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Transcranial magnetic stunning of broilers: a preliminary trial to induce unconsciousness

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Abstract

This study was performed to identify whether non-focal transcranial magnetic stimulation (TMS) with an adapted coil for broilers has the potential for use as a non-invasive stunning method for broilers. Application of the TMS probe resulted in dominance of theta and delta waves and appearance of spikes in the electroencephalogram (EEG) after stimulation. Correlation dimension (CD) analyses of the EEG signals recorded prior to and following the application of TMS suggested that the birds might be unconscious for approximately 15 to 20 s assuming that a reduction in CD to 60% of the baseline value indicates unconsciousness. Other observations included loss of behavioural arousal or muscle tone (muscle flaccidity), and irregular heart rate after TMS. It can be suggested that TMS has the potential to be developed as a stunning method in the future. The technique, evaluated using small number of broilers in this study, requires further improvement and the use of a power supply optimised in future research. Transcranial magnetic stimulation of the brain has potential for application as a non-invasive stunning method for broilers, which could be acceptable to some religious groups opposed to the use of established or conventional stunning methods.

Keywords: *animal welfare, behaviour, brain and heart activity, broilers, single and double coil, transcranial magnetic stunning*

Introduction

A basic requirement for humane slaughter of animals, including poultry, is that they should be stunned, rendered unconscious and insensible, prior to slaughter by exsanguination. Under commercial conditions, poultry species are mostly stunned using an electrified water bath.

The water-bath method is based on application of an electric current through the body of the bird which is hung head-down by the legs in moving shackles. Thereafter, the birds pass through the bath in line. Depending on the dimensions of the bath, several birds are submerged (up to their shoulders) simultaneously in water. In conventional use, a metal strip at the base of the water bath acts as one electrode whilst the earthed shackles form the negative electrode. In this way, the electric current is passed through the bird, from head-to-legs. The water bath is electrically live so that each bird is stunned the moment it makes contact with water (Bilgili 1999; Raj 2003).

Scientists have long been searching for alternatives to electro-narcosis (ie electrical stunning). Transcranial magnetic stimulation (TMS) is a recently developed non-invasive technique used in the field of human psychiatry to treat depression with slowly repeated pulses to the frontal lobe (George 2003) or to induce seizures (Lisbany 2002). However, its greater use is in the measurement of conduction time in the motor pathways of the central nervous system (CNS) (Mills 1999).

Electromagnetic induction was first described by Michael Faraday in 1831. He wound two coils on an iron ring and showed that when the coil was connected on one side an electrical current passed through the coil on the other side. With non-invasive magnetic stimulation, the stimulating coil acts as the first coil, air as the medium for the flow of the magnetic field and the electrical conductivity of the brain tissue as the second coil. A magnetic field is the means by which an electrical current is generated within the tissue that causes depolarisation of the cell membrane. (Fitzgerald *et al* 2006). In practice, a single (circular) or double TMS probe containing a copper coil is placed on the skull and an electric current charged by a TMS generator induces the magnetic stimulus within the surface cortex of the brain. TMS fields extend only a few centimetres to the surface of the cortex, although a double probe allows further penetration into the brain (George 2003). Several measurements were performed under controlled laboratory conditions on individual broilers used in the present study. These investigations were performed in cooperation with scientists from the University of Bristol, UK, using a prototype device (Anil *et al* 2000).

The objective of the study was to determine if transcranial magnetic stimulation with an adapted coil for broilers has the potential for further development to be a non-invasive stunning method for broilers. The effects of TMS were assessed in broilers using electroencephalograms (EEGs) and electrocardiograms (ECGs).

Materials and methods

Study animals

The experiment was performed in a laboratory of the Wageningen UR Livestock Research, The Netherlands, in close proximity to where the broilers were raised. In total, 25 broilers were used for the experiment. During the procedures, the birds were placed one-by-one in a purpose-made restrainer for stunning and recording of the EEG and the ECG. The birds were suspended individually by the feet from shackles and TMS was applied using a single or double hand-held coil on the head of the animal.

At the end of the experiment the broilers were killed, then weighed.

Registration of EEG and ECG and behaviour

Prior to the application of TMS, each broiler was equipped with EEG and ECG recording electrodes in accordance with the method described by Lambooij *et al* (2008). The EEGs and ECGs were recorded for 30 s before and until 5 min after the TMS. The recorder used was a DI 720 data recording module with a WinDaq Waveform browser (Dataq Instruments, Akron, Ohio, USA).

The electrical activity recorded on the EEG can be classified into delta (0–4 Hz), theta (4–7 Hz), alpha (8–13 Hz) and beta (> 13 Hz) frequency bands. Animals are considered to be conscious within the alpha and beta rhythm bands (Kooi *et al* 1978). Predominance of theta and delta waves and appearance of spikes on the EEG leading into an iso-electric line indicate cessation of brain activity (Lopes da Silva 1983).

The behaviour of the animals was monitored for the occurrence of:

- tonic cramp: severe tension of all muscles;
- clonic cramps: uncontrolled severe muscle contractions;
- exhaustion: muscle flaccidity;
- recovery wake up with righting.

Experimental treatments

The TMS Stimulater Magstim Rapid 2 used during this study was patented and manufactured by The Magstim Company Ltd, Carmarthenshire, UK. The company makes the equipment for use with human patients. The device used was designed to deliver a magnetic stimulus to the brain induced via an electric current passing through a copper coil built-in to a probe which is placed on the head in close proximity to the brain. This was possible by using either a double probe placed over the skull or single probe (circular) placed on the rear of the skull. The stimulating coil consists of tightly wound and well-insulated copper windings with safety switches to prevent high temperature and the design was adapted to the head of a 5- to 6-week old broiler.

It was possible to adjust the amount of power generated by the device and the characteristics (duration of stun, number of biphasic pulses per second and the waiting time between cycles) of the impulse generated using a control panel on the stimulator.

To be sure to induce an effect, each TMS application lasted 5 s. After each TMS, the broilers were observed for a maximum of 5 min for their responses to pain stimuli (comb pinch) whilst the birds were in the restrainer. The first comb pinch was administered 30 s after completion of the stun and then 1 min after the stun, Thereafter, a comb pinch was given at 2, 3, 4 or 5 min after stunning. This process was terminated after two successive positive responses in order to limit the distress to the bird. The stimulator was set at 51 or 80% power for the single and the double probe, respectively. The higher the power setting the lower the frequency. Frequencies of 50 and 35 Hz were set at 51 and 80% power, respectively. The number of pulses was also adjustable and set within the range of 250 to 300 pulses in the pulse train.

Ethics

The experiments were approved beforehand by the Ethical Committee of the Animal Sciences Group of Wageningen UR, The Netherlands.

Statistical analysis

The EEG traces were subjected to correlation dimension (CD) analysis. This analysis provides a non-linear (fractal) measure of signal complexity (for algorithm, see Broek *et al* 2005). Correlation dimension analysis is a relatively new technique that has been customised to measure depth of anaesthesia in humans (Broek 2003). The small amplitude, high frequency (awake) EEG signal is more complex than the large amplitude, low frequency (unconscious) signal. Therefore, high CD values are taken to indicate consciousness while low values indicate a state of unconsciousness. It is suggested that chickens are awake, drowsy and asleep at a CD score of 7, 6.6 and 6, respectively (Coenen & van den Broek 2005) and a reduction in CD to 60% of the baseline value is seen in unconscious birds (McKeegan *et al* 2007).

An analysis of variance was used to analyse the heart rate (Genstat 2008).

Results

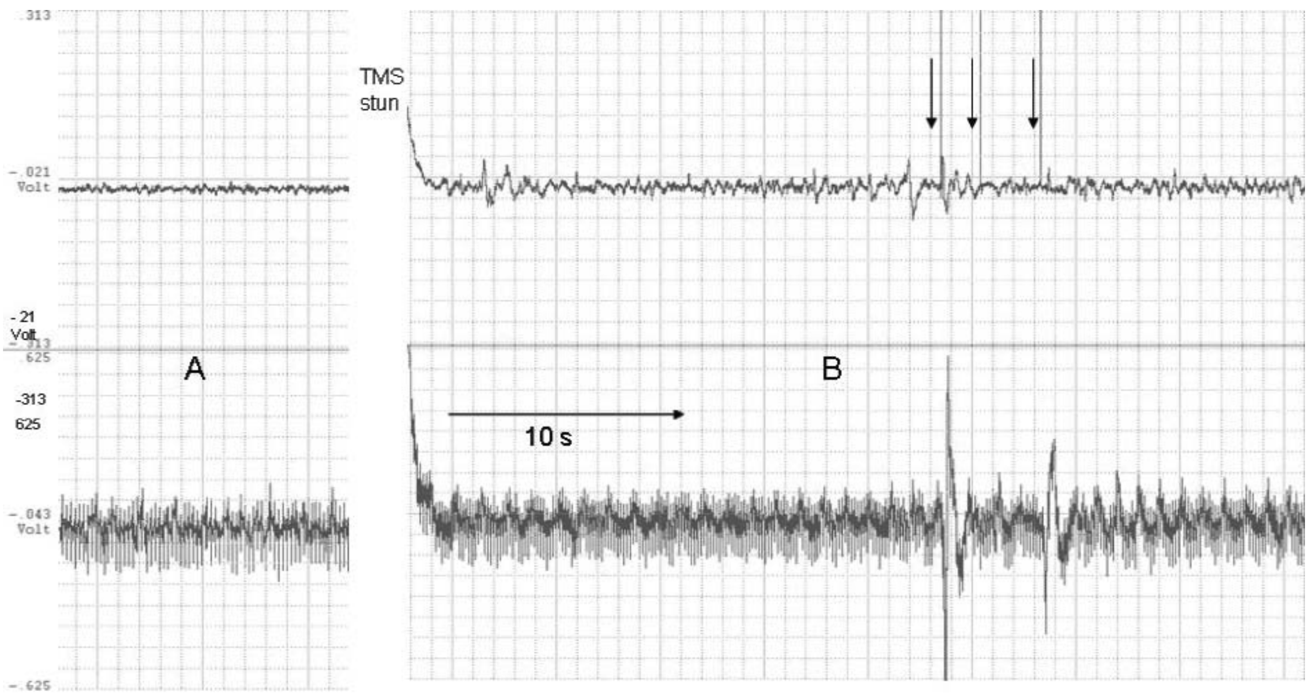
Table 1 shows an overview of the settings used in the 20 successfully completed TMS (one bird was applied twice) during the experiments (results from 6 birds were omitted due to technical difficulties). The bodyweights of the broilers are also shown in Table 1.

Use of the single circular probe resulted in the dominance of theta and delta waves and appearance of spikes on the EEG after TMS. An example is shown in Figure 1. After CD analyses with the EEGs when using a single coil, the birds

Table 1 Overview of technical settings and bodyweight of broilers used during individual stuns using a TMS prototype.

N	Power (%)	Frequency (Hz)	Duration (s)	Number of pulses	Mean (± SEM) weight (kg)
Single coil					
3	51	50	5	250	3.6 (± 0.5)
2	80	35	5	300	3.4 (± 0.3)
Double coil					
3	51	50	5	250	3.4 (± 0.2)
5	80	35	5	250	3.5 (± 0.4)
7	51	50	5	300	3.5 (± 0.5)

Figure 1



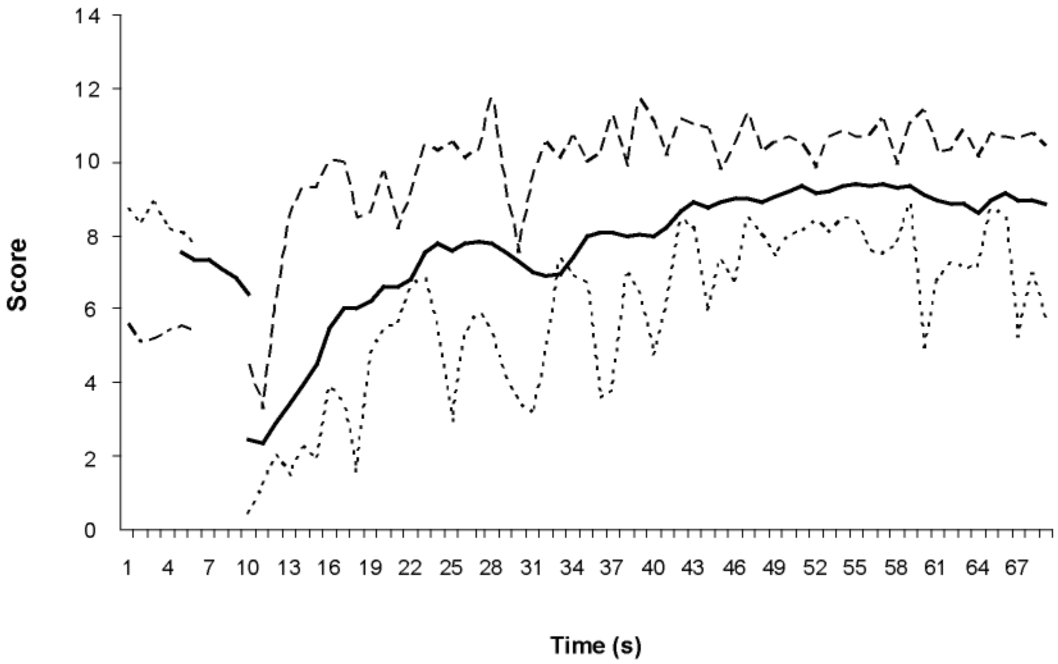
EEG (upper) and ECG (lower) (A) before and (B) after TMS stunning using a double coil with a power of 51% (↓ administration of a comb pinch).

displayed a sharp decrease in score which is interpreted as an indication of unconsciousness and then the birds recovered gradually as indicated by an increasing score (Figure 2). When using the double coil and a power of 80% (250 pulses), the EEGs of the birds showed dominance of theta and delta waves with spikes (Figure 3). As evidence of a profound effect of TMS on brain function, the birds showed a long-lasting change in behaviour seen as a loss of arousal or muscle tone (muscle flaccidity). Using a lower power of 51% (300 pulses) the birds appeared unconscious, recovered slightly and became drowsy again (Figure 4). According to the correlation dimension analyses (Broek 2003) of the EEGs, assuming that a reduction in CD to 60% of the baseline value indicates unconsciousness, it is inferred that the birds could have been rendered unconscious by the TMS for approximately 15 to 20 s.

Three of the five birds reacted to a pain stimulus 30 s after TMS, one at 1 min and one at 2 min. Using the double probe with 80% power, three birds responded at 30 s, two birds at 1 min and one bird did not respond during the measuring time of 5 min. When applying 50% power, three birds responded after 30 s, three after 1 min and two responded after 2 min following TMS.

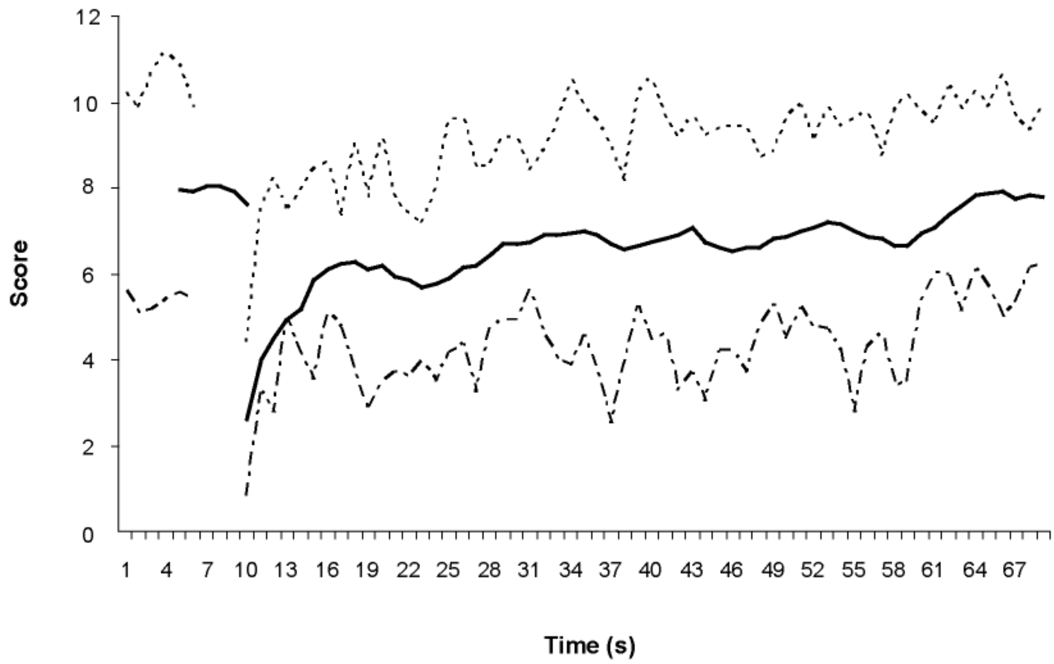
The heart rate was irregular after stunning which can be seen from the results shown in Table 2 and differed significantly ($P < 0.01$) before and after TMS and between the three treatments.

Figure 2



Correlation dimension analyses (van den Broek 2003) of EEG before and after TMS stunning using a single coil. The birds might be unconscious for approximately 15–20 s assuming that a reduction in CD to 60% of the baseline value indicates unconsciousness. Mean (± SD) of five successive values.

Figure 3



Correlation dimension analyses (van den Broek 2003) of EEG before and after TMS stunning using a double coil with a power of 80%. The birds might be unconscious for approximately 10–18 s assuming that a reduction in CD to 60% of the baseline value indicates unconsciousness but were drowsy thereafter. Mean (± SD) of five successive values.

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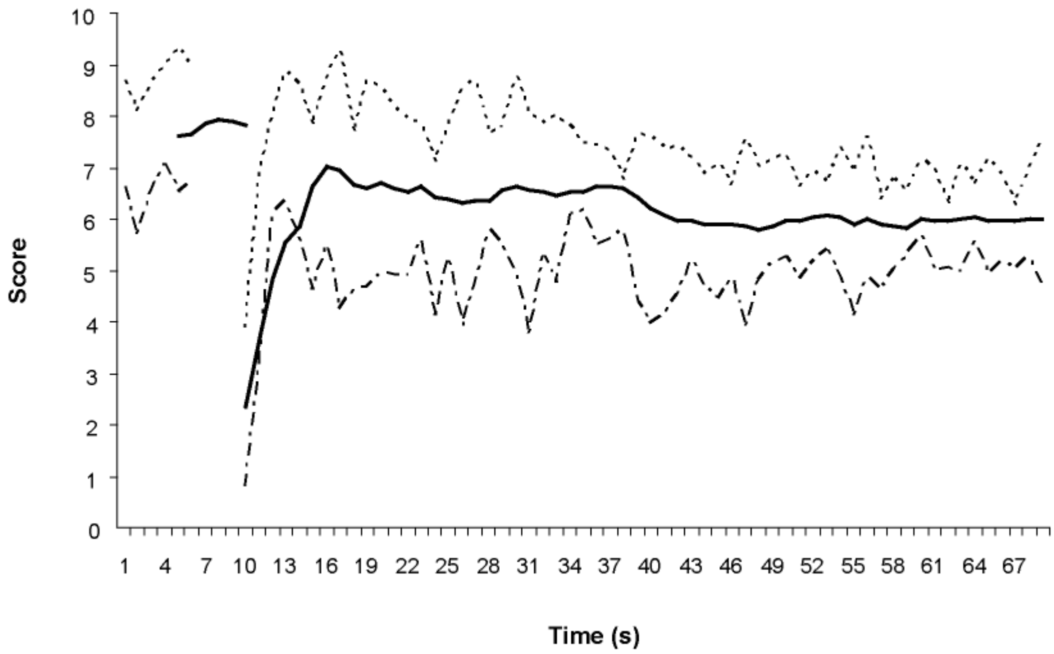
Figure 4



Correlation dimension analyses (van den Broek 2003) of EEG before and after TMS stunning using a double coil with a power of 51%. The birds might be unconscious for approximately 10–15 s assuming that a reduction in CD to 60% of the baseline value indicates unconsciousness but were drowsy afterwards. Mean (± SD) of five successive values.

Table 2 Mean (± SD) heart rate (beats min^{-1}) before and after stunning with the TMS prototype.

	N	Before stunning	After stunning					
			30 s	1 min	2 min	3 min	4 min	5 min
Single coil	5	374 (± 105)^{a}	302 (± 77)^{b,x}	300 (± 56)	200 (± 62)	276 (± 62)	268 (± 45)	258 (± 38)
Double coil 80% power	7	359 (± 64)^{a}	324 (± 71)^{b,y}	373 (± 66)	359 (± 68)	334 (± 63)	329 (± 57)	310 (± 33)
Double coil 51% power	8	343 (± 64)^{a}	353 (± 58)^{b,z}	426 (± 39)	380 (± 54)	366 (± 40)	346 (± 42)	331 (± 40)

Superscripts differ significantly at $P < 0.01$, according the analysis of variance.

Discussion

Electrical stunning is based on the induction of a general epileptiform insult (‘grand mal’ or seizure-like state) by the application of adequate electrical current through the head and brain. The epileptic process is characterised by rapid and extreme depolarisation of the neuronal membrane potential and development of a synchronised electrical activity in the neurones. This can be measured and observed on the recorded EEG as such an insult produces relatively small waves increasing in amplitude in the tonic phase (rigid with epileptiform EEGs), and decreasing in frequency in the clonic phase (high motor activity in muscles), resulting, ultimately, in a period of strong depression of brain electrical activity as indicated by the occurrence of profoundly suppressed or iso-electric EEGs (Lambooij 2004).

A human being is unconscious during the three phases of a general epileptiform insult on the EEG. By analogy, a vertebrate is also considered to be unconscious and insensible during such an insult. The analogy postulate is used to make the existence phenomena in vertebrates plausible (Lopes da Silva 1983). The postulate rests on the homology of brain structures and similarities in behavioural pattern between humans and vertebrates in situations in which humans experience and report positive or negative feelings Moreover, the brain is in a stimulated condition and unable to respond to external stimuli, including pain (Lopes da Silva 1983).

During electro-anaesthesia, unconsciousness may be present, but without a general epileptiform insult (Sances & Larson 1975). It has been suggested that the ascending activating influence of the reticular formation of the brain stem suppresses responses of the telencephalon or cortex (the seat of consciousness) during current delivery. Another suggestion is that the administered current interferes sufficiently with neuronal function at the thalamic (mid brain) level to cause anaesthesia without the occurrence of epileptiform EEGs (Sances & Larson 1975). It has also been observed that overt behavioural unconsciousness or loss of somatosensory potentials can occur without the development of polyspike activity in the EEG in sheep and poultry, respectively. These observations imply that electrical stunning can induce insensibility without producing epileptiform activity (Lambooij 2004). We did not observe a general epileptiform insult after TMS. However, according to analysis of EEG theta, delta, spikes and no response to a pain stimulus were observed which may indicate that the brain is in a stimulated condition and unable to respond to external stimuli, including pain (EFSA 2004) (Figure 1). Earlier studies with poultry (McKeegan *et al* 2007) suggested that a reduction in CD to 60% of the baseline value seen in unconscious birds was an indicator of an unconsciousness level similar to anaesthetised humans: the birds could have been rendered unconscious by the TMS for approximately 15–20 s. Following this, the birds may be in a state of drowsiness and sleep, because the CD score is lower than 6.6 (Coenen & van den Broek 2005).

There is a need for research to develop alternative, ideally non-invasive, stunning methods, such as captive-bolt stunning and electrical stunning by insertion of the skin of the electrodes (Knight & Anil 2003). A non-invasive and potentially pain-free method that does not result in tissue damage before death may also be acceptable for Muslim groups. In practice, an intense magnetic field is generated by passing a large amount of current through a copper coil. The coil is positioned close to the head so that the brain lies within this magnetic field. Bristol University research has provided evidence for insensibility during the TMS application (Anil *et al* 2000). Using similar technology, studies aimed at producing seizure activity and prolonged insensibility without a painful induction are being conducted using new equipment and special coils in animals (Anil *et al* unpublished). In the present study, the period of TMS application was 5 s which is acceptable when the induction of unconsciousness is non-aversive and does not cause anxiety, pain, distress or suffering in conscious animals (EFSA 2004). The coils were not ideal to always be placed correctly and the power supply might not be sufficient to induce the requisite length of unconsciousness to be useful in a slaughterhouse. The coil needs to be improved and the power supply optimised in future research. Transcranial magnetic stimulation of the brain is a potential alternative for use as a stunning method for broilers, however, more research is necessary to develop the method further.

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