NOCICEPTORS IN THE LEGS OF POULTRY: IMPLICATIONS FOR POTENTIAL PAIN IN PRE-SLAUGHTER SHACKLING

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Shackling of commercial poultry involves the insertion of each leg into parallel metal slots and holding the bird inverted for a period of time before stunning and slaughter. Nociceptors signalling noxious stimulation of the skin have been identified in the beak and feathered skin but not in the scaly skin of the leg. The physiological properties of the C-fibre mechano-thermal (CMT) nociceptors in the skin over the tarsometatarsus in the lower leg were studied in response to quantitative mechanical stimulation. The electrical activity was recorded from single C-fibres dissected from the parafibular nerve in anaesthetized animals. The receptive fields of these receptors were small and spot-like, measuring in the region of 1–3 mm in diameter. The threshold to mechanical stimuli ranged from 0.8–15 g using von Frey filaments, and from 3–33 g using a 0.5mm probe mounted on a feedback-controlled stimulator. Stimulus response curves using a ramp-and-hold stimulus were recorded for a number of fibres. After comparing these threshold measurements and the stimulus response data with previous measurements of the force applied to the legs during shackling, it was concluded that shackling is likely to be a very painful procedure.

Keywords: *animal welfare, nociception, pain, poultry, shackling, skin nociceptors*

Introduction

The slaughter of poultry differs from that of other animals in that the live birds are hung upside down by the legs in shackles on a conveyor prior to stunning and slaughter. In the majority of processing plants, the shank (tarsometatarsus) of the bird is manually inserted into the shackle which is usually made of stainless steel bars of 6–10 mm in diameter (Sparrey & Kettlewell 1994). Shackle design varies between manufacturers but the MAFF Code of Practice for the slaughter of poultry (MAFF 1991) describes the preferred design of shackles, which should have parallel slots for the insertion of each leg and a box at right angles on which the birds' feet rest. Sparrey (1994) calculated that the resultant force on each leg of the bird could be 180N applied over an area of 1cm^2. The slot size of the shackle is particularly important because broilers show variable leg sizes, with males having consistently larger legs than females. The pressures required to compress broiler legs into

shackles increases exponentially with deformation and it requires four times as much pressure to compress a 14.5mm leg by 20 per cent to fit into an 11.5mm shackle, as it does to compress the same leg by 10 per cent to fit into a 13mm shackle (Sparrey 1994). It is possible that these forces acting over relatively small areas of the leg would cause pain and distress to the birds and the welfare concerns of shackling have been reviewed by Sparrey and Kettlewell (1994).

There are a number of potential sources of pain following shackling. The pressures placed on the shank could stimulate nociceptors (receptors peferentially sensitive to a noxious stimulus) in the skin and the periosteum of the tarsometatarsus. Hanging heavy birds upside down could put abnormal stresses on the skeleton, and bone breakage is common in end-of-lay hens (Gregory & Wilkins 1990). This potential pain would be increased by the struggling and wing-flapping responses which accompany shackling (Gregory & Bell 1987; Jones *et al* 1998; Gregory *et al* 1989). The ankle joint is well supplied with nociceptors (Gentle 1992) which respond to abnormal movements of the joint and could be a source of pain in shackling. This problem is likely to be exacerbated in birds with articular disease which is common in commercial broiler flocks (Kestin *et al* 1992). In inflammatory articular disease, the joint capsule nociceptors become sensitized and even small movements can become painful (Gentle & Thorp 1994; Gentle 1997). While there is information concerning nociceptors in the beak (Breward 1985; Gentle 1989) and the feathered skin (Necker & Reiner 1980) there is, however, no information on the properties of the nociceptors in the scaly skin of the leg. There is also no information concerning the painful consequences of periosteal stimulation.

This study investigated the physiological properties of C-fibre mechano-thermal (CMT) cutaneous nociceptors present in the scaly skin of the shank of the chicken to determine threshold and response characteristics to mechanical stimulation and to relate their responses to the pressures produced in commercial shackling.

Methods

Preparation

All procedures were conducted under Home Office Project (PPL 60/1632) and Personal (Pil 60/3500) licences. All animals were deeply anaesthetized throughout the duration of the experiment. Eight, 20-week-old, Brown Leghorn hens (*Gallus gallus* var *domesticus*) were the subjects in this study. They had been hatched and reared at the Roslin Institute, UK. Although shackling is normally undertaken in broilers (5 –7 weeks old) or end-of-lay hens (70–80 weeks old), the use of 20-week-old birds of a laying strain in this experiment was a compromise between the young age of the broiler and the smaller size of the layer leg. There was, however, no reason to expect the cutaneous sensory receptors to react differently at any of the relevant ages.

The animals were initially anaesthetized with sodium pentobarbitone (Sagatal™; Rhône Mérieux, Dublin, Ireland) given intravenously (24-30 mg kg^{-1}) which provided sufficient duration of anaesthesia to cannulate the brachial vein; and the birds were maintained under deep urethane (ethyl carbamate) anaesthesia (1.5g kg^{-1} body weight). The level of anaesthesia was maintained throughout the experiment, such that at no time did the animals experience any pain or discomfort and at the end of the recording period the birds were humanely killed with an overdose of Sagatal™ (60 mg kg^{-1}). Heart rate was continuously

monitored throughout the experiment and the body temperature was maintained at 40°C by means of a heated blanket controlled with a rectal probe.

Recording

The physiological properties of the cutaneous nociceptors in the scaly skin of the shank were investigated by recording the neural activity in single sensory afferent nerve fibres dissected from the parafibular nerve. The recording set-up was similar to that used previously to record from sensory receptors in the ankle (Gentle 1992, 1997; Gentle & Thorp 1994) because the parafibular nerve innervates both the ankle and the skin of the shank and toes. The parafibular nerve was dissected from the surrounding connective tissue approximately halfway along the femur. The electrical activity (action potentials) of single sensory nerve fibres was recorded by carefully lifting dissected nerve filaments onto a silver wire electrode. A small strand of the nerve sheath was placed on a second electrode which acted as the indifferent, or ground, electrode. The electrical activity was amplified using an ac preamplifier (Type P15, Grass Instruments, Quincy, USA) which enabled the action potentials to be displayed on a storage oscilloscope (Model 5113, Tektronix Inc, Beaverton, USA) and the electrical activity was stored on a tape recorder (Store 4DS, Racal Recorders Ltd, Hythe, UK). Further analysis of individual nerve activity was performed using the Spike 2™ analysis program with a 1401 Interface (Cambridge Electronic Design Ltd, Cambridge, UK) onto a Viglen computer (Viglen Ltd, Alperton, UK).

To verify that the nociceptor afferent was an unmyelinated C-fibre, the conduction velocities of all the fibres were measured and only those fibres with conduction velocities below 2m s^{-1} were recorded. To measure conduction velocity, the skin was incised about 20mm proximal to the ankle joint and the parafibular nerve was cleared from the surrounding connective tissue. The nerve was placed on stimulating electrodes and electrically stimulated (by square-wave pulses using a DS2 isolated stimulator [Digitimer Ltd, Welwyn Garden City, UK]) to induce an evoked action potential in the nerve. The distance between the stimulating and recording electrode was approximately 100mm and the conduction velocity was calculated from the time required for the evoked action potential to travel from the stimulating electrodes to the recording electrode.

Identification and stimulation of sensory afferent fibres

Following dissection of a nerve filament, the surface of the shank was probed with a hand-held glass rod with a ball-shaped tip 1mm in diameter to identify a clearly discernible slowly adapting mechanoreceptor. The conduction velocity of the nerve fibre was established and, if it was below 2m s^{-1}, the receptive field of the receptor was then stimulated thermally using a feedback-controlled thermal stimulator (Gentle 1989). The surface of the skin was heated using a prefocused quartz light bulb with a built-in reflector and the temperature of the skin measured with a type K thermocouple placed on the skin at the centre of the bulb's focus. The increase in temperature was set at 1°C s^{-1} up to a preset temperature. If the receptor responded to thermal stimulation in the noxious range (45–60 °C), the receptor was classified as a mechano-thermal nociceptor.

After identification of the mechano-thermal nociceptor, the area of skin which produced a response to probing with the glass rod was determined (the receptive field). The minimum stimulus intensity required to produce a response to mechanical stimulation (the threshold) was initially determined with von Frey filaments ranging from 0.1–15 g. Higher thresholds

were determined using a feedback-controlled mechanical stimulator (Gentle 1992). Von Frey filaments differ in size, with the lowest filaments also having the smallest tip diameters, so to control for the size of the probe, quantitative mechanical stimuli were delivered using a 0.5mm diameter tungsten probe. The tungsten probe applied a steadily increasing pressure to the skin up to a predetermined level and held the pressure constant for 10s (ramp-and-hold mechanical displacement). To determine the stimulus response characteristics of the receptors, a series of ramp-and-hold mechanical stimuli were delivered to the skin at increasing stimulus magnitudes from the threshold upwards. To try to avoid sensitization or desensitization of the receptors, there was a 2-min interval between stimuli. Stimulation was stopped when there was either no further increase in the response, or a decline.

Results

A total of 21 CMT nociceptors were identified among the eight birds and their physiological responses recorded. The number of receptors identified in each bird ranged from 1 to 4. The conduction velocities of the fibres ranged from 0.66–2.0 m s^{-1} (mean ± SEM, 1.074 ± 0.102 m s^{-1}). The receptive fields of the majority of these receptors were small and spot-like and ranged from 1–2 mm in diameter. Larger receptive fields were found in four receptors, one in each of four birds, with the largest measuring 4mm in diameter. The receptive fields were often situated in the tissues between scales and stimulating the scale did not produce a response. There were other receptors, for example in parts of the leg with small scales, where the receptive field extended over one or more scales. The positions of the receptive fields of all the receptors examined are shown in Figure 1 where it can be seen that receptors were identified on the whole of the lateral surface of the skin of the tarsometatarsus.

Figure 1 **Lateral view of the skin over the tarsometatarsus showing the positions of the centres of the receptive fields (●) of each of the nociceptors tested in all eight birds.**

Thermal thresholds ranged from 39–61 °C (mean ± SEM, 50.7 ± 1.47 °C) and thresholds to mechanical stimulation using von Frey filaments ranged from 0.8–15 g (mean ± SEM,

5.37 ± 1.67 g). Mechanical thresholds using the 0.5mm diameter probe ranged from 3–33 g (mean ± SEM, 9.87 ± 1.98 g), and the individual values are shown in Figure 2. The dotted line at 36g in Figure 2 indicates the same force delivered by the 0.5mm probe as would be applied during shackling (180N over 1cm^2 [Sparrey 1994]).

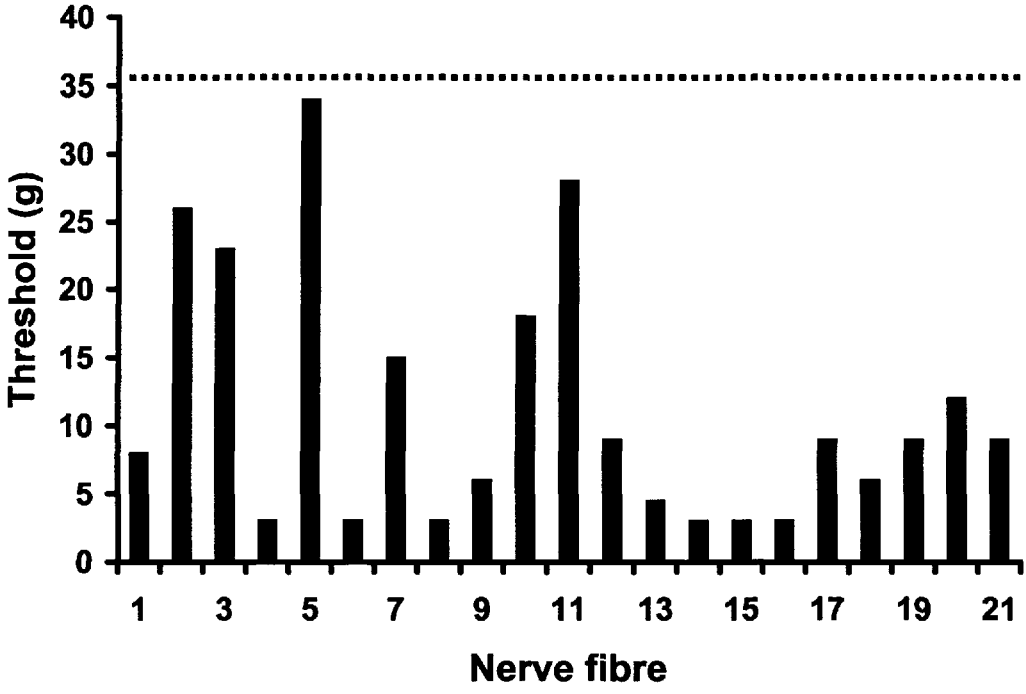

Figure 2 **Mechanical threshold values for each of the nociceptors tested. The dotted line at 36g denotes the same force delivered by the 0.5mm probe as would be applied during shackling.**

The response characteristics of the receptors to a ramp-and-hold mechanical stimulus are shown in Figure 3. The nerve fibres showed no spontaneous activity and responded during a suprathreshold stimulus with a slowly adapting irregular discharge of action potentials. Increasing pressure produced an increase in response, and stimulus response curves for the 11 fibres which were held for long enough for these to be obtained are shown in Figure 4. It can be seen that the CMT nociceptors vary in their thresholds, response maxima and slope of the curves. Most of the fibres respond up to a maximum value and any further increase in pressure results in a decline in response. All of the receptors had response thresholds below 36g and in 7 out of the 11 fibres (63%) the maximum response obtained was also below this stimulus level.

Discussion

Although CMT nociceptors have been described in the beak (Breward 1985; Gentle 1989) and feathered skin (Necker & Reiner 1980) of birds, there have been no detailed studies of

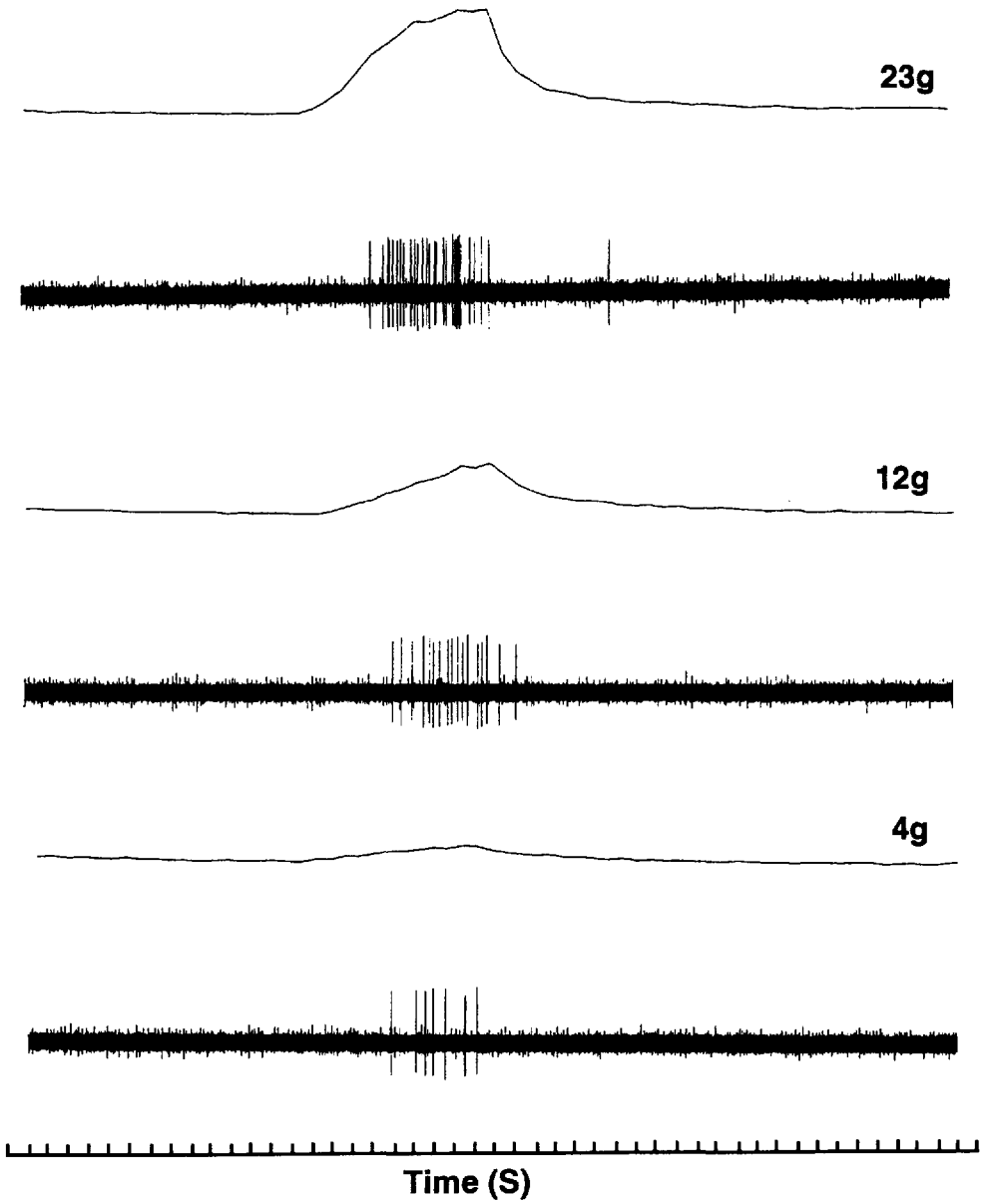

Figure 3 **An example of the responses of a CMT nociceptor to a ramp-and-hold mechanical stimulus. Each upper trace shows the force applied to the skin and each lower trace the action potentials in the nerve fibre. The receptor shows an irregular pattern of discharge with an increase in response with increasing stimulus intensity.**

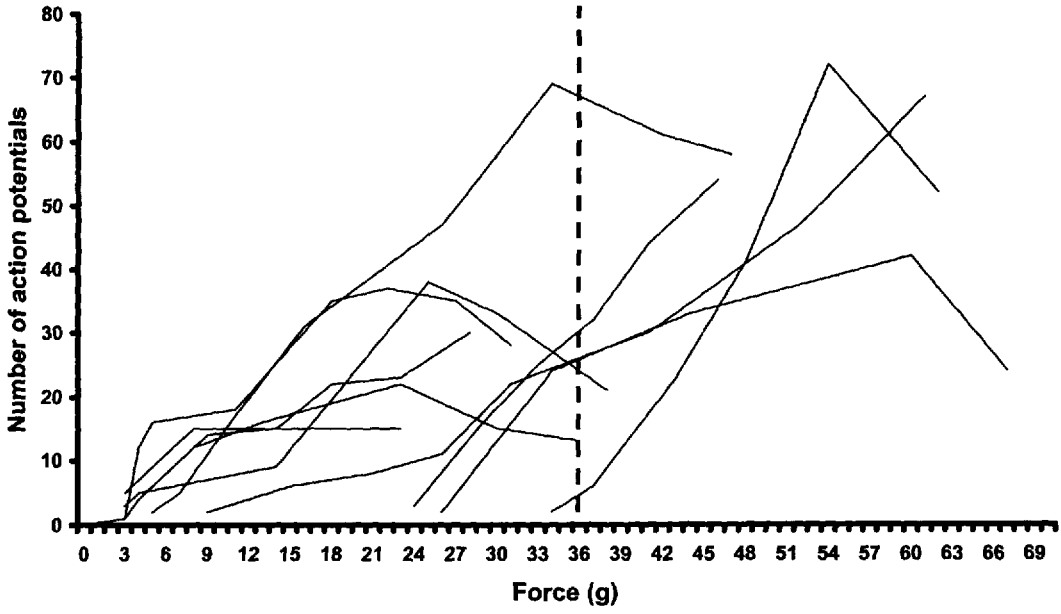

Figure 4 **Stimulus response curves for 11 mechano-thermal nociceptors from all eight birds in response to a ramp-and-hold mechanical stimulus to the receptive field of the receptor on the surface of the skin. The number of action potentials are the number of responses during the ramp-and-hold mechanical stimulus of a 10s duration. The dotted line at 36g denotes the same force as would be applied during shackling.**

the responses of these receptors to mechanical stimulation. The receptive fields of the majority of CMT nociceptors in the leg were similar in size to those found in the beak (1–2 mm in diameter) and, like those in the beak, they also showed no spontaneous activity and responded to the stimulus with a slowly adapting irregular discharge of action potentials.

The results show that nociceptors are present in the scaly skin over the lateral surface of the tarsometatarsus and that they have response thresholds much lower than the cutaneous forces exerted on this part of the leg during shackling. The maximum discharge rates of these nociceptors occur over a wide range of forces and it is clear from Figure 4 that a large proportion of the receptors will be discharging at their maximum rates during shackling. These results have clear implications for pain in the animals during shackling prior to stunning and slaughter. Although discharge rates of nociceptors cannot be directly equated to pain, it is clear from microneurography studies in humans that they are closely related (Torebjörk *et al* 1996). There is a nearly linear relationship between the mean suprathreshold response functions of a population of CMT nociceptors and the median ratings of pain in humans (Torebjörk *et al* 1984). Also CMT nociceptors are activated by small increments of 0.1–0.5 °C on a base temperature of 45°C, which is matched by the human ability to detect such increments as painful (Robinson *et al* 1983). However, heat thresholds of human nociceptors are generally lower than pain thresholds – and it is the graded response to

suprathreshold noxious stimulation which seems to be a more suitable indicator of nociceptive function rather than the mere consideration of thresholds (Handwerker & Kobal 1993).

The relationship between pain and mechanical stimulation is more complex and there is a greater discrepancy between pain thresholds and activation thresholds of C-fibre nociceptors (Van Hees & Gybels 1981). This discrepancy may be due to the activation of fewer nociceptors in the punctate stimulus used in mechanical stimulation studies as opposed to the more widespread heat stimulus of the light bulb used in thermal studies (Torebjörk *et al* 1996). One of the advantages of using humans in pain studies is that the subjects can be asked about their sensory experience – something which is not possible with animals. In the chicken, however, there is evidence from studies on beak trimming (Breward & Gentle 1985; Gentle 1989) and arthritis (Gentle 1992, 1997; Gentle & Thorp 1994) that there is a relationship between the activation of nociceptors and the behavioural evidence of pain (Gentle *et al* 1990, 1991; Gentle & Corr 1995). On the basis of the responses of the CMT nociceptors recorded from the skin over the tarsometatarsus, together with what is known about the area of shackle contact on the leg, it could be concluded that shackling is likely to be a very painful procedure.

In a recent review of shackling in poultry (Sparrey & Kettlewell 1994) the legislative regulations (*Slaughter of Poultry [Humane Conditions] Regulations 1984* [GB Parliament 1984a], provided for in Section 3 of the *Slaughter of Poultry Act 1967*, chapter 24 [GB Parliament 1967] and section 6 of the *Animal Health and Welfare Act 1984* [GB Parliament 1984b]) were discussed. This review stressed that central to the regulations was the intention that birds should not subjected to unnecessary pain in shackling prior to slaughter. It is clear from the work on shackle design (Sparrey 1994) and the current work on the responses of nociceptors in the tarsometatarsus that significant pain is likely to be inflicted on the animals during shackling and while awaiting slaughter. There are at least two solutions to this problem. The first would be to change the shackle design to reduce the force applied to the leg and to relate the size of the shackle to the size of the leg of the bird. At present, there is little quantitative legislation relating to the size of the legs of the birds and the size of shackle grip used (Sparrey & Kettlewell 1994). The second solution is to shackle the birds after stunning or death using, for example, gas-stunning where the birds can be stunned in crates with the minimum of handling before stunning or death.

To place these results on skin nociceptors in the context of the amount of pain suffered during shackling, it must also be remembered that the legs of some birds will be compressed by 20 per cent to insert them into the shackles (Sparrey 1994). This degree of compression will inevitably result in noxious periosteal stimulation. There are close similarities between birds and mammals in nociception and pain (Gentle 1992) and it seems a reasonable assumption that noxious periosteal stimulation is also very painful.

Animal welfare implications

Poultry destined for slaughter have their lower legs (tarsometatarsus) inserted into stainless steel shackles and the birds are hung head down on a conveyer until they reach the stunning bath. This leads to the obvious distress caused by inversion and forced restraint. By recording from the nociceptors in the skin of the tarsometatarsus, it can be demonstrated that the forces exerted by shackling will excite the majority of nociceptors in the skin. The main conclusion which could be drawn from this level of activity in peripheral nociceptors is that shackling is

likely to be very painful. These results, therefore, have major implications for animal welfare legislation relating to the pre-slaughter treatment of poultry.

References

Breward J 1985 *An Electrophysiological Investigation of the Effects of Beak Trimming in the Domestic Fowl* (Gallus gallus domesticus). Unpublished PhD Thesis, University of Edinburgh: Edinburgh, UK

Breward J and Gentle M J 1985 Neuroma formation and abnormal afferent nerve discharges after partial beak amputation (beak trimming) in poultry. *Experientia 41:* 1132-1134

GB Parliament 1967 *The Slaughter of Poultry Act 1967.* Her Majesty's Stationery Office: London, UK

GB Parliament 1984a *The Slaughter of Poultry (Humane Conditions) Regulations 1984. Statutory Instrument 1984 No 2056.* Her Majesty's Stationery Office: London, UK

GB Parliament 1984b *Animal Health and Welfare Act 1984.* Her Majesty's Stationery Office: London, UK

Gentle M J 1989 Cutaneous sensory afferents recorded from the nervus intramandibularis of *Gallus gallus* var *domesticus. Journal of Comparative Physiology A 164:* 763-774

Gentle M J 1992 Ankle joint (Artc. intertarsalis) receptors in the domestic fowl. *Neuroscience 49:* 991-1000

Gentle M J 1997 Sodium urate arthritis: effects on the sensory properties of articular afferents in the chicken. *Pain 70:* 245-251

Gentle M J and Corr S A 1995 Endogenous analgesia in the chicken. *Neuroscience Letters 201:* 211-215

Gentle M J, Hunter L N and Waddington D 1991 The onset of pain related behaviours following partial beak amputation in the chicken. *Neuroscience Letters 128:* 113-116

Gentle M J and Thorp B H 1994 Sensory properties of ankle joint capsule mechanoreceptors in acute monoarthritic chickens. *Pain 57:* 361-374

Gentle M J, Waddington D, Hunter L N and Jones R B 1990 Behavioural evidence for persistent pain following partial beak amputation in chickens. *Applied Animal Behaviour Science* 27: 149-157

Gregory N G, Austin S D and Wilkins L J 1989 Relationship between wing flapping at shackling and red wingtips in chicken carcases. *Veterinary Record 124:* 62

Gregory N G and Bell J C 1987 Duration of wing flapping in chickens shackled before slaughter. *Veterinary Record 121:* 567-569

Gregory N G and Wilkins L J 1990 Broken bones in chickens: effects of stunning and processing in broilers. *British Poultry Science 31:* 53-58

Handwerker H O and Kobal G 1993 Psychophysiology of experimentally induced pain. *Physiological Reviews 73:* 639-671

Jones R B, Satterlee D G and Cadd G G 1998 Struggling responses of broiler chickens shackled in groups on a moving line: effects of light intensity, hoods, and 'curtains'. *Applied Animal Behaviour Science 58:* 341-352

Kestin S C, Knowles T G, Tinch A E and Gregory N G 1992 Prevalence of leg weakness in broiler chickens and its relationship with genotype. *Veterinary Record 131:* 190-194

MAFF 1991 *Code of Practice on the Welfare of Poultry at Slaughter.* Her Majesty's Stationery Office: London, UK

Necker R and Reiner B 1980 Temperature-sensitive mechanoreceptors, thermoreceptors, and heat nociceptors in the feathered skin of pigeons. *Journal of Comparative Physiology A 135:* 201-207

Robinson C J, Torebjörk H E and LaMotte R H 1983 Psychophysical detection and pain ratings of incremental thermal stimuli: a comparison with nociceptor responses in humans. *Brain Research 274:* 87-106

Sparrey J 1994 *Aspects in the Design and Operation of Shackle Lines for the Slaughter of Poultry.* Unpublished MPhil thesis, University of Newcastle upon Tyne: Newcastle upon Tyne, UK

Sparrey J M and Kettlewell P J 1994 Shackling of poultry: is it a welfare problem *World's Poultry Science Journal 50:* 167-176

Torebjörk H E, LaMotte R H and Robinson C J 1984 Peripheral neural correlates of the magnitude of cutaneous pain and hyperalgesia: simultaneous recordings in humans of sensory judgments of pain and evoked responses in nociceptors with C-fibers. *Journal of Neurophysiology 51:* 325-339

Torebjörk H E, Schmelz M and Handwerker H O 1996 Functional properties of human cutaneous nociceptors and their role in pain and hyperalgesia. In: Belmonte C and Cervero F (eds) *Neurobiology of Nociceptors* pp 349-369. Oxford University Press: Oxford, UK

Van Hees J and Gybels J 1981 C nociceptor activity in human nerve during painful and non-painful skin stimulation. *Jouranl of Neurology, Neurosurgery and Psychiatry 44:* 600-607