Electroencephalographic responses of anaesthetised rats to carbon dioxide inhalation

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Abstract

Exposure to high concentrations of CO₂ is a common means of stunning and killing laboratory rodents. However, there is concern regarding the potential for animals to have aversive experiences, such as pain or breathlessness, prior to loss of awareness. This preliminary study evaluated the electroencephalographic (EEG) responses of rats (Rattus norvegicus) to CO₂ inhalation, using a method based on a minimal anaesthesia model previously used to assess nociception in mammals. Fifteen adult female Sprague-Dawley rats were anaesthetised with halothane in oxygen and maintained at a minimal plane of anaesthesia. EEG was continuously recorded throughout a 10-min baseline period followed by sequential exposure to 5, 15, 30 and 50% CO₂. The EEG summary variables median frequency (F50), 95% spectral edge frequency (F95) and total power (P_TO) were derived from the raw EEG. The F50 of the EEG, a sensitive indicator of nociception, increased significantly above baseline during exposure to 15% CO₂, suggesting this concentration was noxious to rats. This is consistent with behavioural aversion in rats at around the same CO₂ concentration. Stimulation of the rat mucosal nociceptors only occurs at CO₂ concentrations of 37% or greater; therefore, it is hypothesised that the observed response was a result of what would have been CO₂-induced aversive respiratory sensation in conscious animals, rather than pain. This study provides some evidence that an anaesthesia model may be utilised to study the nocuous effects of low-moderate CO₂ exposure in rodents.

Keywords: animal welfare, carbon dioxide, electroencephalogram (EEG), euthanasia, negative affective experience, rat

Introduction

Laboratory rats (Rattus norvegicus) and mice (Mus musculus) are used extensively in biological research. The vast majority of these are killed, whether it be at the conclusion of the research project, for the purposes of tissue harvest, or to manage the size and composition of breeding stock (Makowska et al 2009). Exposure to carbon dioxide (CO₂) is a common means of killing laboratory rodents. This induces central nervous system depression and subsequent loss of awareness, followed by respiratory and/or cardiac arrest and death. However, there are concerns that rodents may experience pain, breathlessness or other aversive sensations prior to loss of awareness (Hawkins et al 2006).

Pre-fill methods using high concentrations of CO₂ are likely to cause pain due to mucosal acidification and are generally not recommended. For example, humans report pain in the eyes, nose and throat following exposure to 40–55% CO₂ (Thurauf et al 1991; Peppel & Anton 1993). During forced exposure to high concentrations of CO₂, using either pre-fill or gradual induction, rats and mice displayed behavioural signs, such as increased locomotion, rearing, agitation, gasping, shaking and hyperventilation, suggesting aversive experience prior to loss of awareness (Britt 1987; Coenen et al 1995; Smith & Harrap 1997; Ambrose et al 2000).

Gradual-fill methods are proposed to be better in terms of animal welfare and cited as being conditionally acceptable by organisations such as the American Veterinary Medical Association (Leary et al 2013). However, behavioural studies indicate that rodents are still averse to, and avoid, CO₂ concentrations well below the threshold for mucosal nociceptor activation. For example, both rats and mice withdrew almost immediately from a chamber pre-filled with 25% CO₂ (Leach et al 2002). Additionally, during exposure to a gradually increasing CO₂ concentration in the presence of a food reward, rats withdrew from the chamber when the concentration reached 13.0 to 15.9% (Niel et al 2008). Aversion to low-moderate CO₂ concentrations is a problem, given that concentrations of 30% or