Br. vet. J. (1991). **147**, 322

CHANGES IN THE SOMATOSENSORY EVOKED POTENTIALS AND SPONTANEOUS ELECTROENCEPHALOGRAM OF HENS DURING STUNNING IN ARGON-INDUCED ANOXIA

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SUMMARY

This study examined the time to loss of consciousness in hens during stunning in argon-induced anoxia. Somatosensory evoked potentials (SEPs) and the spontaneous electroencephalogram (EEG) were recorded in 12 culled hens prior to and during stunning in less than 2% oxygen (air displaced by argon). An additional 20 hens were stunned with a similar concentration of oxygen and the time to loss of posture, eye closure, and the onset and duration of clonic and tonic convulsions were recorded. A further 10 hens were immersed in less than 2% oxygen for 15–17 s and their response to comb pinching was tested as soon as they had been transferred to atmospheric air.

It is concluded that the birds had not lost the primary response in their SEPs by the time they started convulsing, but the reduction in the amplitude of the SEPs, changes in their spontaneous EEG and a negative response to comb pinch before the start of the convulsions indicated that the birds were unconscious when they convulsed.

INTRODUCTION

Previous investigations in this laboratory have indicated that carbon dioxide- or argon-induced anoxia could be useful methods for stunning chickens. It has been found that batch stunning broilers in carbon dioxide- or argon-induced anoxia resulted in better carcass and meat quality (Mohan Raj *et al.*, 1990c) and a lower incidence of broken bones when compared with electrical stunning (Mohan Raj *et al.*, 1990a). It was also found that the convulsions that occur during stunning in argon resulted in rapid early post-mortem glycolysis without affecting the meat quality, and it was suggested that this could provide an opportunity for filleting the carcasses at an earlier time *post mortem.*

It is important to establish whether the birds are conscious or unconscious during the convulsive phase. A previous investigation using somatosensory evoked potentials (SEPs) and spontaneous EEG as indicators of cerebral function revealed that hens experienced

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suppressed brain function at about 30 s after exposure to 45% carbon dioxide. Since the convulsions started about 15 s later, it was concluded that the hens were unconscious during the convulsive phase (Mohan Raj *et al.*, 1990b). The absence of an evoked response indicates a profound form of brain failure and provides an unequivocal diagnosis of insensibility following stunning (Gregory & Wotton, 1990). However, it should be noted that the presence of an evoked response implies that the afferent pathways to the higher brain centres are intact, but not necessarily whether the animal is aware of the stimulus. For example, evoked responses are present during anaesthesia in chickens (Gregory & Wotton, unpublished observations).

This study was carried out to determine if the birds are unconscious when they are convulsing during argon stunning.

MATERIALS AND METHODS

The experiment was divided into three parts. Twelve hens were implanted with electrodes as described in the previous report (Mohan Raj *et al.*, 1990b) and used to investigate the time to loss of SEPs and spontaneous EEG during argon stunning. Twenty hens were used to determine the time of the occurrence of loss of posture, eye closure, onset and durations of clonic and tonic convulsions during exposure to argon. An additional 10 hens were used to determine the response to a comb pinch after 15–17 s exposure to less than 2% oxygen. At this time the convulsive phase would have started at least in a small proportion of the birds.

Each bird was stunned separately and the experimental procedures were the same as described in an earlier report (Mohan Raj *et al.*, 1990b), except for the design of the stunning apparatus. In this study, a wooden box (62×62×62 cm) with a perspex window and a lid was used as the stunning apparatus. This stunning box was filled with argon to achieve less than 2% oxygen and the concentration of oxygen was continuously monitored (Servomex gas analyser, Model 1175). A hen implanted with EEG recording and somatosensory stimulating electrodes according to Gregory & Wotton (1989), was restrained inside a lidless close fitting hexagonal box to avoid any damage to the electrodes during convulsions. Just prior to stunning, the SEPs (3.4 V for 2 ms at 2 stimuli/s) and EEG were recorded for 2 min while the hens were in air and in the restrained position. The restrained bird was then placed inside the stunning apparatus which had been prefilled with argon. The spontaneous EEG and SEPs were continuously recorded for 2 min during exposure.

Consecutive averages of eight successive stimuli given during 4 s were analysed to identify the presence or absence of the primary response in the SEPs. Preliminary analysis of the data from two batches of hens showed that the birds convulsed before the total abolition of SEPs occurred. Thus, a third batch of 10 hens was used to test for the response to comb pinching after exposure to less than 2% oxygen for 15–17 s (7–5 s before the start of convulsions). The head withdrawal response to comb pinching was also tested prior to exposure to argon. Comb pinching was performed at the anterior end of the comb with the nails of the index finger and thumb of the left hand, while gently holding the head of the bird with the right hand.

The spontaneous EEG recordings were subjectively evaluated for the changes in the waveforms and their time of occurrence in relation to the time of exposure to anoxia. In addition, the EEG traces of eight hens were also subjected to analysis by Fast Fourier

Transformation (FFT). Epochs of 5 s duration were sampled at a frequency of 200 Hz and these epochs were subsequently split into 1 s epochs to determine the changes, as they occurred rapidly during the post-exposure period. A mean of five 1 s epochs during the pre-exposure period (between 90 and 95 s after the start of the SEP stimuli) was considered as a representative control for each hen. Each 1 s epoch, up to 50 s during exposure, was analysed to evaluate the changes in the EEG due to argon-induced anoxia. The RMS power (root mean square power, hereafter termed RMS) of the epochs was determined to compare the changes induced by the anoxia. The term EEG suppression used in this study refers to the decrease in amplitude or RMS of the signals. The amplitude spectrum of the FFT was also computed for a frequency range of 1–30 Hz (DADiSP Worksheet) and the relative power content as a percentage of the total was determined for the following frequency ranges: 1–4 Hz; 5–7 Hz; 8–13 Hz and 14–30 Hz. After the derivation of the power spectrum of individual hens the average was taken for the eight hens.

In addition, the data were subjected to an unpaired *t*-test to determine the statistical significance of any differences between the time to occurrence of behavioural events, loss of SEPs and changes in the waveforms of the EEG evaluated subjectively.

RESULTS

The results of the subjective evaluation of the spontaneous EEG traces and the time of occurrence of the behavioural events are presented in Table I. The subjective evaluation of the EEG traces showed that the low frequency–high amplitude waves and a marked reduction in amplitude of the waveforms appeared at 11 s and 17 s respectively. These times were very close to the time to loss of posture and eye closure respectively (11 s and

Table I
The sequence and time of occurrence of events during stunning of chickens in argon-induced anoxia

Events	*Time of occurrence(s)*		*95% Confidence interval*	
	mean	*SD*	*lower*	*upper*
Spontaneous EEG				
Low frequency and high amplitude	11	3	9	12
EEG suppression	17	3	17	19
Onset of quiescent EEG	62	6	57	66
Loss of SEPs	29	8	24	34
Behavioural events				
Loss of posture	11	2	10	12
Eye closure	18	3	17	19
Onset of clonic phase	22	4	20	24
Duration of clonic phase	12	3	10	13
Onset of tonic phase	35	5	35	39
Duration of tonic phase	23	8	20	27

Time to loss of SEPs in this table refers to total abolition of both the primary and the cortical response.

18 s). The time to loss of SEPs was 29 s and it was significantly longer than the time to start of clonic convulsions (22 s; P=0.003). Figure 1 would indicate that the response of the higher centres to the afferent signals was reduced in 9–16 s, and after 24 s of exposure it was totally lost. The amplitude of the SEPs decreased by 50% after 16 s exposure to argon (Fig. 1).

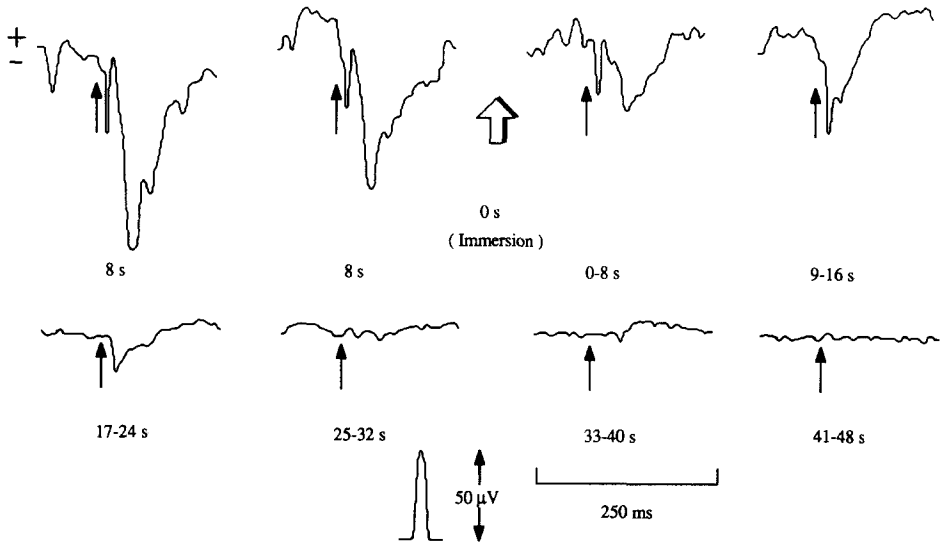

Fig. 1. The effect of argon-induced anoxia on the somatosensory evoked potentials of a hen. ↑ indicates the event marker for the stimulus. Each trace is an average of 16 stimuli delivered at the rate of two per second.

The relative change in the RMS of the EEG signals is presented in Fig. 2. Due to the individual bird variation in the time of onset of convulsions and the smoothing of the line (with a 5 point smoothing in the graph), the relative change in the mean RMS of the EEG (n=8), as indicated by the solid line in Fig. 2, did not correspond with the timings reported in Table I. Due to these differences in methodology, the solid line shown in Fig. 2 tends to suggest that the EEG suppression occurred only at 28 s, which, in comparison with the values shown in Table I, was 11 s longer. The relative changes in the RMS of a hen which showed fewer convulsions (indicated by the dotted line in Fig. 2) were used as an example to illustrate the changes. In that particular hen, the RMS value gradually decreased until 8 s post-exposure, after which the RMS increased briefly by 34% between 9 and 11 s due to the appearance of slow waves. At 12 s post-exposure, the EEG contained only 34% of the RMS of the pre-exposure control, and subsequently the power gradually decreased, leading to a quiescent phase. The RMS of all eight hens followed a similar trend, but the time of occurrence of the decrease differed between 12 and 17 s, and the time to onset of convulsions ranged from 17 to 33 s. This variation tended to mask the suppressive effect in the mean trace during this period.

Figure 3 illustrates the amplitude spectra of the individual frequency ranges of the EEG signals (average of eight hens). The 1–4 Hz range gradually increased up to 22 s after exposure to argon. When the convulsions started, this frequency range fell to a

minimum with a concurrent increase in the 8–13 Hz range. After the clonic convulsive episode, the 1–4 Hz range gradually increased until 45 s post-exposure. The 5–7 Hz frequency range decreased by 35% during the initial 5 s period, but by 10 s it had returned to a level which was very close to that of the pre-exposure EEG. After this increase, this frequency range gradually decreased to 45% of the pre-exposure control levels within 30 s. The 8–13 Hz frequency range increased by 10 s post-exposure and during the clonic convulsive phase, i.e. between 22 and 30 s post-exposure. The 14–30 Hz frequency range increased slightly during the initial 5 s period, but it momentarily decreased by about 20% at 10 s post-exposure. Subsequently, this frequency range increased by 40% during the clonic and tonic convulsive episodes and remained higher than the content of the pre-exposure control for up to 50 s post-exposure.

All the hens in the third batch showed a positive response to comb pinching just prior to exposure to argon, and when retested after exposure to less than 2% oxygen for 15–17 s none of the birds showed a positive response.

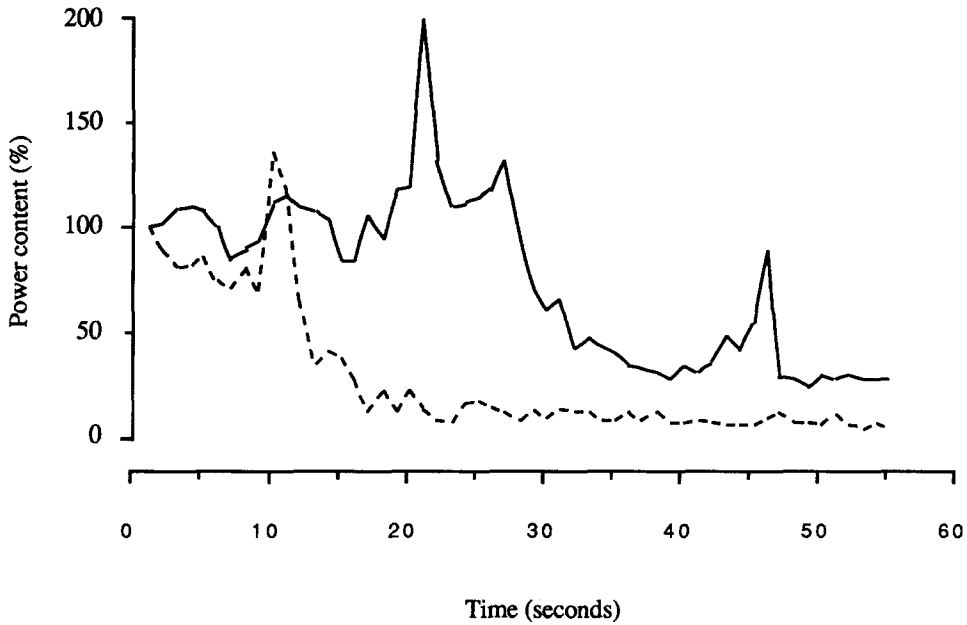

Fig. 2. The changes in the RMS of the EEG signals of hens during stunning with argon-induced anoxia. ———, Average of 8 hens; - - -, RMS of a single hen which showed fewer convulsions.

DISCUSSION

Based on the changes in the EEG waveform, the effects of anoxia have been characterized into four phases in the cat (Baumgartner *et al.*, 1961): (1) first, the *free interval* during which there was no change in the EEG; (2) an *activation period* during which the EEG showed arousal type waveforms (15–40 Hz and the presence of spike waves) between the 10th and 20th s of breathing 100% nitrogen. This change is thought to be due to cortical

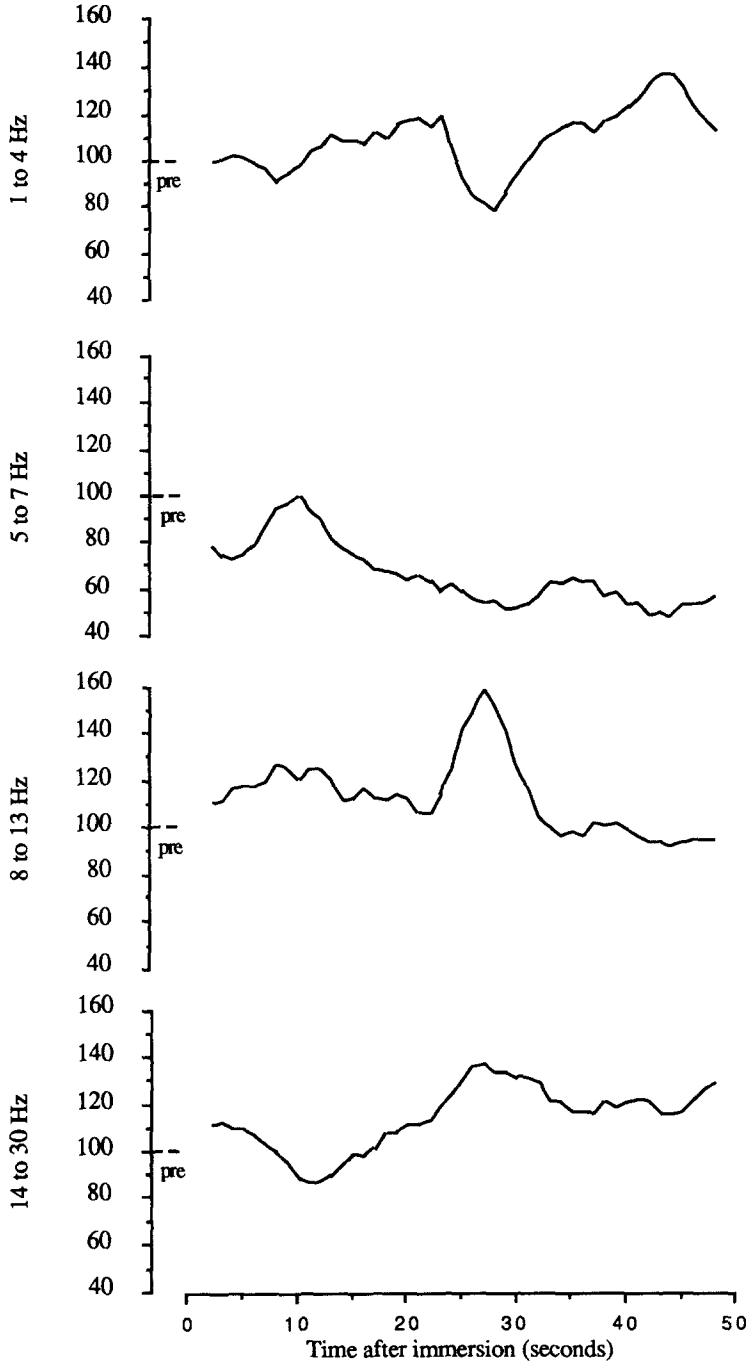

Fig. 3. Relative changes (%) in the frequency spectra of the EEG signals of hens (n=8) during stunning with argon-induced anoxia.

activation caused by the reticular discharges induced by the carotid and aortic chemoceptors responding to changes in the P_{aO_2} and P_{aCO_2}; this was followed by (3) a *delta period* during which the EEG showed slow rhythms between 1 and 5 Hz due to cortical synchronization. This period was found to be shorter in true anoxia in comparison with incomplete anoxia (1–2% oxygen) and hypoxia (8% oxygen); (4) lastly, a *null period* characterized by complete electrical silence of the EEG. These four stages occurred in the same sequence but lasted considerably longer and showed greater variation in incomplete anoxia and mild hypoxia compared with complete anoxia. The average survival times of the EEG (time to isoelectric EEG) in the cat were reported to be 36 s, 50 s and 185 s, respectively, in anoxia, incomplete anoxia and hypoxia (Baumgartner *et al.*, 1961).

During anoxia induced by nitrogen inhalation, the time of appearance of the delta period (slow waves in the EEG) coincided with the loss of consciousness in man (Kasamatsu, 1952) and with significant changes in the firing pattern of cortical neurons recorded individually with microelectrodes in the cat (Kasamatsu *et al.*, 1956). During the transition from the activation period to the delta period in the cat, half of the cortical neurons have been reported to have lost their spontaneous discharge, even though evoked sensory neuronal discharges (e.g. by optic nerve stimulation) could be elicited even during the first part of the null period (Baumgartner *et al.*, 1961).

In this study, in which hens were exposed instantaneously to argon, neither the behaviour of the birds nor the subjectively observed changes in the EEG waveforms showed any arousal. However, the frequency spectra indicated that during the initial 5 s after exposure to argon there was an increase of up to 10% in the > 13 Hz frequency range (without any increase in the amplitude), and this could represent a degree of arousal. This was probably due to the handling of the birds during immersion into argon.

Both subjective and FFT analysis showed that the slow waves (1–8 Hz in the case of FFT analysis) appeared at about 11 s post-exposure and these slow waves predominated for only 3–4 s, which is characteristic of true anoxia (Baumgartner *et al.*, 1961). These slow waves are due to the effect of anoxia on the cerebral cortex and they are the first indication of cortical dysfunction (Gastaut & Gastaut, 1958). The onset of slow waves has been attributed to depletion of cortical acetylcholine (Crossland & Richter, 1950), the neurotransmitter associated with reticular activation of the cortex and by implication the high frequency activity found in the EEG of conscious animals. This depletion of acetylcholine may be due to the reduction in the rate of its resynthesis. The hypocapnia that occurs during anoxia probably causes cerebral vasconstriction, and this in association with diminished arterial oxygen tension of the anoxia interferes with the oxidative metabolic processes of the nerve cells (Ernsting, 1956). Extrapolating from human experience, this delta period may represent loss of sensibility in hens.

In the majority of the hens after the initial EEG suppression, spike discharges occurred and this resulted in an increase in the RMS up to 28 s post-exposure (solid line in Fig. 2). When the birds were dead, shaking their heads or moving the electrode leads indicated that physical artefacts were of very low amplitude in the EEG, and so they would have accounted for only a small part of the increase in the RMS. The time of occurrence of this epileptiform activity coincided with the time of occurrence of the clonic convulsions. However, the dotted line in Fig. 2 indicates the extent of EEG suppression that had occurred in a hen which did not show any clonic convulsions. In this hen there was a reduction of 66% in the RMS at 12 s post-exposure and this could indicate a severe

dysfunction of the higher centres. Although the reduced level of RMS activity at which consciousness is lost is not known, if a 50% reduction in RMS could be an arbitrary unit representing failure of the higher centres, it was found that it occurred in an average of 14 s (range=6–22; n=8). It has been reported that the survival time of cerebral cortex during anoxia is only 14–15 s in the cat (Sugar & Gerard, 1938).

In addition to the slow waves, the sharp decline in the RMS of the EEG could be viewed as further evidence of a rapid induction of insensibility in hens, since the suppression of EEG indicates reduced neuronal activity. This point is further emphasized by the results of the averaged SEPs (Fig. 1). These became suppressed between 9 and 16 s, and were absent between 17 and 24 s after exposure, except for the earliest negative going potential. This early potential represents the first telencephalic response or may even represent the thalamo-telencephalic afferent volley, and would be expected to have greater resistance to anoxia than the potentials of longer latencies. At the time of loss of SEPs the average RMS of the EEG signal was only 20% (n=8) of the pre-exposure period. A similar reduction in the power of the telencephalic signals would also arise during the hypoxaemia produced by exsanguination and it has been reported that the RMS content of ECoG was reduced 90% in the 30–40 s when exsanguination was performed after electrical stunning of calves (Bager *et al.*, 1990).

The persistence of the high frequency signals (> 13 Hz), though very much suppressed, beyond the time to loss of SEPs appears to be non-specific with no relevance to the state of consciousness of the hens. For example, decapitation of anaesthetized rats showed similar suppression of the electrocorticogram, simultaneously with rapid accumulation of intracellular NADH (Mayevsky, 1978). The oxidation of NADH helps to provide energy for the active metabolic processes in the cell including the sodium–potassium pump, thus its accumulation would indicate failure of normal metabolism and electrical activity. In this study, the disappearance of slow waves and an immediate onset of EEG suppression would indicate that the oxidative metabolic process was seriously interfered with. By implication this would mean that the suppressed EEG with residual high frequency activity represents a period of severe metabolic crisis and as such it is unlikely to represent a state of sensibility.

The onset of convulsions can be interpreted as further evidence of cortical dysfunction. It has been reported that depression of the electrical activity induced by anoxia extends progressively from the telencephalon to the diencephalon and then to the mesencephalon (Ernsting, 1965). Anoxic convulsions result from the release of the caudal reticular formation from the suppression by higher centres, particularly the cerebral cortex and rostral reticular formation (Dell *et al.*, 1961; Ernsting, 1965). Thus the onset of convulsions can be viewed as failure of the cortical control and activity. Anoxia results in suppression of the rostral reticular formation and this helps to produce a loss of consciousness, synchronization of the cortical activity and the loss of suppression of the caudal reticular formation, and hence the onset of convulsions (Dell *et al.*, 1961; Ernsting, 1965).

It is concluded that the changes in the waveforms of EEG and SEPs, and the absence of response to a comb pinch indicated that the hens had lost consciousness before the convulsions began. The hens were evidently unconscious when the convulsions occurred during stunning in argon-induced anoxia.

ACKNOWLEDGEMENT

This research was conducted in collaboration with British Oxygen Company Limited, UK. The authors wish to thank Dr C. C. Daly for his advice in the FFT analysis.

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(Accepted for publication 12 November 1990)