

## Chronic neurophysiological and anatomical changes associated with infra-red beak treatment and their implications for laying hen welfare

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### Abstract

The long-term effects of infra-red beak treatment (IRBT) in laying hens were investigated by examining beak-nerve function and anatomy over a range of ages. In IRBT and control (intact) birds that were ten, 30 or 50 weeks old, the responses of single sensory nerve fibres were recorded from the intramandibular nerve, which provides sensation to the lower beak. The beaks were also measured and subject to microscopic and radiographic examination. The responses of 386 nerve fibres were recorded including mechanoreceptors, thermoreceptors and nociceptors. Receptive field positions indicated that the entire lower beak was sensitive to thermal and mechanical stimuli, regardless of age or treatment. There was no evidence of a treatment effect on nociceptive thresholds at any age. Beak measurements demonstrated that application of IRBT at day-old affected, on average, 36% of beak area, and resulted in a 44% reduction in beak length by four weeks of age. Radiographs produced no evidence of adverse or pathological change in relation to IRBT. Microscopic evaluation of beak-tip anatomy revealed evidence of healing including re-epithelialisation, fibrovascular hyperplasia and bone remodelling. By four weeks of age there was limited nerve regeneration in IRBT beaks, including re-population of mechanoreceptors in some birds. In older birds, re-innervation and scarring was visible, but no neuromas or abnormal proliferations of nerve fibres were observed at any age. Collectively, the results suggest that IRBT does not result in chronic pain or other adverse consequences for sensory function.

**Keywords:** animal welfare, beak trimming, infra-red beak treatment, laying hen, pain, nociceptors

### Introduction

Beak trimming, involving the partial amputation of the beak, is routinely carried out to prevent or control feather pecking and cannibalism in the egg industry. Beak trimming is normally carried out in the first week of life and the most common method used in the past was the application of a hot-blade which simultaneously cuts and cauterises the beak. A new approach, infra-red beak treatment (IRBT), has recently been widely adopted in which the beak is subject to a localised, non-contact, high intensity infra-red energy source; after approximately two weeks the beak has healed proximal to the damaged area and the tip of the beak is lost. The avian beak is a complex structure consisting of bone, dermal and epidermal cellular layers and a smooth, hardened, keratinised layer (the rhamphotheca). The cellular layers contain mechanoreceptors (Herbst and Grandry corpuscles), blood vessels and free-nerve endings including nociceptors (Kuenzel 2007). Removal of up to 50% of the upper and lower beak (the extent of beak trimming expressed as a percentage of the total length of the intact upper bill, as measured from the distal

portion of the nasal opening to the bill tip) will result in a loss of rhamphotheca, mechanoreceptors, nociceptors, other free-nerve endings (eg thermal receptors) and the bill-tip organ (Gentle & Breward 1986).

Welfare concerns regarding beak trimming are numerous and include loss of normal beak function (reduced ability to feed, drink and preen) as well as short-term pain and debilitation. There is ample evidence for temporarily reduced feeding and bodyweight after beak trimming (Blokhuis *et al* 1987; Glatz & Lunam 1994; Gentle *et al* 1997) while negative, non-significant or positive effects on preening and plumage condition have been reported (Duncan *et al* 1989; Lee & Craig 1991; Sandilands & Savory 2002). Acute pain after beak trimming has been investigated in electrophysiological, behavioural and physiological studies. Based on evidence of nerve injury discharge during trimming (Gentle 1991), reduction in beak usage (guarding) after trimming (Gentle *et al* 1991) and increased heart rate (Glatz 1987), it appears that removal of the beak tip does result in short-term pain, although it has been suggested that this occurs after an initial pain-free period of up to 26 h (Gentle 1991; Gentle *et al* 1991).

The development of neuromas (a proliferative mass of nerve processes that develop at the severed end of a nerve) has been associated with beak trimming, following their discovery in the beaks of birds 15 days after they had been beak trimmed at five weeks of age (Breward & Gentle 1985). While neuromas are not necessarily painful, their presence is associated with abnormal spontaneous neural activity which could result in chronic dysaesthesia or pain (Breward & Gentle 1985; Gentle 1986). Later findings suggest that neuromas are more likely to form when older birds are beak trimmed (Gentle *et al* 1997) or when beaks are severely (as opposed to moderately) trimmed (Lunam *et al* 1996). Thus, age of beak trimming and its extent are key factors affecting the presence of neuromas and scar tissue in mature hens. It has been suggested that beak-trimmed birds may experience something akin to phantom limb syndrome (Breward & Gentle 1985), in which sufferers have a vivid sensory experience of the missing area which may be accompanied by chronic pain. In a recent publication, however, Kuenzel (2007) argues that based on comparisons of avian somatosensory cortex with that of humans and monkeys, such sensations are unlikely to occur in moderately beak-trimmed birds.

Because the widespread use of IR beak trimming is relatively recent, the vast majority of available information on the welfare consequences of beak trimming relates to the procedure when carried out using the traditional hot-blade method. Thus, little information is available describing the anatomical, neurophysiological and behavioural consequences of IR beak treatment. A few studies (Marchant-Forde & Cheng 2006; Dennis *et al* 2009) have examined the effects of IR beak treatment with preliminary conclusions that the welfare consequences of this approach are similar to or less severe than traditional methods. As described above, the likelihood of long-term abnormal sensory and potentially painful consequences resulting from beak trimming relate to age and extent of trimming, and it is not unreasonable to assume that method of trimming could also be important. Dennis *et al* (2009) suggest that IRBT “may be a wellbeing-friendly alternative to hot blade beak trimming in laying hens”. However, before such claims can be verified, there is a need for improved knowledge of the long-term neurophysiological and anatomical consequences of the IR approach.

The first aim of this study was to characterise the neurophysiological properties of sensory afferents in the beaks of IR beak-trimmed and control birds at a range of ages representative of the commercial life of the laying hen. Secondly, we aimed to describe the gross structure and histopathology of the beaks of IR-treated and control birds at a range of ages, to examine beak re-growth, regeneration and re-innervation.

## Materials and methods

### Subjects and husbandry

Laying hens were obtained from a commercial unit (three separate batches of 20, [each containing ten IRBT and ten control birds], aged ten, 30 and 50 weeks of age). The birds had been either subject to a standard IRBT procedure in a commercial hatchery (treatment) or left intact (control). The birds were housed in a commercial cage layer house until collected for the experiment. On arrival at the university, the birds were housed in pairs (treated and control kept separately) in pens (2.1 × 1 m; length × width) which allowed visual and auditory contact with neighbours. Each pen was furnished with deep wood-shaving litter and a nest box, and food and water was provided *ad libitum*. The electrophysiological experiments were carried out with authorisation from the Home Office through Project and Personal Licences. In addition to the age groups outlined above, post mortem measurements (see below) were carried out on a further 40 birds aged day-old or four weeks of age (four groups of treatment (n = 5) and control (n = 5) birds at each age).

### Animal preparation and electrophysiological recordings

After cannulation of the brachial vein, the birds were maintained under urethane (ethyl carbamate) anaesthesia (1.5 g kg<sup>-1</sup> bodyweight) for the duration of the experiment. Body temperature was maintained by means of a heated blanket thermostatically controlled by a rectal probe. The anaesthetised bird was placed on the heating blanket and its head was securely fixed horizontally by pressing into a bed of dental putty (President Putty Soft, Coltene, Switzerland) before fixing in place with cyanoacrylate adhesive (Vetbond, 3M, St Paul, MN, USA). The intra-mandibular nerve, which supplies the cutaneous afferent innervation of the lower beak, was exposed in the intra-mandibular canal. The surrounding skin was sutured to a metal ring (diameter 10 mm) to create a raised circular opening to which was added warmed liquid paraffin to prevent the nerve from drying. The nerve was freed from surrounding connecting tissue before being supported on a black perspex platform. The nerve was de-sheathed and single-unit recordings were made from small nerve bundles dissected from the main nerve trunk using sharpened watchmakers' forceps. These were lifted onto one pole of a bipolar silver wire electrode with the other pole connected to a small strand of the nerve sheath. The electrical activity was amplified using an AC pre-amplifier (P15, Grass Instruments, West Warwick, USA) and was recorded after being digitised through an A/D interface (1401plus, Cambridge Electronic Design, Cambridge, UK) and stored in a data acquisition and analysis programme (Spike 2 Version 4.01, Cambridge Electronic Design).

After dissection of a nerve bundle, the lower beak was subject to a gentle mechanical search stimulus (hand-held glass probe, tip diameter; 1 mm). When mechanically

**Table 1** Counts of receptor type investigated by age and treatment, presented as totals for each group of ten birds.

Age (weeks)	Control/treatment	Rapidly adapting mechanoreceptors	Slowly adapting mechanoreceptors	Nociceptors	Thermoreceptors	Other
10	C	20	7	8	6	3
	T	30	11	7	16	8
30	C	22	18	6	15	5
	T	27	17	10	15	0
50	C	21	11	7	18	2
	T	26	15	6	27	2
<b>Total</b>		<b>146</b>	<b>79</b>	<b>44</b>	<b>97</b>	<b>20</b>

C: control intact; T: IRBT.

sensitive units were identified, the size (small; 1–2 mm<sup>2</sup>, medium; 2–5mm<sup>2</sup>, large; > 5 mm<sup>2</sup>) and position of their receptive field was determined qualitatively and where possible, mechanical threshold was determined using calibrated nylon monofilaments (von Frey hairs) ranging from 0.1 to 48 g. Occasionally, the position of the receptive field (eg inside beak) did not allow its mechanical threshold to be determined. The distance from the lower beak tip to the proximal edge of the receptive field was also measured. Mechanoreceptors were noted as being rapidly or slowly adapting. Slowly adapting units with small receptive fields (candidate nociceptors) were checked for noxious thermal sensitivity using a thermal stimulator (pre-focused MR11/12V/10W quartz light bulb [Pro Life, Milton Keynes, UK] with a built-in reflector) oriented vertical to the beak surface. The temperature applied was measured with a thermocouple placed in the centre of the bulb's focus on the surface of the beak. The rate of temperature increase, final temperature and the duration of the final temperature could all be varied. In the present experiment, a ramp and hold stimulus was used with the hold 1 s in duration. Where possible, the thermal threshold of polymodal nociceptors was determined and a stimulus-response curve constructed. Spontaneously active units were subject to cold stimulation (the tip of a cotton bud soaked in iced water) to identify thermoreceptors. At the end of the experiment, birds were killed humanely with a lethal injection of sodium pentobarbitone without regaining consciousness.

#### Beak measurements

Digital photographs were taken of the birds' heads against an appropriate scale. Image analysis software (Image J, see <http://rsbweb.nih.gov/ij/>) was used to make the following measurements in duplicate: length of upper beak from proximal limit of nasal cavity to beak tip; length of lower beak from edge of soft tissue to beak tip, head area including beak, beak area. Because of scale differences, the proportion of beak area to total head area was calculated and this proportion was used to examine differences in beak size between control and treated birds. To examine beak shape, the percentage difference between upper and lower beak length was also calculated in each age group.

#### Radiography

Radiographs were taken of the birds' heads at four, ten, 30 and 50 weeks of age. To maximise definition, the birds were placed on film cassettes which contained high-definition screens and mammography film (Agfa Detail R Mamoray screens, Agfa-Gevaert Ltd, Brentford, UK).

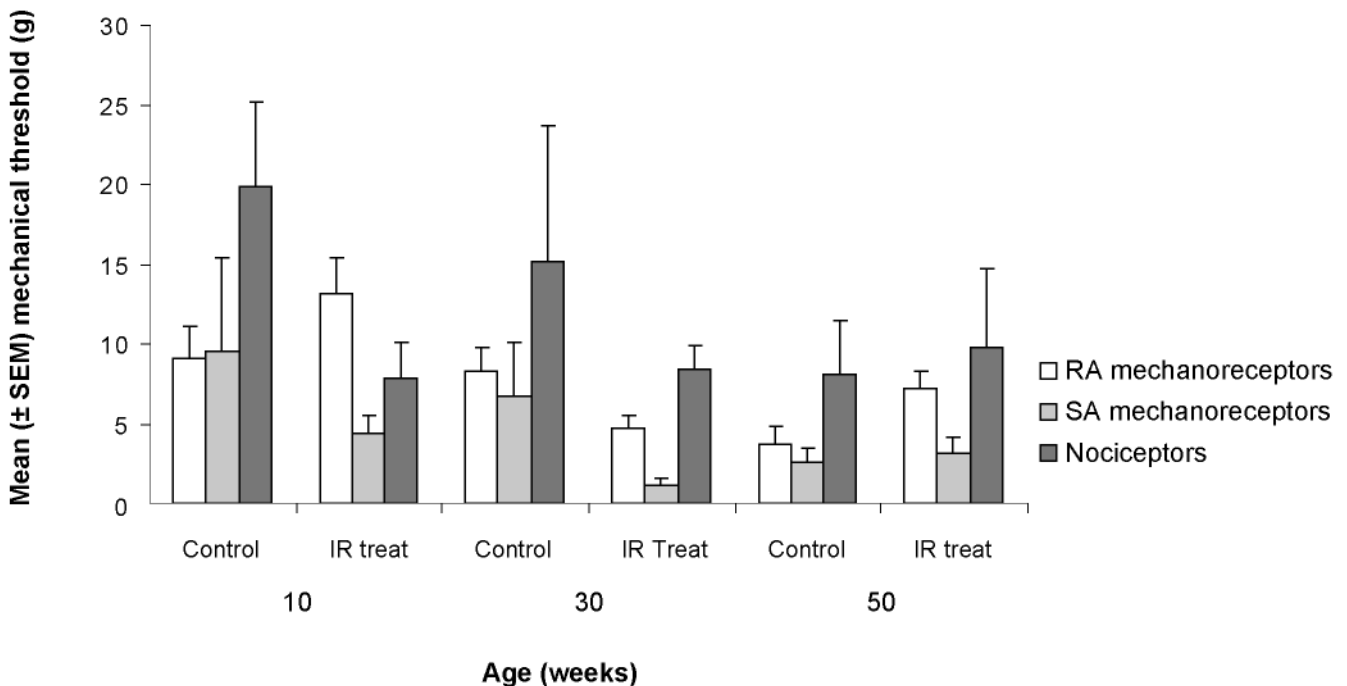
#### Histopathology

Tissue samples (the lower mandible) were fixed for a minimum of 72 h in 10% buffered neutral formalin then decalcified in EDTA for a minimum of two weeks. The samples were then trimmed and processed to paraffin wax on a Tissue Tek VIP 1000 (Sakura Finetek, USA) tissue processor, before being embedded into wax blocks on a Tissue Tek TEC Embedding Centre. The wax blocks were sectioned at 4 µ on a ThermoShandon Finesse Microtome (Thermo Electron Corporation, USA). Sections were incubated for 1 h in a 60°C incubator and then stained by haematoxylin and eosin and, in four-week old birds, by immunohistochemistry using S100 to highlight nerve tissue. Sections were mounted using DPX mounting medium to produce a permanent preparation. A particularly detailed examination of beaks at four weeks of age was carried out as, because of their small size, this group were not able to undergo electrophysiological investigations.

#### Analysis

After log-transformation, age- and treatment-related effects (and their interactions) on response thresholds of mechanoreceptors and nociceptors were carried out using a General Linear Model (Minitab Version 15, 2007). Where possible, thermal stimulus response curves for nociceptors were constructed. Conduction velocities were not measured due to the proximity of the recording and stimulus sites; however the action potential durations of a range of unit types were measured to give an indication of fibre type (each measure consisting of the average of five half-peak amplitude durations as described in Gee *et al* 1999). *T*-tests were used to make simple comparisons between beak length measurements at various ages.

Figure 1



Mean ( $\pm$  SEM) mechanical thresholds of rapidly (RA) and slowly adapting (SA) mechanoreceptors and nociceptors in control and treated birds at ten, 30 and 50 weeks of age.

## Results

### Electrophysiological recordings

The activity of 386 sensory afferents was recorded; the numbers of each receptor type (according to age and treatment) are shown in Table 1. Mechanical, thermal and nociceptive receptive fields were encountered over the entire lower beak including all outer and inner beak surfaces and beak tip of both intact and treatment birds.

Rapidly adapting (RA) mechanoreceptors were encountered very commonly during nerve dissection, and in total the responses of 146 RA units were recorded. Some RA units responded to touch with one or two spikes (Herbst units responding to vibration), others responded with a short burst (Grandry units). Grandry units responded to a moving stimulus with a train of impulses and some responded during the onset and offset of stimulation. Rapidly adapting units had mechanical thresholds ranging from 0.1 to 48 g (overall mean [ $\pm$  SD], 7.8 [ $\pm$  7.8 g]). Their receptive fields were generally of medium to large size and were often elliptical. The average half-peak amplitude duration for six randomly chosen rapidly adapting units was 0.26 [ $\pm$  0.05] ms (range 0.20 to 0.33 ms).

An age effect on RA mechanical thresholds was apparent; there was a significant overall trend ( $F = 6.41$ ,  $P = 0.002$ ) for an increase in sensitivity (decrease in mechanical threshold) with age (Figure 1). There was no treatment effect but a significant age/treatment interaction was identified ( $F = 4.45$ ,  $P = 0.014$ ). *Post hoc t*-test comparisons revealed

a significant difference between the mechanical thresholds of control or treatment RA units at 30 weeks (thresholds of treatment birds were lower than those of controls;  $P = 0.032$  [Figure 1]), and 50 weeks (effect reversed with treatment RA units exhibiting significantly higher mechanical thresholds than control units;  $P = 0.029$  [Figure 1]).

Slowly adapting (SA) mechanoreceptors were encountered less commonly than RA units, and in total 79 were recorded. Their mechanical thresholds ranged from 0.1 to 20.4 g (3.7 [ $\pm$  4.3] g). SA units showed considerable variability in their dynamic and static discharge characteristics and their rate of adaptation. SA units with small, medium and large receptive fields were observed. Overall, SA units had significantly lower mechanical thresholds than RA receptors (*t*-test,  $P = 0.024$ ; Figure 1). The average half-peak amplitude duration for six randomly chosen slowly adapting units was 0.28 ( $\pm$  0.05) ms (range 0.23 to 0.36 ms). Analysis of mechanical thresholds between control and treatment SA units yielded no significant results for age or treatment, but there was a significant age/treatment interaction ( $F = 3.73$ ,  $P = 0.03$ ). *Post hoc* comparisons showed that at 30 weeks treatment units had significantly lower thresholds than controls ( $P = 0.026$ ; Figure 1).

Nociceptors (afferents responding preferentially to noxious mechanical and/or thermal stimulation) were relatively rare and 44 were identified. Mechanical thresholds for these units ranged from 1.1 to 48 g (11.5 [ $\pm$  11.6] g). All exhibited slowly adapting responses to mechanical stimulation and had small, spot-like receptive fields. As would be expected,

**Table 2** Mean proportional measure of beak area in relation to total head area in treated and control birds at each age with 95% confidence intervals.

Age	Control	95% Confidence Interval	Treated	95% Confidence Interval
1 day old	7.22	6.52–7.92	7.82	6.94–8.70
4 weeks old*	11.16	10.57–11.76	9.80	9.38–10.22
10 weeks old*	12.31	11.16–13.47	10.61	9.20–12.01
30 weeks old*	10.33	9.06–11.60	9.51	8.57–10.46
50 weeks old*	10.69	9.74–11.63	9.00	8.20–9.79

\* Indicates a significant difference between treated and control birds at  $P = 0.05$ .

nociceptive units had significantly higher mean mechanical thresholds than RA mechanoreceptors ( $t$ -test,  $P = 0.002$ ). There were no treatment or age effects on the mechanical thresholds of nociceptors. There was, however, a non-significant trend for nociceptors in ten- and 30-week old treated birds to have lower mechanical thresholds than controls (Figure 1). Twenty-three of the nociceptors were polymodal and responded to noxious heat, thermal thresholds ranged from 42 to 54°C (46.9 [ $\pm$  3.6]°C). Thermal stimulus-response curves were constructed for nine units and four units showed a peak in response at 53°C. There was no evidence of a treatment effect on thermal thresholds, though numbers for comparison were small. Nociceptors which did not respond to heat were high-threshold mechanical nociceptors whose response thresholds ranged from 4.1 to 48 g. Half-peak amplitude spike duration was determined for all nociceptors, and the average was 0.57 ( $\pm$  0.27) ms (range 0.24 to 1.44 ms). Nociceptive units were never spontaneously active initially, though a small number exhibited spontaneous firing after repeated mechanical or thermal stimulation. This effect was seen in nociceptors in both control and treatment birds.

Thermally sensitive receptors were regularly encountered and 97 were recorded. They were characterised by ongoing spontaneous activity which took various forms (regular, bursting, rapid or slow firing). Responses to cold were variable; cold stimulation resulted in inhibition of activity (eleven units, likely to be warmth receptors) or, more commonly, in a burst of activity usually followed by inhibition (cold receptors) before the eventual return of spontaneous firing. Approximate receptive fields could be identified and were distributed throughout the beak in treatment and control birds. No evidence for mechanical sensitivity was found in these receptors, except one which was a thermally sensitive mechanoreceptor. The average half-peak amplitude duration for six randomly chosen thermoreceptors was 0.27 ( $\pm$  0.09) ms (range 0.20 to 0.40 ms). All spontaneously active units encountered were tested for thermal (cold) sensitivity; and in total 20 units exhibiting ongoing spontaneous activity did not respond to cold (ten treatment, ten control). These units also did not exhibit any

response to mechanical stimulation and while they may have been thermoreceptors responsive to non-noxious warmth (not tested), their nature remains uncertain. Overall, their average half peak amplitude spike duration was 0.41 ( $\pm$  0.17) ms (range 0.21 to 0.85 ms).

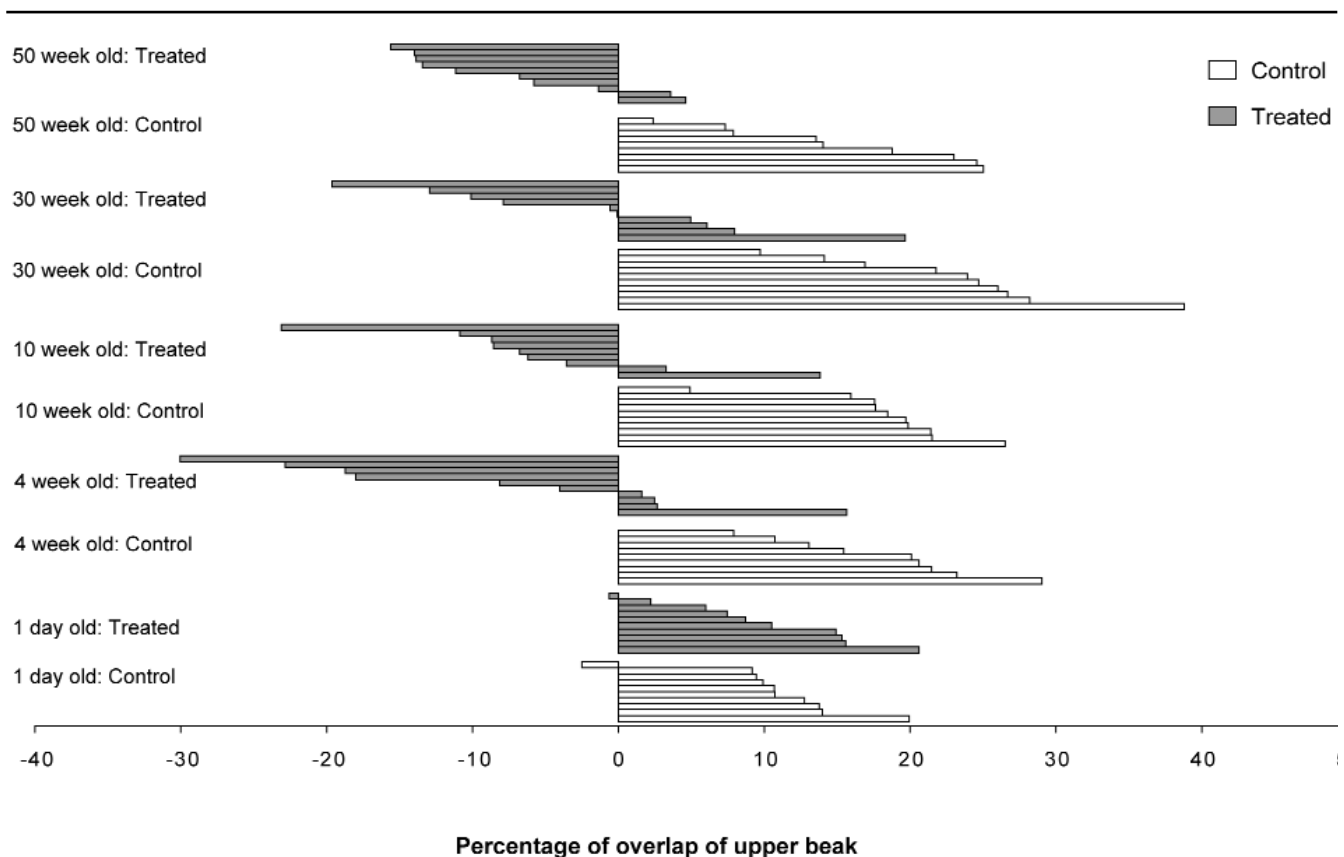
#### Beak measurements

IRBT birds had an obviously different, blunter beak shape compared to controls at all ages except day-old. Proportional measures clearly showed decreased beak size (area) in IR-treated birds compared to intact controls (see Table 2) and this difference was highly significant at four ( $P < 0.001$ ), ten ( $P = 0.04$ ) and 50 weeks ( $P = 0.005$ ). There was no difference at day-old, because at that time the IR-treated birds had not yet shed the IR-affected tissue. At day-old, the percentage beak length (based on distance from proximal nares to beak tip) affected by the IR treatment was 65% for the upper beak and 51% for the lower beak. Total beak area affected by IRBT was 36%. At four weeks, the mean percentage beak length loss was 44% for the upper beak and 26% for the lower beak (note this reflects beak growth in intact birds), by 50 weeks this had reduced to 25% (upper beak). In treatment groups it was evident that in the majority of birds the lower beak was longer than the upper beak (the opposite was the case in controls) resulting in a beak step, as indicated by percentage differences (see Figure 2).

#### Radiography

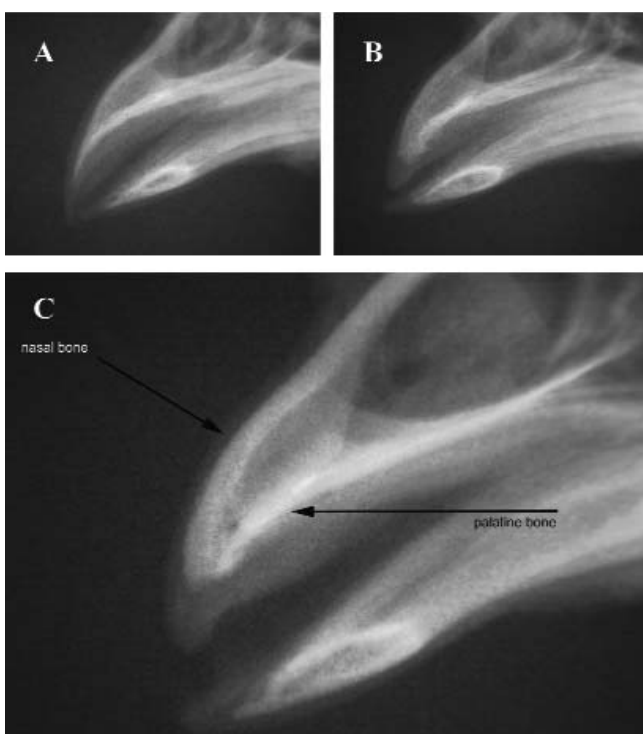
Control and treated birds were compared and evidence of differential sclerosis and/or lucency of the beak were sought. In the control birds, the sharp (hooked) beak outline, palatine bone and nasal cavity were clearly visible (Figure 3[a]). In IR-treated birds the blunted beak shape was evident, but the palatine bone was visible as a sharp line (as in controls) and the nasal cavity was unaffected (Figure 3[b]). Detailed evaluation provided evidence of marginal thickening of the palatine bone in some IR-treated birds at 50 weeks of age (Figure 3[c]), but this is likely to be a normal bone remodelling response and would have no clinical consequences. Overall, no evidence of adverse or pathological change (eg exuberant periosteal reaction) was evident in IR-treated birds compared to controls.

Figure 2



Percentage overlap of upper beak in treated and control birds at each age. A positive percentage difference indicates that the upper beak is longer than the lower beak (as is the case in normal, intact beaks), while a negative percentage difference indicates a beak step where the lower beak protrudes from beneath the upper beak.

Figure 3



Radiographs of the beak tips of intact (A) and IRBT (B) beaks at 50 weeks of age. C shows marginal thickening of the palatine bone in an IRBT bird.

### Histology

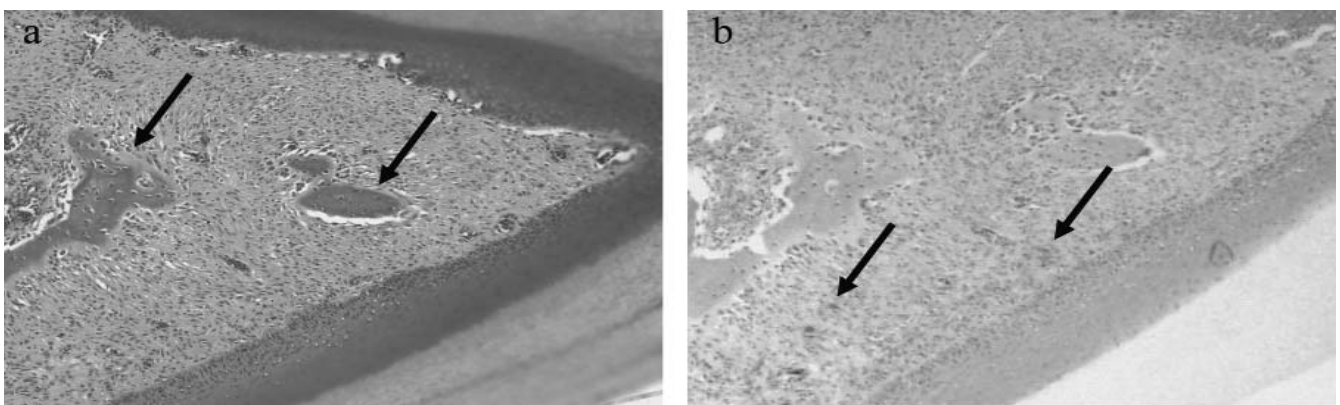
Histological sections of tips of the lower beaks of six four-week-old birds that had been IR beak treated had sufficient tissue presented in an orientation that was suitable for interpretation. There was evidence of re-epithelialisation, fibrovascular hyperplasia and bone remodelling in the tips of all six treated lower beaks (Table 3), consistent with an appropriate healing response (Figure 4). For comparison, interpretable tissue from the tips of beaks was available from eight four-week-old control (intact) birds (Table 3).

Mechanoreceptors (Herbst corpuscles) were visible within the area of healing at the tips of lower beaks in two of six beak-treated birds (Table 3). In one bird (G9A), one Herbst corpuscle was visible at the tip of the lower beak in the plane of section, compared to two Herbst corpuscles in the adjacent tissue. In another bird (G7A), 12 Herbst corpuscles were visible at the tip of the lower beak, compared to one Herbst corpuscle in the adjacent tissue (Figure 5). The average number of Herbst corpuscles at the tips of the lower beaks in treated birds at four weeks of age was  $2.2 (\pm 4.8)$  (range 0–12;  $n = 6$ ). In eight four-week-old control birds, moderate to abundant Herbst corpuscles were visible at the tips of the lower beaks and the average number of Herbst corpuscles was  $6.9 (\pm 2.2)$  (range 4–11;  $n = 8$ ). Thus, there

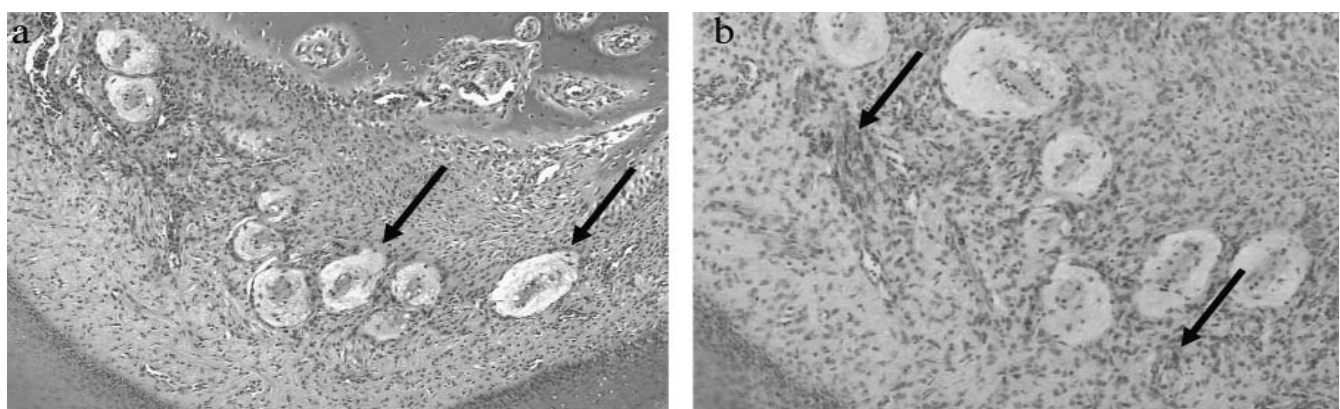
**Table 3** Histological findings in lower-beak tips from IR-beak-treated and control birds at four weeks of age.

Bird ID	IRBT	Complete epithelialisation	Fibrovascular hyperplasia	Bone remodelling	Number of Herbst corpuscles in lower beak tip	Number of Herbst corpuscles in adjacent tissue	Density of nerves in beak tip <sup>†</sup>	Density of nerves in adjacent tissue <sup>†</sup>
G1A	+	+	+	+	0	0	0	1
G3A	+	+	+	+	0	0	1	1
G4A	+	+	+	+	0	0	2	2
G5A	+	+	+	+	0	2	NI	NI
G7A	+	+	+	+	12	1	2	2
G9A	+	+	+	+	1	2	NI	NI
Y43A	-	+	-	-	7	2	2	2
Y56A	-	+	-	-	8	3	3	2
Y59A	-	+	-	-	4	2	2	2
Y17A	-	+	-	-	7	0	3	2
Y02A	-	+	-	-	7	4	3	2
Y23A	-	+	-	-	11	4	3	3
Y51A	-	+	-	-	7	3	NI	NI
Y60A	-	+	-	-	4	2	1	2

<sup>†</sup> Density of nerves was scored semi-quantitatively as absent (0), sparse (1), moderate (2) and abundant (3) based on positive staining for S100. NI = S100 staining not interpretable.

**Figure 4**

Histological section of the tip of the lower beak in an IR-beak-treated bird (G3A) at four weeks of age. In (a) there is re-epithelialisation, fibrovascular hyperplasia and bone remodelling (arrows) at the site of healing. No Herbst corpuscles are visible. H&E. Original magnification 100x, while (b) shows immunohistochemistry for S100 (brown staining) demonstrating sparse repopulation by small nerve fibres (arrows). Avidin-biotin peroxidase staining. Original magnification 100x.

**Figure 5**

Histological section of the tip of the lower beak in an IR-beak-treated bird (G7A) at four weeks of age. In (a) multiple Herbst corpuscles are visible among fibrovascular hyperplasia at the site of healing at the beak tip. H&E. Original magnification 100x, while (b) shows immunohistochemistry for S100 (brown staining) demonstrating a moderate density of small nerve fibres at the site of healing at the beak tip (arrows). Avidin-biotin peroxidase staining. Original magnification 200x.

was repopulation of Herbst corpuscles at the tips of the lower beaks in a relatively low proportion of beak-treated birds at four weeks of age, although repopulation by numerous Herbst corpuscles was present in one of six birds. Histological sections in four of the six beak-treated birds and seven of the eight control birds were stained by immunohistochemistry for S100, a marker for neural crest cells that enhances the visibility of nerve axons in histological sections. The density of nerves at the beak tips and in the adjacent tissue was scored semi-quantitatively as absent (0), sparse (1), moderate (2) and abundant (3). There was sparse to moderate repopulation with small nerve fibres in the tips of three of the four lower beaks from birds that had been beak treated (mean density 1.3 [ $\pm$  1.0];  $n = 4$ ), compared to sparse to abundant small nerve fibres in all seven lower beaks from control birds (mean density 2.4 [ $\pm$  0.8];  $n = 7$ ; Table 3). Thus, there was a lower density of nerve fibres at the tips of beaks in IRBT birds at four weeks of age compared to control birds of the same age. Of particular note, no structures resembling microneuromas or other abnormal proliferations of nerve fibres were observed in any birds.

In older birds, the degree of keratinisation of the beaks made sectioning very difficult and therefore only a limited number of usable sections of the lower-beak tip were obtained. Histological sections were examined from ten-, 30- and 50-week-old IRBT birds and control birds. Re-epithelialisation, fibrous scarring and bone remodelling were evident at the tip of the beak in the treated birds and there was no evidence of neuromas at any age. Herbst corpuscles were visible at the tip of the beak in some of the treated birds and in untreated birds. Repopulation by small nerves was also evident at the tip of the beak in the treated birds.

## Discussion

The sensory receptors identified in this study correspond closely to previous reports of the properties of afferents recorded from the intramandibular nerve in the chicken (Breward & Gentle 1985; Gentle 1989, 1991). This is, however, the first study to examine the neurophysiological consequences of infra-red beak treatment, and the first investigation of afferent response characteristics in intact birds at a range of ages. The findings also provide further evidence of the highly innervated nature of beak tissue.

Different sensory receptors are innervated by nerve fibres with different diameters and degrees of myelination. Cutaneous mechanoreceptors are innervated by A $\beta$  fibres, which are 6–12  $\mu\text{m}$  in diameter and conduct at 33–75  $\text{m s}^{-1}$ . Thermoreceptors and free-nerve endings transmitting touch and pressure, and some nociceptors are innervated by small myelinated A $\delta$  fibres, which are 1–5  $\mu\text{m}$  in diameter and conduct at 3–30  $\text{m s}^{-1}$ . Nociceptors and warmth receptors are innervated by unmyelinated, slow conducting C fibres, which are 0.2–1.5  $\mu\text{m}$  in diameter and conduct at 0.5–2  $\text{m s}^{-1}$ . Due to the proximity of the recording and stimulus sites, it was not possible to measure conduction velocities of the nerve fibres during recordings. This problem has been

encountered by others in similar preparations (Necker & Reiner 1980; Gentle 1991). In the absence of conduction velocities, sensory afferent types can be identified by their response properties and fibre innervation type can be inferred by examining action potential duration which is known to relate to fibre diameter (Rose *et al* 1986). The average half-peak amplitude duration has been used for this purpose (Gee *et al* 1999) and was determined for the units recorded in the current study. The durations observed matched the response properties as expected, the shortest spike durations were found in rapidly adapting mechanoreceptors (A $\beta$ ), followed by thermoreceptors and slowly adapting mechanoreceptors (A $\delta$ ). Nociceptor-spike durations indicated that these were both A $\delta$ - and C-fibre innervated and relate well to published durations (Gee *et al* 1999).

The receptor types identified (mechanoreceptors, thermoreceptors and nociceptors) had similar characteristics to those described previously, and receptive fields extending to the inner and outer beak tip were observed in treatment birds at all ages. For both RA and SA mechanoreceptors, the range of mechanical thresholds found here was wider than previously reported. This was evident particularly for SA mechanoreceptors (mean threshold here 3.7 g compared to 0.62 g; Gentle 1989), which may be due to the greater age range examined in the current work. It is also possible that some units classed as SA mechanoreceptors could have been high threshold mechanical nociceptors (as suggested by Gentle 1989), contributing to an increased mean mechanical threshold for this group. Gentle (1989) also reported that a large number of SA mechanoreceptors exhibited thermal sensitivity. This was not the case in the current experiment (only one SA receptor with thermal sensitivity was identified), but this may relate to the fact that non-noxious heat was not applied routinely as a stimulus. Mechanoreceptor response abnormalities associated with nerve regeneration such as multiple receptive fields, unusually small or large receptive fields and pronounced fatigue on repetitive stimulation, as described by Mackel *et al* (1985), were not observed in any of the preparations.

Age effects on mechanical sensitivity were found in RA mechanoreceptors only, with a trend for an increase in sensitivity at 30 and 50 weeks compared to ten weeks. Treatment effects on mechanoreceptor thresholds were found in both RA and SA receptors. RA mechanoreceptor thresholds in treatment birds were lower than controls at 30 weeks, indicating increased mechanical sensitivity. This does not support the notion that beak trimming/treatment may work by reducing sensory feedback from the beak. However, the potential biological significance of this result is weakened by the fact that this effect was reversed at 50 weeks (treatment birds less sensitive than controls). In SA mechanoreceptors, there was also some evidence for a treatment effect on mechanical thresholds, suggesting increased mechanical sensitivity (lower mechanical thresholds) in treatment birds. The effect was evident at 30 weeks (incidentally matching the finding for RA receptors) and



was abolished by 50 weeks. The changes observed do not support the notion that beak treatment reduces sensory feedback, and do not concur with Breward and Gentle (1985) who reported no effects of beak trimming on mechanoreceptor thresholds. Thus, the findings here for changes in RA and SA mechanical thresholds may relate specifically to IRBT, to the specific age groups concerned, or may be artefactual, and it is difficult to speculate on their biological significance (if any).

Nociceptors were relatively rare but several were encountered in both treatment and control birds in all age groups. As expected, they had higher mechanical thresholds than mechanoreceptors and some responded to noxious heat stimulation (polymodal nociceptors). Their thresholds and response curves closely matched those reported previously (Necker & Reiner 1980; Breward & Gentle 1985; Gentle 1989). Treatment effects on nociceptor thresholds are of particular interest because of concerns that beak trimming/treatment is effective because it results in pain being induced during routine pecking. There was no evidence of a treatment effect on either mechanical or thermal nociceptive thresholds at any age. This agrees with previous findings with regard to nociceptive thermal responses; Breward and Gentle (1985) found no systematic reduction in thresholds in hot-blade beak-trimmed birds. Although there was a numerical trend for nociceptors in ten- and 30-week-old treated birds to have lower mechanical thresholds than controls, this was not statistically significant. Breward and Gentle (1985) reported that 37% of nociceptors in beak-trimmed birds responded to stimulation with an abnormal bursting pattern; this effect was not observed in any of the nociceptors recorded here. Other potential abnormalities in nociceptor response, such as spontaneous activity and long afterdischarge were not observed in control or treatment birds. A few nociceptors became spontaneously active after repeated thermal or mechanical stimulation, but this effect was observed in nociceptors in both control and treatment birds and has been reported previously in intact beaks (Necker & Reiner 1980). Together, the findings indicate that beak treatment had no significant effect on nociceptor thresholds or response characteristics, providing no evidence that IRBT is associated with hyperalgesia (increased sensitivity to pain) or allodynia (painful response to a non-noxious stimulus) after ten weeks of age. Whether these effects are present before ten weeks of age can only be speculated upon as younger, smaller birds present a significant challenge to obtaining electrophysiological recordings. It should be noted that there is some behavioural evidence of short-term pain (longer time taken to approach feed and initiate feeding, showing less beak-related behaviours and lower feed intake for up to nine weeks) after IRBT (Marchant-Forde & Cheng 2006).

The most striking finding reported by Breward and Gentle (1985), in relation to beak-trimmed birds, was the large number of abnormal spontaneously active fibres observed. These were attributed to neuromas which had formed in the

beak stumps of trimmed birds. In the current study, particular attention was paid to spontaneously active units. Thermoreceptors exhibit ongoing firing at room temperature (Gentle 1989) so all spontaneously active units were tested for their response to cold, and the vast majority responded appropriately. Warmth and cold receptors were found equally in control and treatment birds and their responses mirrored those described in the literature. A small number of spontaneously active units (20, less than 6% of the afferents encountered) did not respond to thermal or mechanical stimulation. While their nature remains unclear, they were relatively rare and were encountered equally in control and treatment birds. It is therefore considered to be unlikely that these represent abnormal neural activity arising from neuromas. The lack of abnormal spontaneous activity in the current study probably relates to the fact that the birds examined by Breward and Gentle (1985) were hot-blade trimmed in adulthood, whereas the birds examined here were treated at day-old (which has been previously shown to avoid persistent neuroma formation: Lunam *et al* 1996; Gentle *et al* 1997). The fact that the birds were IR beak-trimmed rather than hot-blade trimmed could also have had an effect.

Beak measurement data indicated that IRBT resulted in changes in beak length and shape and as the birds were commercially sourced we can assume this is commercially relevant. The extent of the trim in terms of beak length reduced with age as regrowth occurred but treated beaks were still significantly shorter than controls at all ages except day-old and 30 weeks. As well as the obvious blunting of the hooked beak, there was a trend for a beak step (lower beak protruding by up to 30%) in treated birds. The functional consequences of this (if any) are not clear, but this finding makes electrophysiological recordings from the lower beak all the more relevant, since for the majority of trimmed birds, it is the lower beak which will first make contact with pecked objects.

Histopathological investigations revealed that by four weeks of age there was evidence of repopulation of tactile receptors and re-innervation of treated beaks (though only in some birds and to a lesser extent than controls). Re-epithelialisation, fibrovascular hyperplasia and bone remodelling were observed, all indicative of an appropriate healing response. The presence of mechanoreceptors from four weeks of age onwards supports the electrophysiological findings which provided evidence of mechanical sensitivity in regrown beak tips from ten weeks of age. In older birds, repopulation of tactile receptors and innervation were visible along with evidence of fibrous scarring. Importantly, no neuromas or abnormal proliferations of nerve fibres were observed in the beaks at any age and this concurs with the lack of abnormal spontaneous activity in the electrophysiological studies. While it could be argued that this result should be treated with caution as serial sections were not examined as in previous studies examining beak neuromas (Lunam *et al* 1996), the absence of neuromas is supported by a lack of related neurophysiological responses.

Previous workers have reported a lack of neuromas when beak trimming (hot blade) was carried out at day-old (Dubbeldam *et al* 1995; Lunam *et al* 1996; Gentle *et al* 1997; Cheng 2006). In a recent report, Lunam (in Glatz & Hinch 2008) reported the presence of persistent traumatic neuromas in birds that had been hot-blade and IR beak treated at day-old. This was unexpected and does not match previous findings for hot-blade trimming, leading to the conclusion that the application of excess heat or excess tissue removal was responsible for neuroma formation in those experiments, which may not be typical of routine IRBT.

### Animal welfare implications

Infra-red beak treatment is now applied routinely in the egg industry, and therefore the welfare implications of this type of beak trimming are of significant interest. The findings of this study suggest that infra-red beak treatment at day-old does not have chronic adverse neurophysiological consequences. Receptive fields for all receptor types (mechanoreceptors, thermoreceptors and nociceptors) were encountered in the regenerated beaks of treated birds at all ages suggesting substantial re-innervation. Importantly, nociceptor thresholds did not differ between treated and control birds and there was no evidence that IRBT was associated with abnormal spontaneous activity which could be attributed to neuromas after ten weeks of age. Histopathological findings confirmed re-innervation and tactile receptor repopulation by four weeks after beak treatment and provided no evidence of neuroma formation in IR-treated birds. Collectively, these results suggest that infra-red beak treatment of day-old chicks is unlikely to be associated with chronic pain after ten weeks of age, though the potentially painful effects of IRBT in the first weeks of life warrant further investigation. Given that the purpose of beak trimming is to reduce or eliminate damaging bird-to-bird pecking (which can be a very substantial welfare issue), it could be argued that IRBT represents a refinement compared to previous approaches and that the welfare cost that it imposes is outweighed by the resulting benefits.

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