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Neuroma formation and abnormal afferent nerve discharges after partial beak amputation (beak trimming) in poultry

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Summary. Following partial amputation of the beak recordings were taken of the electrical activity from single afferent fibers of the intramandibular nerve. A total of 192 single afferent fiber units were isolated of which 47 were classified as nociceptors, with an abnormal pattern of discharge, and 89 were abnormal spontaneously active units. Following amputation neuromas were developing by 15 days after surgery and they were well formed by 20 to 30 days. The presence of neuromas together with abnormal spontaneous activity originating from them raise serious welfare questions concerning beak trimming.

Key words. Poultry; beak trimming; intramandibular nerve; neuroma; afferent electrical activity.

The mutilation of farm animals to prevent them developing damaging behavioral vices is an emotive subject but there is little scientific evaluation of these in relation to the animal's welfare. Partial amputation of the beak is often performed in laying and breeding chicks and sometimes in adult birds to prevent or control cannibalism and feather pecking. Removal of part of the beak stimulates nociceptors[1] and creates feeding difficulties[2].

Previous studies of peripheral nerve injury and subsequent neuroma formation in the mouse and rat have suggested that abnormal activity arising from the regenerating axons is implicated in post-amputation stump pain[3]. However, with the exception of one clinical report[4], no experimental work has been performed on amputated stumps. The beak of the chicken is extensively innervated and has numerous cutaneous sensory receptors which have been classified as low-threshold mechanoreceptors, cold receptors and nociceptors[1,5]. These nociceptors have properties very similar to those described in mammals including man[6–12] and they are excited during the process of beak trimming. It is, however, not known what kind of information the damaged beak conveys to the central nervous system. The aim of this study was to investigate the long-term neurological consequences of beak trimming by examining both anatomically and physiologically the nerves running to the stump of the beak.

Methods. Adult Brown Leghorn hens, weighing from 1 to 1.5 kg, were anesthetized with sodium pentobarbitone (Sagatal, May & Baker Ltd) given i.v. The response of individual birds to barbiturate anesthesia is variable so that the level of anesthesia was monitored using the comb-pinch reflex (dose 24–30 mg). About ⅓ of the upper and lower beaks were removed using a commercial heated blade debeaker (Cope & Cope Ltd). The debeaker consisted of a lower metal bar on which the beak was placed and a movable electrically heated upper blade. The upper blade is manually pushed against the beak where it cuts through the beak cauterizing the stump at the same time. At intervals ranging from 1 to 83 days after beak trimming they were anesthetized (Ethyl carbamate, 1.5 g/kg given i.v.) and recordings were made from single afferent fibers of the intramandibular nerve. The intramandibular nerve, which supplies the cutaneous afferent innervation of the lower beak, was exposed in the mandibular canal. The nerve was supported on a black perspex platform in a pool of liquid paraffin. Fine filaments of the nerve, containing single active fibers, were dissected using fine watchmakers forceps and placed over silver wire recording electrodes.

To investigate the anatomical consequences of beak trimming 20, 5-week-old, Brown Leghorn chickens were anesthetized (sodium pentobarbitone) and ⅓ of the upper and lower beak was removed in the same way as the birds used for the electrophysiology. Two birds were killed at various intervals after beak trimming (1, 3, 6 and 24 h and 3, 6, 10, 15, 20, 30 days), the beaks were removed and fixed in formalin-acetoalcohol. The tissue was decalcified, embedded in paraffin wax and serially sectioned at 10 µm. All sections were collected and the slides were stained with either Hematoxylin/Eosin or Bodian's Protargol or Masson's Trichrome.

Results and discussion. A total of 192 single afferent fiber units were successfully isolated from 36 beak-trimmed birds. 28 of these units were classified as low threshold mechanoreceptors[5,13], with properties similar to those encountered in the intact beak. 47 units were classified as nociceptors[1], differing only slightly from normal nociceptors in thresholds and stimulus-response properties, but showing an abnormal pattern of discharge. Normal nociceptors exhibited a persistent discharge during sustained suprathreshold mechanical or thermal stimulation and in the case of thermal stimulation the discharge increased with increased temperature. Thermal thresholds of normal nociceptors ranged from 40° to 48 °C (mean 43.0, SE 0.66) and mechanical thresholds from 1.4 to 50 g[1]. Unlike the regenerating C-heat receptors reported in the cat[14] there was no systematic reduction in thermal thresholds in the nociceptors found in the amputated stump (thresholds 36–58 °C, mean 46.6, SE 0.83). In the intact beak the nociceptors responded to a suprathreshold thermal stimulus with a continuous train of impulses[1] with only 6% of the units showing a bursting (grouped) discharge. Whereas 37% of the nociceptors recorded from the amputated stump

Spontaneously firing units recorded from the intramandibular nerve of beak-trimmed chickens. The ongoing activity was recorded, then each unit was tested for its responsiveness to heat, cold and mechanical stimulation. 53 units were successfully isolated; they are divided into eight groups on the basis of their responsiveness to the different stimulus modalities

Number of units	Stimulus			Spontaneous firing pattern	Time after operation (days)	RF location (mm)
	Heat	Cold	Mechanical			
3	↑	↑	↑	I, B	5–38	2–12
5	↑	N.E.	↑	I	7–30	0–11
1	↑	N.E.	N.E.	B	22	7
1	N.E.	↑	N.E.	I	31	0
25	N.E.	N.E.	N.E.	R, I, B	13–83	N.L.
11	↓	↑	N.E.	R.I.	6–83	0–4
6	↓	↑	↑	I, R	12–83	0–4
1	↓	↑	↓	B	31	3

↑ = Stimulus provokes an increase in the firing rate of the unit. ↓ = Stimulus provokes a decrease in the firing rate of the unit. N.E. = Stimulus has no effect on the firing rate of the unit. R = Regular firing pattern. I = Irregular firing pattern. B = Bursting firing pattern. Receptive field (RF) location refers to the distance (measured to the nearest 0.5 mm) from the distal end of the beak stump to the distal edge of the receptive field. N.L. = Receptive field not located.

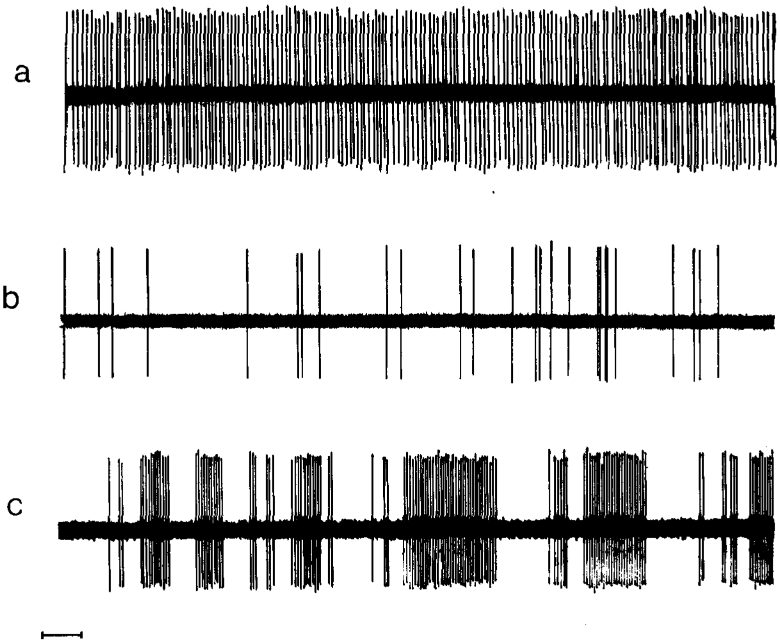

Figure 1. Three examples of afferent spontaneous activity recorded from the intramandibular nerve of the amputated beak. *a* Unit with a regular (9 Hz) discharge pattern, recorded 36 days after amputation. The receptive field was located 1 mm proximal to the tip of the amputation stump. *b* Unit with an irregular discharge pattern, recorded 37 days after amputation. The receptive field could not be located using heat, cold or mechanical stimulation. *c* Unit with a bursting discharge pattern, recorded 31 days after amputation. The discharge pattern within each burst is regular. The receptive field was located 3 mm proximal to the tip of the amputation stump. Time calibration bar = 1 s in *a* and *c*, 5 in *b*.

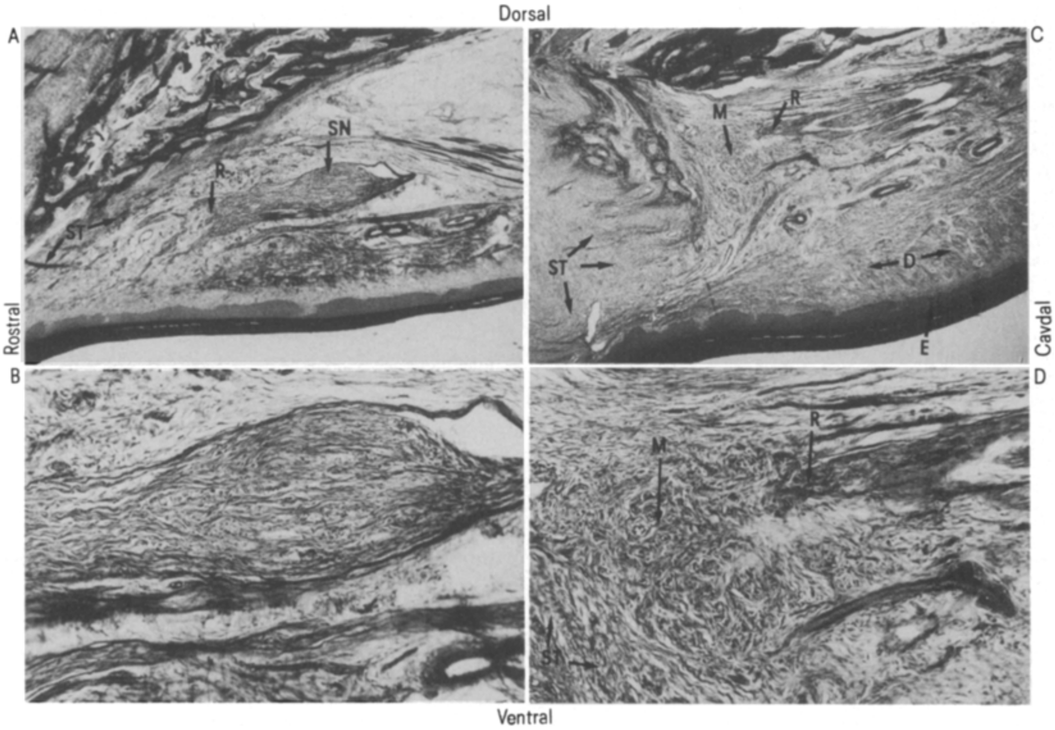

Figure 2. Photomicrographs of 10 µm thick sections of the upper beak of the chicken 30 days after beak trimming. All sections stained with trichrome method. *A* Lower power (× 100) section showing a neuroma formed at the stump of the nerve (SN) with a complex mass of regenerating fibers (R) growing towards the scar tissue (ST) at the end of the beak. *B* A higher magnification of the stump neuroma (× 400) shown in *A*. *C* Low power (× 100) section showing regenerating fibers forming a complex neuroma (N) adjacent to the scar tissue at the end of the beak. *D* A higher magnification (× 400) of the neuroma shown in *C*. E = epidermis, D = dermis, B = maxilla bone.

responded with a bursting pattern of discharge. The mechanoreceptors and nociceptors present in the amputated beak stump, like those in the intact beaks, did not discharge in the absence of stimulation.

The most characteristic abnormality encountered in the beak stump was the presence of large numbers of spontaneously active units. A total of 96 such units were recorded, 53 of which are reported here. For each of these 53 units their receptive fields were located and the unit was tested for responsiveness to heat, cold and mechanical stimuli using the techniques described previously[1]. The pattern of spontaneous discharge was basically either regular, irregular or bursting (fig. 1). The effects of heat, cold and mechanical stimulation on the rate of response varied from unit to unit. There were eight classes of stimulus-modality responses (table) ranging from an excitatory effect (i.e. increase in discharge rate) to all three modalitites (heat, cold, mechanical) to total unresponsiveness to any stimulus. Three spontaneously active units had response characteristics similar to cold receptors i.e. a regular spontaneous discharge which was excited by a cold stimulus, inhibited by heat and unaffected by mechanical stimulation. The remaining 50 units were completely abnormal in their discharge and response characteristics. They were recorded from the beak stump at 5–83 days after the initial amputation with their receptive fields located on the distal tip of the stump and at varying distances (up to 12 mm) proximal to it. This spontaneous activity is markedly similar to that observed in the experimental neuroma preparation developed initially by Wall, Devor and coworkers[15–17] in the rat and later extended to the mouse and cat[18,19].

Beak trimming results in both cutting and cauterizing the beak, and a significant but variable amount of the remaining beak was damaged by the cautery. The nerves in the beak were damaged by the high temperature of the cautery blade for a distance of 2–3 mm from the cut end. By 6 days after trimming the damaged portion of the nerve had been completely degenerated. At 10 days there was evidence of nerve regrowth with some enlargement of the end of the nerve. This regeneration and regrowth of the nerve fibers continued so that by 15 days clear neuroma was present at the end of the nerve stump together with numerous bundles of regenerating fibers. These regenerating fibers continued to grow but, because of the adjacent scar tissue, were unable to innervate dermal structures and consequently the fibers grew back on themselves to form a complex mass of intertwining regenerating nerve fibers together with the surrounding tissue. In some nerves there was a simple terminal neuroma. In others a neuroma was formed at the original stump of the nerve (fig. 2A, B) in association with a large and complex neuroma formed adjacent to the scar tissue which forms the end of the beak. Some nerves did not appear to have a neuroma at the original stump but, instead, had a complex neuroma adjacent to the scar tissue (fig. 2C, D).

The activation of specific nociceptors in humans[20] and spontaneous discharges originating from stump neuromas are implicated in acute and chronic pain syndromes. From previous work[1] it is clear that the process of beak trimming results in the activation of specific nociceptors in the beak at the time of surgery. From the work presented here it is clear that neuromas are formed as a result of the amputation and that these neuromas probably give rise to abnormal spontaneous nervous activity.

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Axillary 5α-androst-16-en-3-one in men and women: relationships with olfactory acuity to odorous 16-androstenes[1]

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Summary. Axillary 5α-androst-16-en-3-one (5α-androstenone) levels were found to be significantly higher in men than in women but do not vary between left and right axillae, are not related to age, handedness or degree of hirsutism (in women) nor to anosmia to this steroid. In men (but not in women), levels are related linearly to axillary cholesterol concentrations but not to squalene. Olfactory thresholds for 5α-androstenone varied widely, the lowest recorded being 0.2 ppb, but there was no difference in thresholds between men and women. Women (70%) found the smell 'repellant' but anosmia did not differ greatly between men and women (9–20%). Anosmia to the smell of 5α-androst-16-en-3α-ol was most marked in women (90%) rather than in men (45%). Axillary 5α-androstenone values were generally consistent with the 'musky' or 'strong' smells of male axillary extracts, compared with the 'sweet' smell of those from female subjects.
Key words. Axilla; 5α-androst-16-en-3-one; 5α-androst-16-en-3α-ol; anosmia; cholesterol; squalene; olfactory threshold.

The subject of olfactory communication in humans has always been of interest but especially so now that many of the odors emitted have been characterized, and also because these odors might possibly act as olfactory cues in social behavior (for review, see Doty[4] and Gower[5]). One of the obvious sources of such volatile substances are the axillae, and recent research has focused on the components of axillary sweat. At least two odorous steroids are known to be present, 5α-androst-16-en-3-one (5α-androstenone) and 5α-androst-16-en-3α-ol (an-α)[6–8], the former being generally thought to have a urine-like smell and an-α a