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Assessment of unconsciousness during carbon dioxide stunning in pigs

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Abstract

The aim of this study was to assess unconsciousness in pigs during exposure to CO_2 through changes in the middle latency auditory evoke potentials (MLAEP) of the central nervous system (CNS), blood parameters (pH, carbon dioxide partial pressure [pCO_2], oxygen partial pressure [pO_2], oxygen saturation [SatO_2] and bicarbonate [HCO^-_3]), behaviour and the corneal reflex. The MLEAP did not decrease significantly until after 60 s exposure to CO_2. The blood parameters (decreased pH, pO_2 and SatO_2 and increased pCO_2 and HCO^-_3) changed 53 s after the onset of immersion. The burst suppression index (BS%) and the A-line ARX index (AAI) from the MLEAP recovered basal levels at 136 and 249 s, respectively. The first blood parameter to return to basal levels was HCO^-_3 at 76 s of exposure, followed by SatO_2 at 180 s, pH and pO_2 at 210 s and pCO_2 at 240 s. During exposure to the gas, pigs exhibited lateral head movements and sneezing (10.3 s), gasping (23.5 s) and vocalisation (26.1 s). Furthermore, all pigs demonstrated muscular excitation after between 19 and 39 s exposure, when the AAI and BS% values were not significantly different from basal values. It was suggested, therefore, that these excitatory movements represent conscious movement, indicative of aversion to the gas. According to our results, loss of consciousness began, on average, after 60 s inhalation of 90% CO_2. During exposure to the gas, decreased brain activity was seen, immediately following the changes in blood parameters. Following exposure, the restoration of blood parameters to basal levels allows a return to normal brain activity.

Keywords: *animal welfare, auditory evoke potential, aversion, blood gas, carbon dioxide stunning, pigs*

Introduction

Carbon dioxide (CO_2) is a gas that, when inhaled at high concentrations, depresses brain activity and induces loss of consciousness (Gregory *et al* 1987; Raj & Gregory 1996; Raj 1999). Its use in pig abattoirs has increased markedly, due to positive effects on meat quality when compared with electrical stunning (Velarde *et al* 2000, 2001). In commercial conditions, animals are immersed into a concentration gradient of the gas, such that, as the cage is lowered into the well, the CO_2 concentration continues to rise until it reaches 80–90% at the bottom of the well.

The inhalation of high concentrations of CO_2 induces hypercapnic hypoxia in the animal and leads to changes in blood parameters, such as pH, carbon dioxide partial pressure (pCO_2), oxygen partial pressure (pO_2), oxygen saturation ($SatO_2$) and bicarbonate concentration (HCO^-_3) (Lomholt 1998; Martoft *et al* 2001). Consequently, there is a decrease in the pH of cerebrospinal fluid (CSF) and the animal loses consciousness (Gregory 1987).

CO_2 stunning provides a number of animal welfare advantages, as it precludes the need for animal restraint thereby allowing group stunning, which reduces handling stress prior to stunning (Velarde *et al* 2000; EFSA 2004). Nonetheless, it has also come under a degree of criticism; loss of consciousness is not immediate (Raj & Gregory 1995), and is dependent upon the CO_2 concentration and the speed at which animals are immersed into the greatest concentration of gas at the base of the well (Troeger & Woltersdorf 1991; Raj & Gregory 1996). Signs of aversion, such as retreating and attempting to escape have been described in pigs during the inhalation of CO_2 (Raj & Gregory 1996; Velarde *et al* 2007). The effects of CO_2 are two-fold; firstly, it causes irritation of nasal mucosal membranes (Gregory *et al* 1990) and, secondly, it is a strong respiratory stimulator that provokes hyperventilation

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(Gregory *et al* 1987), and suffocation prior to loss of consciousness (EFSA 2004).

During inhalation, loss of posture has been considered the first behavioural indicator of the onset of unconsciousness (Raj & Gregory 1996). At this point, pigs demonstrate muscular excitation. Forslid (1987) found that low frequency activity in the EEG (> 4 Hz) became the dominant signal prior to the start of convulsions and suggested that pigs were unconscious before this muscular excitation phase. Conversely, Hoenderken (1983), who also analysed changes in the EEG, stated that unconsciousness appeared after the muscular excitation period and, as a result, these body movements were voluntary escape attempts. This lack of agreement is due to difficulties determining the exact moment at which the loss of consciousness occurs from the amplitude and frequency of the EEG.

Auditory evoked potentials (AEP) have been suggested as being a more precise indicator of the level of consciousness than the EEG (Thornton *et al* 1989). It consists of a series of waves representing the processing of the auditory stimulus within nuclear structures of the auditory pathway in the brain (Martoft *et al* 2001). The part of the signal occurring in the interval, 10 to 100 ms after auditory stimulus presentation, is the middle latency auditory evoke potential (MLAEP) and has been used to evaluate changes in neural activity and assess the depth of anaesthesia in humans (Jensen *et al* 1996, 1998; Litvan *et al* 2002) and pigs (Martoft *et al* 2002). From the MLAEP, the A-line ARX index (AAI) and the burst suppression index (BS%) can be estimated to assess unconsciousness during states of anaesthesia (Jensen 1999; Litvan *et al* 2002). The AAI is a numerical index, ranging from 0 to 99 that quantifies MLAEP variations of amplitude and latency. Higher values are related to awareness, while decreases in AAI indicate a gradual loss of consciousness (Jensen 1999). The BS% indicates the percentage of isoelectric activity during the preceding 30 s and also ranges from 0 to 100 (Litvan *et al* 2002).

Under commercial conditions, the absence of a corneal reflex has been used to assess the state of unconsciousness. However, this reflex is indicative only of brain-stem activity and its relationship with cortical function is not clear (Anil & McKinstry 1991).

The aim of this study was to assess unconsciousness during the induction and awakening from CO_2 anaesthesia, using AAI, BS%, blood gas parameters and corneal reflexes.

This experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of IRTA.

Materials and methods

Animals

Twenty-five commercial, crossbred female pigs weighing between 25 and 35 kg were used. Four days before the start of the study, the pigs were transported from their farm of origin to the IRTA facilities and housed in three pens, maintaining the same groups as the farm of origin. The pens (1.80 × 2.20 m; length × width) were provided with straw and were adjacent to the experimental facilities. Water and food were available *ad libitum* and animals were fasted 12 h prior to the experimental procedure.

Experimental procedure

The study was carried out during three consecutive weeks. Each day, two pigs were exposed individually to 90% CO_2 in a CO_2 Dip-Lift (BUTINA Aps, Copenhagen, Denmark). This system consists of a 195 × 61 × 90 cm (length × width × height) cage which was lowered into a 260 cm deep well. The CO_2 exposure cycle lasted 76 s, and consisted of the first 23 s (during which time the cage was lowered), the following 30 s (while the cage remained at the bottom of the well at the highest concentration), and the final 23 s (during which time the cage was raised).

Each pig to be stunned was placed in sternal recumbency in a net restrainer in order to minimise discomfort to the animal. The restrained animal's limbs were approximately 10 cm above the ground. Blood gases, auditory evoked potentials and behavioural measures were recorded during the 10 min prior to CO_2 exposure, during exposure to CO_2, and for a further 10 min after exposure. Once CO_2 immersion was completed, the presence of a corneal reflex was also monitored continuously.

Blood gases

The day prior to exposure to CO_2, the seven pigs for which blood parameters were to be recorded were tranquilised with an intramuscular injection of 0.1 mg kg^{-1} Azaperone (Esteve Veterinaria, Barcelona, Spain) and anaesthetised intravenously with a 0.3 mg kg^{-1} injection of Propofol (Braun Medical, Barcelona, Spain) into the auricular vein. Once anaesthetised, an 18 G × 10 cm catheter (Vygon, Spain) was placed into the carotid artery through a puncture in the deepest point of the groove formed between the medial sternocephalic and lateral brachiocephalic muscles. The catheter was led out through the skin via a small incision and fixed to it with suture thread. After surgery, the animals were placed in individual recovery pens and supplied with *ad libitum* water and feed until 12 h prior to the start of the experiment. During this period, the position of the catheter was checked regularly and a saline solution applied to guard against tract blockage.

Prior to each pig being immersed in the well, the catheter was connected to an extended catheter (300 cm long × 2.5 mm internal diameter) (Vygon, Spain). The basal sample was taken immediately prior to cage descent. During CO_2 exposure, three more samples were taken: i) when the pig reached the bottom of the well (23 s); ii) when the pig began its ascent (53 s) and iii) at the end of the exposure cycle (76 s). During recovery, six more samples were collected at 15, 45, 75, 105, 135 and 165 s after the end of CO_2 exposure. Blood samples were preserved in ice in 2 ml syringes with heparin and analysed, one hour after collection, using gas testing equipment (ECOSYS II-Eschweiler Compact BGA, Germany) for determination of pH, pCO_2, pO_2, $SatO_2$ and HCO^-_3 levels.

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Middle latency auditory evoked potentials (MLAEP)

The MLAEP was recorded through three surface electrodes (ALARIS AEP™ Monitor Electrodes, AMBU, Denmark) placed at various points on the shaved skull of the pig. The positive electrode was placed between the frontal and parietal bones. The negative electrode was placed on the dorsal part of the occipital bone, and the reference electrode was placed between the positive and the negative electrodes (Figure 1). Once the electrodes were fixed, the impedance was measured. Values below 5 KOhms (KΩ) were considered adequate for the recording. If the impedance was higher, the skull was reshaved and/or the electrodes replaced. The pig was then fitted with headphones that provoked a bilateral click train lasting 2 ms with a repetition frequency of 9 Hz. The electrodes were connected to an AEP-monitor (ALARIS AEP™ Danmeter, Denmark) to record the EEG signal. The MLAEP was extracted from the EEG between 20 and 80 ms after each auditory stimulus, using an autoregressive model with exogenous input (ARX) adaptive method (Jensen *et al* 1998). The A-Line ARX Index (AAI), and the burst suppression (BS%) were calculated from the extracted MLAEP wave (Jensen 1999; Litvan *et a*l 2002). For the data analysis, the basal data recorded during the 10 min prior to CO_2 exposure were averaged and compared to the average value each second during exposure to CO_2 and 10 min afterwards.

Animal behaviour and reflexes

The behaviour of the pigs during exposure to CO_2 was recorded with two video cameras placed in the cage. The first camera recorded a dorsal view of the animal and the other recorded a cranial view of the face of the animal. Each animal was marked individually and behavioural parameters scored were: i) lateral head movement and sneezing (Hartung *et al* 2002); ii) gasping, a very deep breath through a wide open mouth which may involve stretching of the neck: considered to be an indicator of onset of suffocation (Lambooij *et al* 1999); iii) vocalisation (EFSA 2004), shouts or snores emitted by the animal; iv) muscular excitation, repeated muscular movement of the whole body, including head movement upwards; v) gagging, low frequency inhalations with the neck towards the front legs and occasional emitting of sounds similar to snoring.

All recording times were synchronised with the time at which the pigs began their descent into the well.

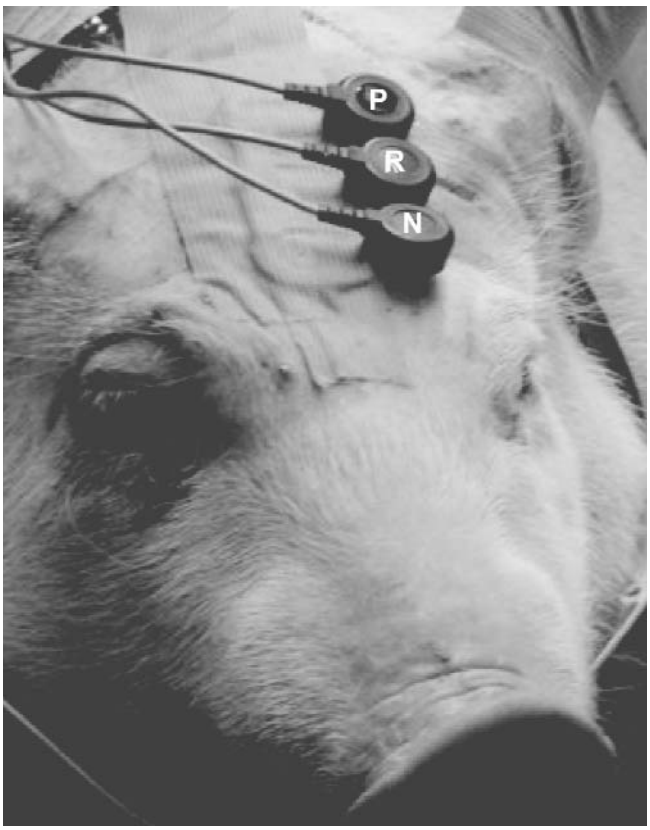
After CO_2 immersion, the presence of a corneal reflex was monitored by palpating the cornea with a pencil at 5 s intervals until the reflex was recovered.

Ten minutes after the end of the study, the pigs were euthanased by exposure to 90% CO_2 for seven minutes.

Statistical analysis

A statistical analysis with Proc Means of the Statistical Analysis System (SAS Institute Inc, Cary, NC, USA 2001) was performed for the corneal reflex and behavioural data (lateral head movement and sneezing, gasping, vocalisation, muscular excitation and gagging). The data obtained from MLAEP (AAI and BS%) were analysed using linear general models, with Proc Mixed proceeding for repeated measures of SAS. In both cases, the variables were submitted to symmetrical composition covariance structure (CS). When the variance analysis showed significant differences ($P < 0.05$), the comparison of least square mean values (LSMEANS) was adjusted to Tukey multiple comparison test. Since data obtained from blood parameters (pH, pCO_2, pO_2, $SatO_2$ and HCO^-_3) were not normally distributed, the statistical analysis for these data was carried out using general models. These data were analysed by the Proc GENMOD of SAS in relation to the effective time period with negative binomial distribution (Cameron & Trivedi 1998). The residual maximum likelihood was used as a method of estimation. The least square means of fixed effects (LSMEANS) were used when analysis of variance indicated differences. The significance level was fixed in all cases at $P < 0.05$.

Figure 1



Position of electrodes: (P) positive; (N) negative and (R) reference.

Results

Blood gases

The blood pH prior to CO_2 exposure was 7.42 (± 0.04). During CO_2 inhalation, the pH decreased and differed significantly ($P < 0.05$) from the basal pH, 53 s from the start of CO_2 exposure through to 180 s of post-inhalation recovery

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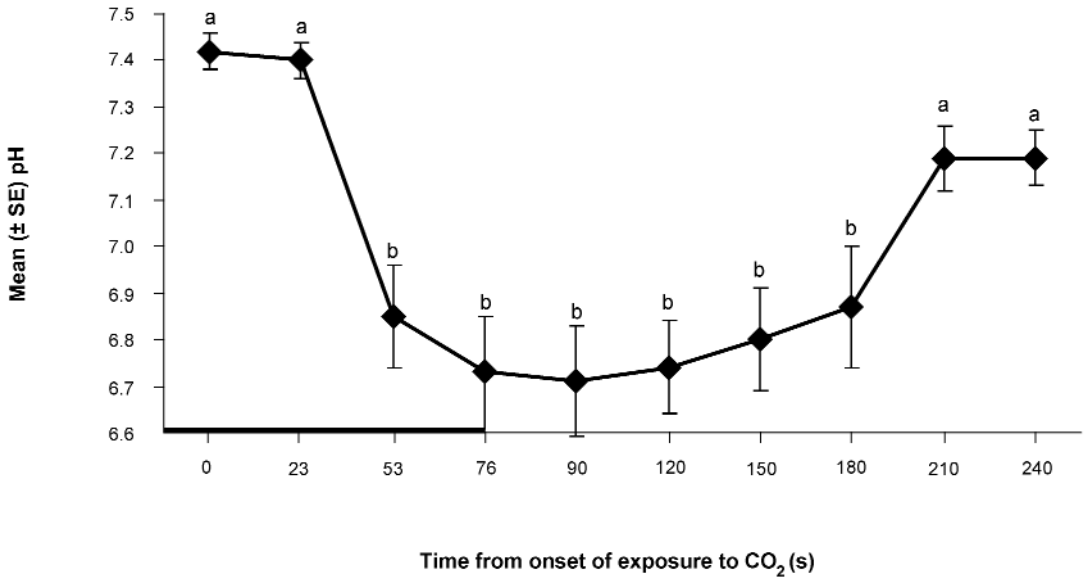
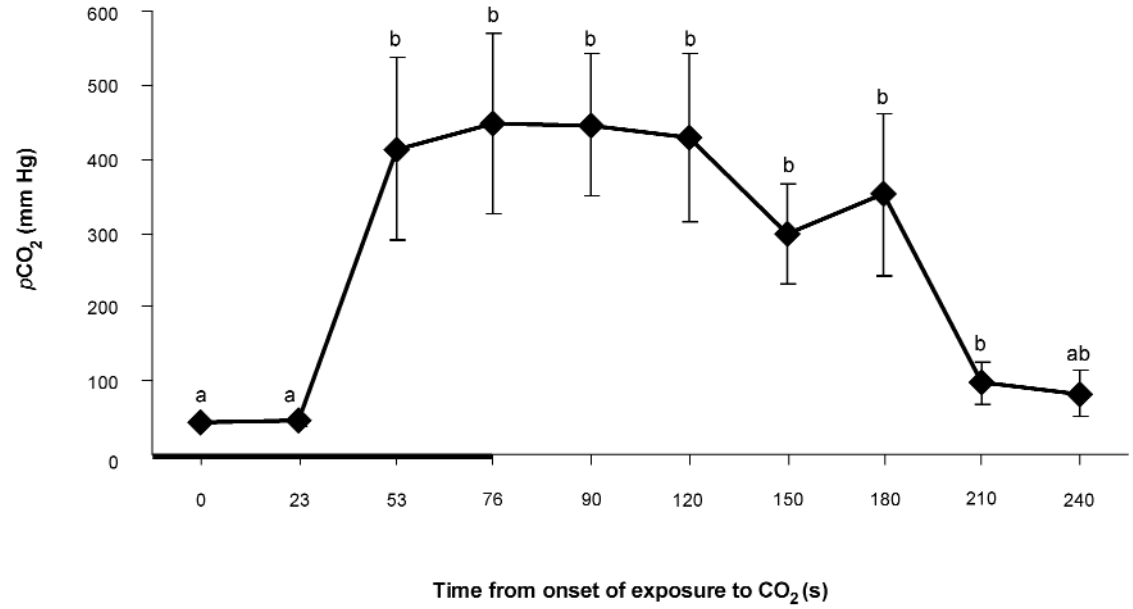
Figure 2



Mean (± SE) pH values before, during and after exposure to 90% CO_2 for 76 s. Black line signifies CO_2 exposure. Values with differing superscripts differ significantly ($P < 0.05$).

Figure 3



Mean (± SE) pCO_2 values before, during and after exposure to 90% CO_2 for 76 s. Black line signifies CO_2 exposure. Values with differing superscripts differ significantly ($P < 0.05$).

(Figure 2). The pH reached its lowest level 15 s after the end of immersion, with a value of 6.71 (± 0.12). Basal pCO_2 was 43.0 (± 5.48) mm Hg. It increased during CO_2 inhalation and differed significantly ($P < 0.05$) from the basal value 53 s after of the onset of CO_2 through until 180 s of post-inhalational recovery (Figure 3). The pCO_2 peaked in value, 76 s into gas immersion, at a point very close to the end of the exposure, at 447.0 (± 122.20) mm Hg. Basal $pO2$ was 79.0 (± 8.16) mm Hg. It decreased during immersion and differed significantly ($P < 0.05$) from values from 53 s after the onset of exposure to CO_2 (Figure 4) through until 180 s of post-inhalational recovery. The pO_2 reached its lowest level 15 s before the end of exposure to CO_2, with a value of 20.3 (± 3.80) mm Hg. The $SatO_2$ basal value was

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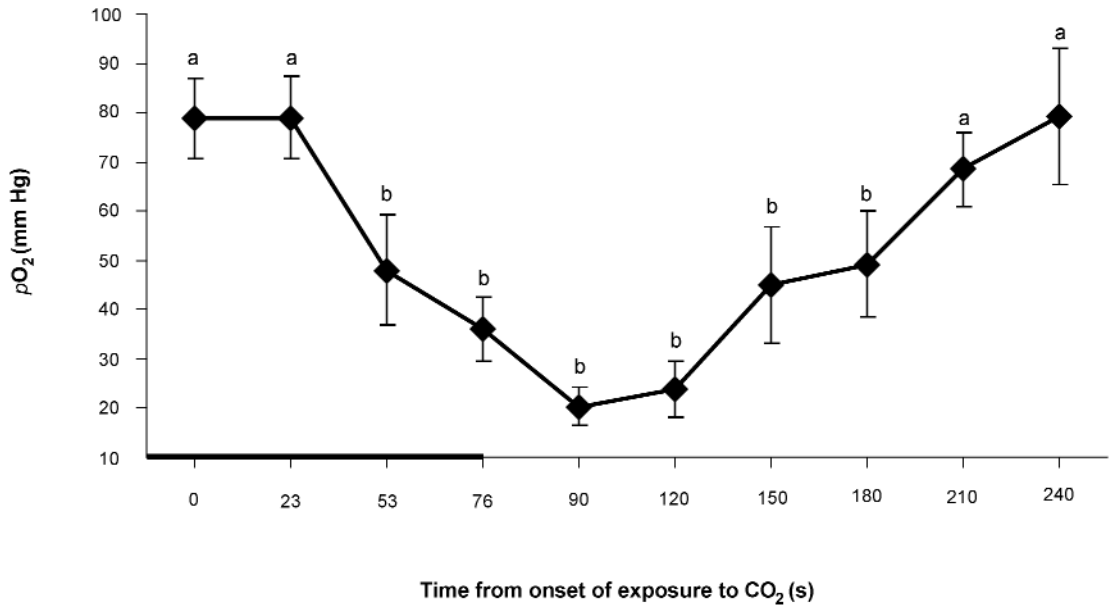
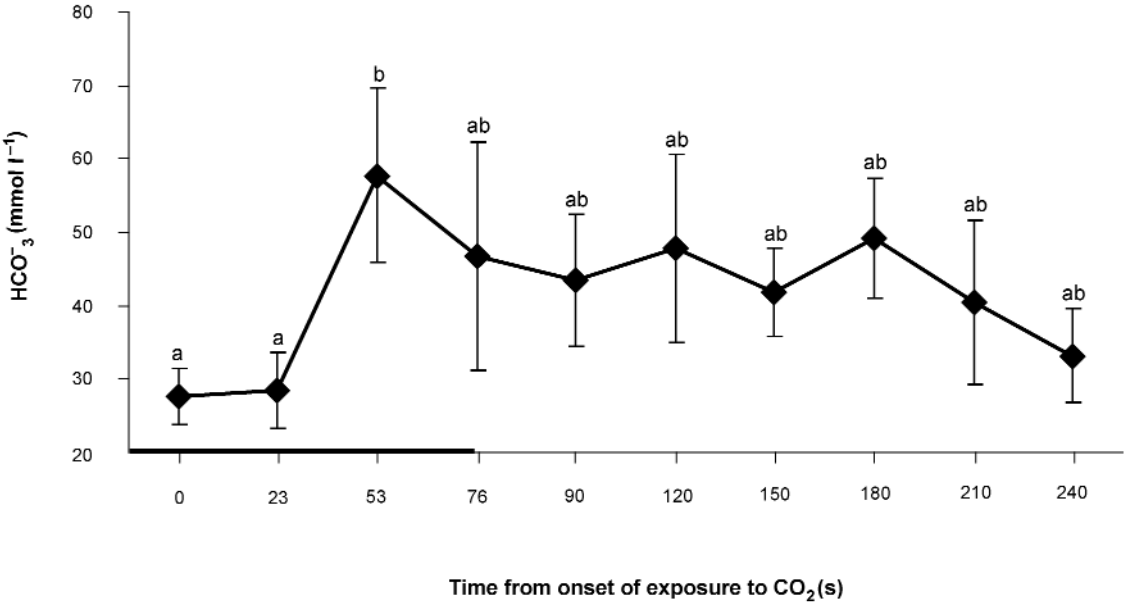
Figure 4



Mean (± SE) pO_2 values before, during and after exposure to 90% CO_2 for 76 s. Black line signifies CO_2 exposure. Values with differing superscripts differ significantly ($P < 0.05$).

Figure 5



Mean (± SE) HCO^-_3 values before, during and after exposure to 90% CO_2 for 76 s. Black line signifies CO_2 exposure. Values with differing superscripts differ significantly ($P < 0.05$).

94.0 (± 2.88)%. It decreased during immersion and differed significantly ($P < 0.05$) from the basal $SatO_2$ from 53 s after the onset of exposure to CO_2, through until 180 s of post-inhalational recovery. The $SatO_2$ reached its lowest value 15 s after the end of exposure, with a value of 11.0 (± 8.59)%. Finally, basal HCO^-_3 was 28.0 (± 3.88) mmol l^{-1} (Figure 5). A significant increase was only seen at 53 s after the onset of exposure to CO_2, with a value of 57.8 (± 11.93).

MLAEP

The MLAEP recording was successful in 19 out of 25 pigs. For the six remaining animals, electrode leads became disconnected during CO_2 exposure. Average AAI and BS%

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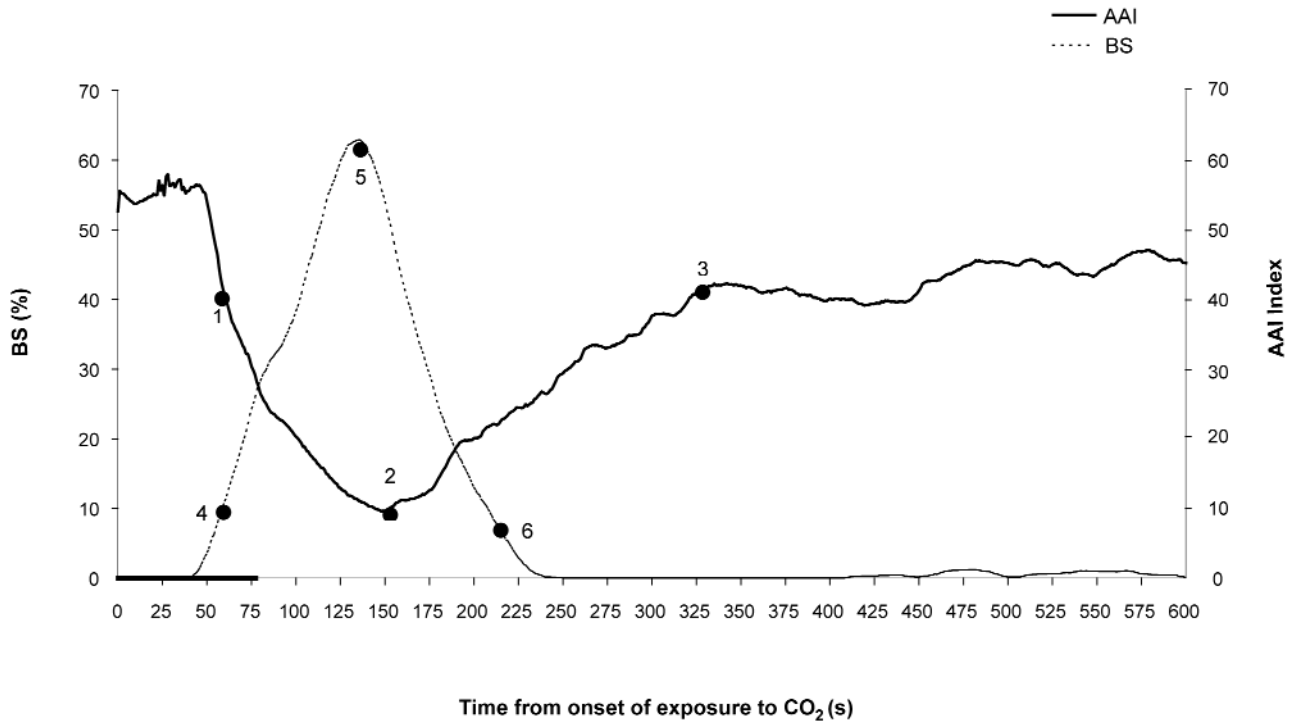
Figure 6



AAI and BS% mean values during exposure and recovery in pigs immersed in 90% CO_2 for 76 s. The times at which the AAI (1) and BS% (4) showed differences with basal levels, the minimum AAI (2) and maximum BS% (5) and the times at which AAI (3) and BS% (6) returned to basal levels are shown.

Table 1 The percentage of animals showing lateral head movements and sneezing, muscular excitation, gasping and vocalisation and the mean (± SE) time to show these behaviours during exposure to 90% CO_2.

Behaviour	Percentage	Time (s)
Lateral head movement and sneezing	53	10.3 (± 1.15)
Start of muscular excitation	100	19.1 (± 1.25)
Gasping	95	23.5 (± 1.45)
Vocalisation	89	26.1 (± 1.80)
End of muscular excitation	100	38.7 (± 1.62)

indices during induction to unconsciousness and recovery are shown in Figure 6. The mean basal AAI index was 52.6 (± 1.41). It began to decrease approximately 52 s after the start of induction, and by 60 s the AAI index differed significantly ($P < 0.05$) from the basal value, with a reading of (40.6 ± 2.74). The AAI index continued to decrease after the end of the CO_2 cycle, reaching its lowest value 72 s after the end of exposure, with a value of 9.6 (± 2.74) (Figure 6). The AAI value then began to gradually increase but remained significantly different from basal levels until 249 s after the end of immersion.

The BS% basal value was 0 (± 0.0) indicating an absence of iso-electrical electroencephalographic activity. It began to increase at 45 s of CO_2 exposure and showed significant differences ($P < 0.05$) at 59 s (9.4 [± 1.75]). It continued to increase until 60 s after the end of the exposure (62.8 [± 1.75]), when it peaked in value. Thereafter, it decreased, although it remained higher than the basal value until 136 s after the end of exposure.

Animal behaviour and physiological reflexes

The percentages of pigs demonstrating specific behaviours during CO_2 exposure, along with the time to perform them, can be seen in Table 1. During exposure to CO_2, 53% of animals exhibited lateral head movements with an average time of 10.3 (± 1.15) s. Thereafter, all the animals demonstrated muscular excitation that began at 19.1 (± 1.25) s and ended at 38.7 (± 1.62) s. During this phase, 95% of animals were seen to be gasping at 23.5 (± 1.45) s and 89% vocalised at 26.1 (± 1.80) s. Gagging was observed in 52% of animals, which did so for the first time at 78.3 (± 4.73) s from the start of immersion.

At the end of exposure, 82% of animals had no corneal reflex. The reflex returned in these animals 27.0 (± 3.92) s later. The outstanding 18% of animals that had a positive corneal reflex at the end of immersion, lost it 18.1 (± 2.49) s later before regaining it at 49.8 (± 2.07) s after the end of gas immersion.

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Discussion

The loss and recovery of consciousness in pigs exposed to 90% CO_2 for 76 s was assessed via means of auditory evoked potentials and corneal reflexes. In addition, stunning physiology was also assessed in terms of blood parameters, such as pH, pCO_2, pO_2, $SatO_2$ and HCO^-_3 and aversion to CO_2 inhalation was assessed through behavioural studies.

The results show that inhalation of 90% CO_2 leads to a progressive increase in blood pCO_2, inducing hypercapnia at 53 s after the onset of immersion. Lomholt (1998) and Martoft *et al* (2002) reported that pCO_2 increased quicker than in the present study, with it becoming significantly higher than basal levels at 40 s after the onset of immersion. However, in these studies, animals were immersed in the greatest CO_2 concentration immediately (approximately 5 s), whereas here, immersion lasted for 23 s and followed a CO_2 concentration gradient that increased with the descent towards the bottom of the well; as is the case commercially. The algorithm used in the present study comprised an optimised artefact rejection method which was able to eliminate the influence from the electromyogram (EMG) during the excitation phase. Therefore, the data recorded in this study should have very little increased delay during the excitation phase.

During CO_2 inhalation, the O_2 of erythrocytes becomes displaced by CO_2 and, as a direct consequence, pO_2 and $SatO_2$ decrease progressively. Kokholm (1990) noted hypoxia when the pO_2 and $SatO_2$ dropped below 60 mm Hg and 90%, respectively. In our study, pigs showed hypoxia at the same time as hypercapnia, at 53 s after the start of exposure. It is suggested, therefore, that both hypercapnia and hypoxia induce unconsciousness in pigs during the inhalation of CO_2. Lomholt *et al* (1998) also found a significant increase in HCO^-_3 during CO_2 inhalation, with values similar to those observed here. Murillo (1995) reported that the respiratory acidosis is characterised by a small increase in the HCO^-_3 concentration in blood.

Conversely, during the recovery from unconsciousness, the HCO^-_3, pH, $pO2$ and $SatO_2$ returned to basal levels earlier than pCO_2.

In the MLAEP, the BS% and AAI values changed during exposure to CO_2. According to these results, the depressive effect of CO_2 exposure is not immediate. When pigs are exposed to 90% CO_2, with a descent time of 23 s, the loss of brain functionality is not evident until the first minute of induction. However, if the descent time is shortened, it is likely that the time to unconsciousness would also be reduced. The changes in brain activity occurred after changes in the blood parameters; evidence that once blood acidosis has been established, the effect on cerebrospinal pH and, hence, brain activity, is fairly immediate.

On the other hand, results from the MLAEP were similar to those found by Martoft *et al* (2002) who also studied evoked potentials in pigs exposed to high concentrations of CO_2. In fact, these authors observed that the period of marked depression (AAI $\leq$ 15) after the end of immersion occurs at approximately 60 to 90 s after the end of this point, with values similar to those found in the present study.

Pigs exposed to 90% CO_2 exhibited side-to-side head movements, sneezing, gasping, muscular excitation and vocalisation during the opening minutes, when brain activity is not depressed and pigs are still conscious. The first behaviour observed was movement of the head and sneezing, on average, 10 s after exposure. Hartung *et al* (2002) postulated that these movements were a clear indication that the animal had detected the gas and found it to be aversive. According to Manning and Schwartzstein (1995), sneezing occurs as a result of CO_2-sensitive nasal chemoreceptors. Gasping and vocalisation were also exhibited by most of the pigs at 23 and 26 s, respectively. Vocalisation has been described as being an indicator of aversion in pigs (Raj & Gregory 1995; EFSA 2004). Gasping indicates suffocation or respiratory arrest (Gregory 1987; Raj 1999). Another behavioural indicator of aversion to CO_2 exposure (Raj & Gregory 1995; Velarde *et al* 2007) is withdrawal movements. However, the pigs in the present study, were unable to perform this movement as they were restrained. Pigs exhibited muscular excitation at between 19 and 39 s exposure to the gas. Hoenderken (1983), by means of an EEG, stated that unconsciousness occurs after this excitation and, hence, these body movements represented voluntary escape attempts. Moreover, Forslid (1987) found that the onset of iso-electric EEG appeared later (53 s) than the onset of muscular movement (28 s). However, he also reported that pigs' muscular movements were preceded by the development of low frequency activity (delta waves) in the neocortical EEG power spectrum and, therefore, the animals were already unconscious at the time.

In addition, during this period, the blood pH and pCO_2 did not differ significantly from basal values. Thus, when we take into account the blood parameters and MLAEP results obtained in the present study, it becomes more likely that pigs are still conscious when muscular movements occur. Consequently, muscular movements observed during CO_2 exposure are more likely to be related to escape attempts (Raj & Gregory 1995; Raj 1996) because of fear, pain or suffocation (EFSA 2004).

The AAI continued to decrease until 72 s after the end of CO_2 immersion whereas the BS% value peaked 60 s after the end of immersion; the period at which the state of anaesthesia is deeper. Blood gas returned to basal levels earlier (between 76 and 165 s after the end of CO_2 immersion) than brain activity (249 and 136 s for AAI and BS%, respectively). In fact, after blood oxygenation, this oxygen must pass the blood-brain barrier and enter the cerebrospinal fluid in order to re-establish the O_2/CO_2 equilibrium and, thus, the pH.

The absence of a corneal reflex is used commercially to assess the effectiveness of stunning as it has been described as the first reflex to disappear during induction to unconsciousness with CO_2 and the first to reappear during recovery (Holst 2001). When the AAI dropped below 15, all the animals had an absence of a corneal reflex and when it rose above 40, the reflex was always present. While the AAI was decreasing and BS% increasing, those animals which had not lost the corneal reflex, finally did so (approximately

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18 s later) thereby confirming a post-induction effect of CO_2 on the corneal reflex, as stated by Panella *et al* (2008). However, all animals recovered their corneal reflex before the AAI and BS% reached minimum and maximum values, respectively. This casts an element of doubt over the effectiveness of the corneal reflex in assessing the consciousness of animals immersed in high concentrations of CO_2. Other authors, such as Panella *et al* (2008) and Forslid (1987), suggested there was great variability in the presence of the corneal reflex between animals after CO_2 exposure.

The study reveals that after 76 s of exposure to 90% CO_2, it takes 325 s to return to a normal level of brain activity. However, in order to avoid pain and stress during slaughter, the pig should be stuck while the animal is in deep anaesthesia. In related AAI studies of humans, dogs and rats, deep anaesthesia was correlated with an AAI index of less than 20 (Jensen *et al* 1998; Thornton *et al* 1989; Litvan *et al* 2002). In the present study, the AAI index for pigs was less than 20 until 104 s after the end of CO_2 exposure. In this instance we suggest this time of 104 s to be the maximum stun-stick interval to avoid pain and suffering during slaughter.

Conclusions and animal welfare implications

When we take the MLAEP into account, the loss of consciousness when a commercial dip-lift stunning system is used, occurs on average at 60 s exposure to 90% CO_2 by volume in atmospheric air. During this time, pigs exhibited side-to-side head movements, sneezing, gasping, muscular excitation and vocalisations. The fact that these behaviours occur when the animal is conscious is evidence that induction to CO_2 anaesthesia is not immediate and pigs suffer from fear, pain and/or stress during immersion into gas. The period of muscular excitation occurs before significant changes are detected in blood pH, pO_2, $SatO_2$, pCO_2 and HCO^-_3 and brain function (AAI and BS%) that would indicate voluntary movements of the animal. This means, from an animal welfare perspective, it is advisable to search for alternatives to the use of CO_2 in stunning pigs in abattoirs.

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References

Anil MH and McKinstry JL 1991 Reflexes and loss of sensibility following head-to-back electrical stunning in sheep. *The Veterinary Record 128*: 106-107

Cameron AC and Trivedi PK 1998 *Regression Analysis of Count Data.* Cambridge University Press: Cambridge, UK

EFSA 2004 Welfare aspects of animal stunning and killing methods. *Scientific report of the Scientific Panel of Animal Health and Welfare on a request from the Commission. Question.* Adopted on the 15th of June 2004. Brussels, Belgium. http://www.efsa.eu.int/science/ahaw/ahaw_opinions/495/opinion_ahaw_02_ej4 5_stunning_report_v2_en1.pdf.

Forslid A 1987 Transient neocortical, hippocampal and amygdaloid EEG silence induced by one minute inhalation of high concentration CO_2 in swine. *Acta Physiological Scandinavica 130*: 1-10

Gregory NG, Moss B and Leeson R 1987 An assessment of carbon dioxide stunning in pigs. *The Veterinary Record 121*: 517-518

Gregory NG, Raj ABM, Audsley ARS and Daly CC 1990 Effects of carbon dioxide on man. *Flieschwirtschaft 70*: 1173-1174

Hartung J, Nowak B, Waldmann KH and Ellerbrock S 2002 Carbon dioxide anaesthesia of pigs for slaughter: Influence of EEG, distribution of catecholamines and clinical reflexes. *Deutsche Tierärztliche Wochenschrift 109*: 135-139

Hoenderken R 1983 Electrical and carbon dioxide stunning of pigs for slaughter. In: Eikelenboom G (ed) *Stunning of Animals for Slaughter* pp 55-63. Martinus Nijhoff Publishers: Boston, USA

Holst S 2001 CO_2 stunning of pigs for slaughter, practical guidelines for good animal welfare. *47th International Congress of Meat Science and Technology*. 27-31 August 2001, Krakow, Poland

Jensen EW, Lindholm P and Henneberg SW 1996 Autoregressive modelling with exogenous input of middle-latency auditory-evoked potentials to measure rapid changes in depth of anaesthesia. *Methods of Information in Medicine 35*: 256-260

Jensen EW, Marianne N and Oteen WH 1998 On-line analysis of middle latency auditory evoked potentials (MLAEP) for monitoring depth of anaesthesia in laboratory rats. *Medical Engineering & Physics 20*: 722-728

Jensen EW 1999 *Monitoring depth of anaesthesia by auditory evoked potentials.* PhD Thesis, Faculty of Health Sciences, University of Southern Denmark, Winsløwparken 19, 3 DK-5000 Odense C, Denmark

Kokholm G 1990 Simultaneous measurements of blood pH, pCO_2, pO_2 and concentrations of haemoglobin and its derivates - a multicenter study. *Scandinavian Journal of Clinical Laboratory Investigation Supplementum 203*: 75-86

Lambooij E, Gerritzen MA, Engel B, Hillebrand SJW, Lankhaar J and Pieterse C 1999 Behavioural responses during exposure of broiler chickens to different gas mixtures. *Applied Animal Behaviour Science 62*: 255-265

Litvan H, Jensen EW, Revuelta M, Henneberg SW, Paniagua P, Campos JM, Martínez P, Caminal P and Villar Landeira JM 2002 Comparison of Auditory Evoked Potentials and the A-line ARX index for monitoring the hypnotic level during sevoflurane and propofol induction. *Acta Anaesthetic Scandinavica 46*: 245-252

Lomholt L 1998 *Changes in blood gases and acid-base values during high concentration pre-slaughter CO_2 stunning of pigs.* PhD Thesis, Department of Anatomy and Physiology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark

Manning HL and Schwartzstein RM 1995 Pathophysiology of dyspnea. *New England Journal of Medicine 333*: 1547-1553

Martoft L, Jensen EW, Rodriguez BE, Jorgensen PF, Forslid A and Pedersen HD 2001 Middle-latency auditory evoked potentials during induction of thiopentone anaesthesia in pigs. *Laboratory Animals 35(4)*: 353-363

Martoft L, Lomholt L, Kolthoff C, Rodríguez BE, Jensen EW, Jorgensen PF, Pedersen HD and Forslid A 2002 Effects of CO_2 anaesthesia on central nervous system activity in swine. *Laboratory Animals 36(2)*: 115-126

Murillo D 1995 Control of respiration. In: AG Sacristan (ed) *Veterinary Physiology* pp 409-417. McGraw-Hill Interamericana: Madrid, Spain

Panella N, Dalmau A, Fàbrega E, Font I, Furnols M, Gispert M, Tibau J, Soler J, Velarde A, Oliver MA and Gil M 2008 Effect of supplementation with $MgCO_3$ and L-tryptophan on the welfare and on the carcass and meat quality of two halothane pigs genotype (NN and nn). *Livestock Science 115*: 107-117

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Raj ABM and Gregory NG 1995 Welfare implications of the gas stunning of pigs 1. Determination of aversion to the initial inhalation of carbon dioxide or argon. *Animal Welfare 4*: 273-280

Raj ABM 1996 Aversive reactions of turkeys to argon, carbon dioxide, and a mixture of carbon dioxide and argon. *Veterinary Record 138*: 592-593

Raj ABM and Gregory NG 1996 Welfare implications of gas stunning of pigs 2. Stress of induction of anaesthesia. *Animal Welfare 5*: 71-78

Raj ABM 1999 Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. *The Veterinary Record 144*: 165-168

SAS 2001 *SAS for Windows Release v8.2*. SAS Institute Inc: Cary, North Carolina, USA

Thornton C, Konieczko KM, Knight AB, Kaul B, Jones JG, Dore CJ and White DC 1989 Effect of propofol on the auditory evoked response and oesophageal contractility. *British Journal Anaesthesia 63*: 411-417

Troeger K and Woltersdorf W 1991 Gas anaesthesia of slaughter pigs. Stunning experiments under laboratory conditions with fat pigs of known halothane reaction type: meat quality and animal protection. *Fleischwirtschaft 71*: 1063-1068

Velarde A, Cruz J, Gispert M, Carrión D, Ruiz-de-la-Torre JL, Diestre A and Manteca X 2007 Aversion to carbon dioxide stunning in pigs: effect of the carbon dioxide concentration and the halothane genotype. *Animal Welfare 16*: 513-522

Velarde A, Gispert M, Faucitano L, Alonso P, Manteca X and Diestre A 2001 Effects of the stunning procedure and the halothane genotype on meat quality a incident of haemorrhages in pigs. *Meat Science 58*: 313-319

Velarde A, Ruiz-de-la-Torre JL, Stub C, Diestre A and Manteca X 2000 Factors affecting the effectiveness of head-only electrical stunning in sheep. *The Veterinary Record 147*: 40-43

https://doi.org/10.1017/S0962728600027834 Published online by Cambridge University Press