expression of c-fos (a marker of neuronal activity) in spinal nociceptive neurons following peripheral stimulation (Svendsen et al 2007). Ambrose (1998) reported writhing in rats (Rattus norvegicus) in response to IP sodium pentobarbital injection. Abdominal writhing is a recognised sign of pain in rats that can be experimentally induced through injecting a known irritant, such as acetic acid, into the peritoneal cavity (Siegmund et al 1957). Further, plasma cortisol was elevated in rats that were decapitated following IP sodium pentobarbital anaesthesia, relative to control rats decapitated without prior anaesthesia (Vahl et al 2005; Wu et al 2015). Elevations in plasma stress hormone concentrations are frequently used as indices of distress induced by painful or noxious procedures. However, such measures are not specific to pain, instead providing a measure of the overall noxiousness of an experience, including both physical and emotional components (Mellor et al 2000). Elevations in plasma stress hormone concentration have been shown to occur in rats administered IP physiological saline solution prior to decapitation, relative to those that did not receive IP saline (Baeck et al 2015; Wu et al 2015), suggesting that the process of IP injection itself is stressful, independent of the compound being delivered.