Effects of Unilateral Cranial Sympathectomy Either Alone or With Sensory Nerve Sectioning on Pedicle Growth in Red Deer (*Cervus elaphus*)

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ABSTRACT In a previous study (Li et al. [1993], J. Exp. Zool., *267:*188–197) sensory nerve sectioning had no effect on the timing of pedicle growth. The aim of the present study was to determine whether sensory nerve sectioning in conjunction with sympathectomy would influence pedicle growth. Twelve intact male red deer calves were allocated to treatment before any pedicle growth as follows: 1) unilateral sensory nerve removal (USX, n = 5), 2) unilateral superior cervical ganglionectomy (SGX, n = 4), or 3) both USX and SGX (SG/USX, n = 3). The calves were observed weekly. In all cases the untreated side was the control. Pedicle initiation was measured with a pedicle detector and after initiation, growth was measured with a ruler. When the treated pedicles reached a length of 60 mm the calves were killed and tissues from the pedicle were examined immunohistochemically for nerves. No large bundles of nerves were observed in the treated pedicle although a few fine fibres were present. All calves grew pedicles. There were no significant differences in the timing of pedicle initiation either within treatment or between treatments. All denervated pedicles grew faster than controls and were consequently higher at examination. The fact that pedicle growth took place despite reduced innervation indicates that a continuous neural connection is not a pre-requisite for normal pedicle growth.

Pedicles are bony protuberances which develop around the time of puberty from antlerogenic regions on the frontal bones of young male deer (Goss, '83). The pedicles are permanent, but from them the deciduous antlers develop, subsequently cast, and regenerate each year during the stag's life. Although the histogenesis of the pedicles has received some study recently (Li et al., '93a), the factors which control histogenesis are not well understood. The sensory nerve supply to the antlerogenic region is from the zygomaticotemporal and supraorbital branches of the trigeminal nerve (Wislocki and Singer, '46). Bubenik ('82, '90b) and Bubenik ('90a) considered that a direct neural link between the antlerogenic region on the frontal bone and the central nervous system was essential for post-natal pedicle development. To test this hypothesis Li et al. ('93a) sectioned the two branches of the trigeminal nerve in young male red deer prior to pedicle initiation. They found that the absence of sensory nerve supply did not prevent pedicle growth or any part of the antler cycle. A sympathetic nerve supply to the head from the superior cervical ganglion (Wika, '80) was not manipulated nor was the auriculopalpebral branch of the facial nerve which provides some sensory nerve supply in some deer (Adams, '79). Consequently it was possible that Li et al. ('93a) had erred in their conclusions in that it was possible that the pedicles of the deer were not completely denervated. The aim of the present study was to determine whether pedicle development took place in young stags whose sensory and sympathetic nerves supplies were removed prior to pedicle initiation.

MATERIALS AND METHODS

Animals

Twelve 4-month-old red deer stag calves were randomly allocated into three groups (n = 4/group) on April 9, 1992 as follows. Group 1 was subject to unilateral removal (USX) of the sensory nerve supply to the antlerogenic region (right side), group 2 was subject to superior cervical ganglionectomy (sympathectomy SGX, right side), and

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group 3 was subject to both sensory nerve removal and superior cervical ganglionectomy (SG/USX, right side only). All deer were kept outside at pasture through the study from April until October 1992. At surgery the animals weighed 46.1 ± 2.0 kg (mean ± standard deviation), and none had begun to grow pedicles.

Surgery

USX

The surgical procedure was the same as published previously (Li et al., '93a) except as detailed. A third incision was made 40 mm caudal to the eye over the zygomatic process of the temporal bone. The facial nerve was located and traced until the auriculo-palpebral branch was located, and a 15-mm section of this branch was removed. The wound was closed with Michel clips.

SGX

The method was essentially similar to that published by Appleton and Waites ('55) with the following modifications. The initial incision was parallel to the paramastoid process of the occipital bone rather than the angle of the ramus of the jaw and extended from proximal to the zygomatic process of the temporal bone to the jugular vein. This angle of approach permitted the jugulohyoid muscle to be reflected (rather than sectioned as in the original method), with the epihyoid bone and the superior cervical ganglion thereby exposed.

SG/USX

The surgical procedures were as described above. All animals were subject to SGX before USX.

Confirmation of effective surgical removal

Sections of sensory nerves and the cervical ganglion were placed in 10% buffered formalin for subsequent histological examination. The tissues were stained with haematoxylin and eosin and silver staining, mounted, and viewed with a microscope at ×100–400 power.

Measurements

Each stag was weighed weekly. Pedicle initiation was defined by using a pedicle detector, a device which indicates pedicle volume by water displacement (Li et al., '93b). This device has a sensitivity of 0.34 mm^3, which is effectively 0.7 mm of pedicle height. After pedicle initiation, defined as two consecutive measurements made with the detector 1 week apart which differed significantly ($P < .05$), the pedicle growth was measured weekly with a ruler.

Tissue collection

When the right (treated) pedicle reached about 6 cm in length, the stag was killed, and a 1-cm transverse section of tissue, inclusive of integument and bone, was removed from each pedicle 20 mm proximal to the growing tip. The tissues were handled, processed, incubated, and viewed using the same techniques as Li et al. ('93a). Briefly neurofilaments were localized using a specific antibody raised against a 200-kD neurofilament polypeptide and subsequent fluorescence viewed and photographed with a Zeiss Axioplan fluorescence microscope.

At slaughter, dissections were carried out on all the operated sites to determine the extent of any nerve regeneration. Pieces of tissue resembling nerve fibres were removed and fixed in 10% buffered formalin for subsequent histological examination.

Data analysis

ANOVA was carried out on live weight at pedicle initiation, the timing of pedicle initiation, pedicle height at the timing of tissue sampling, and pedicle growth rate. The main effects were side (treated compared with control) and denervation treatment; the interaction between the main effects was also considered.

RESULTS

Confirmation of denervation

Histological examination of all sensory nerves removed at the time of surgery indicated that sensory denervation was successful. In seven of the eight animals allocated to superior cervical ganglionectomy, surgery was successful, as determined by histology of the tissues removed, but in one animal from the SG/USX group a lymph node was erroneously removed instead of the superior cervical ganglion. Therefore data from that animal were added to the USX group and omitted from the SG/USX group.

Examination of the neurofilaments in the material sampled at slaughter revealed that antineurofilament antibody bound strongly and with good specificity to neural tissue in the skin and vascular layers of the pedicle sections. Nerve intact control pedicles (on the left of each animal) were characterised by many large bundles of nerves close to blood vessels and around hair follicles and sebaceous glands (Figs. 1, 2). All dener-

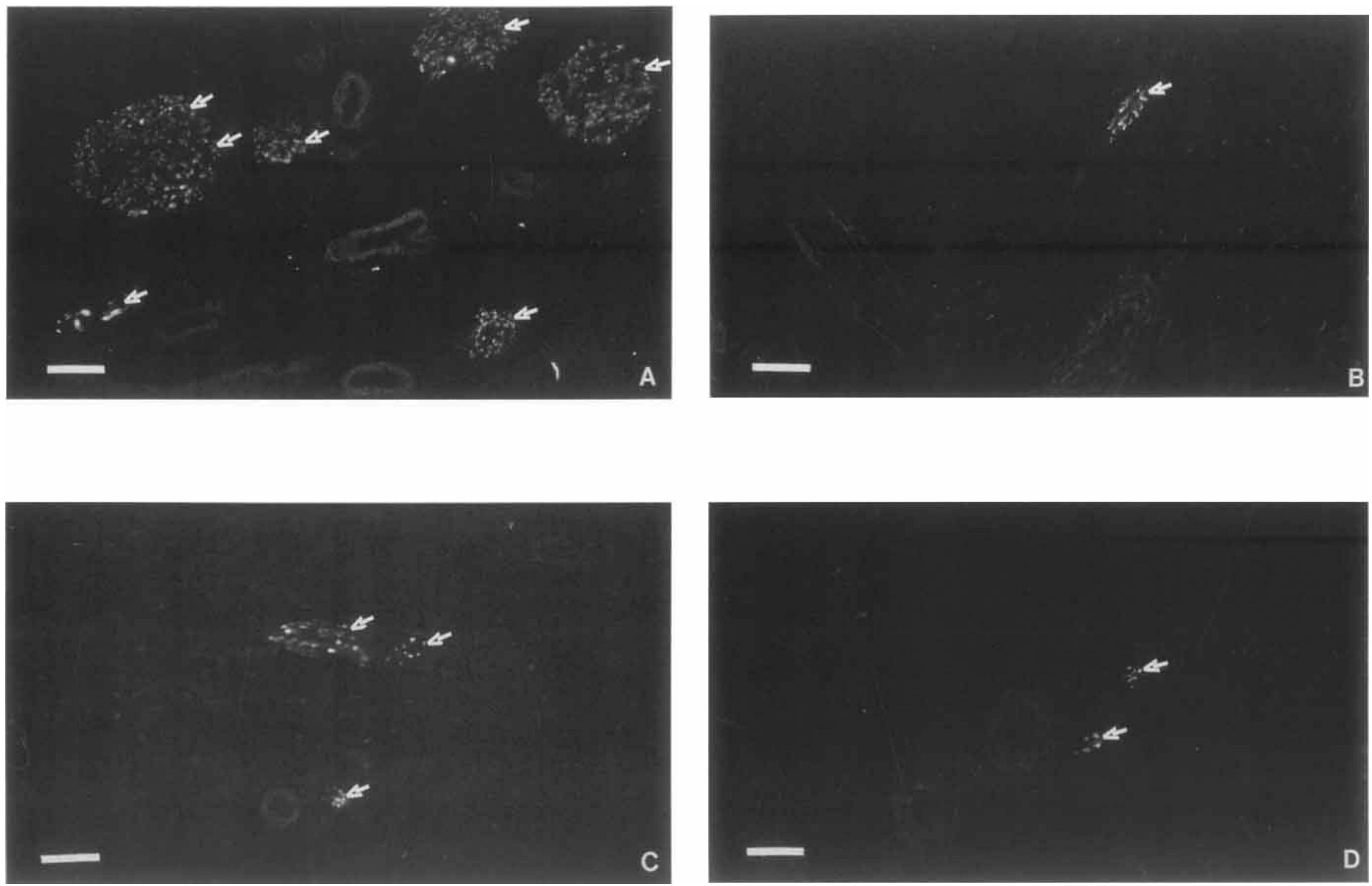

Fig. 1. Neurofilaments in the vascular layer of the pedicle. **A:** Left pedicle with all nerves intact. **B:** USX (sensory denervated pedicle). **C:** SGX (sympathectomised pedicle). **D:** SG/USX (sensory denervated and sympathectomised pedicle). Arrows indicate nerve bundles. Bar = 0.05 mm.

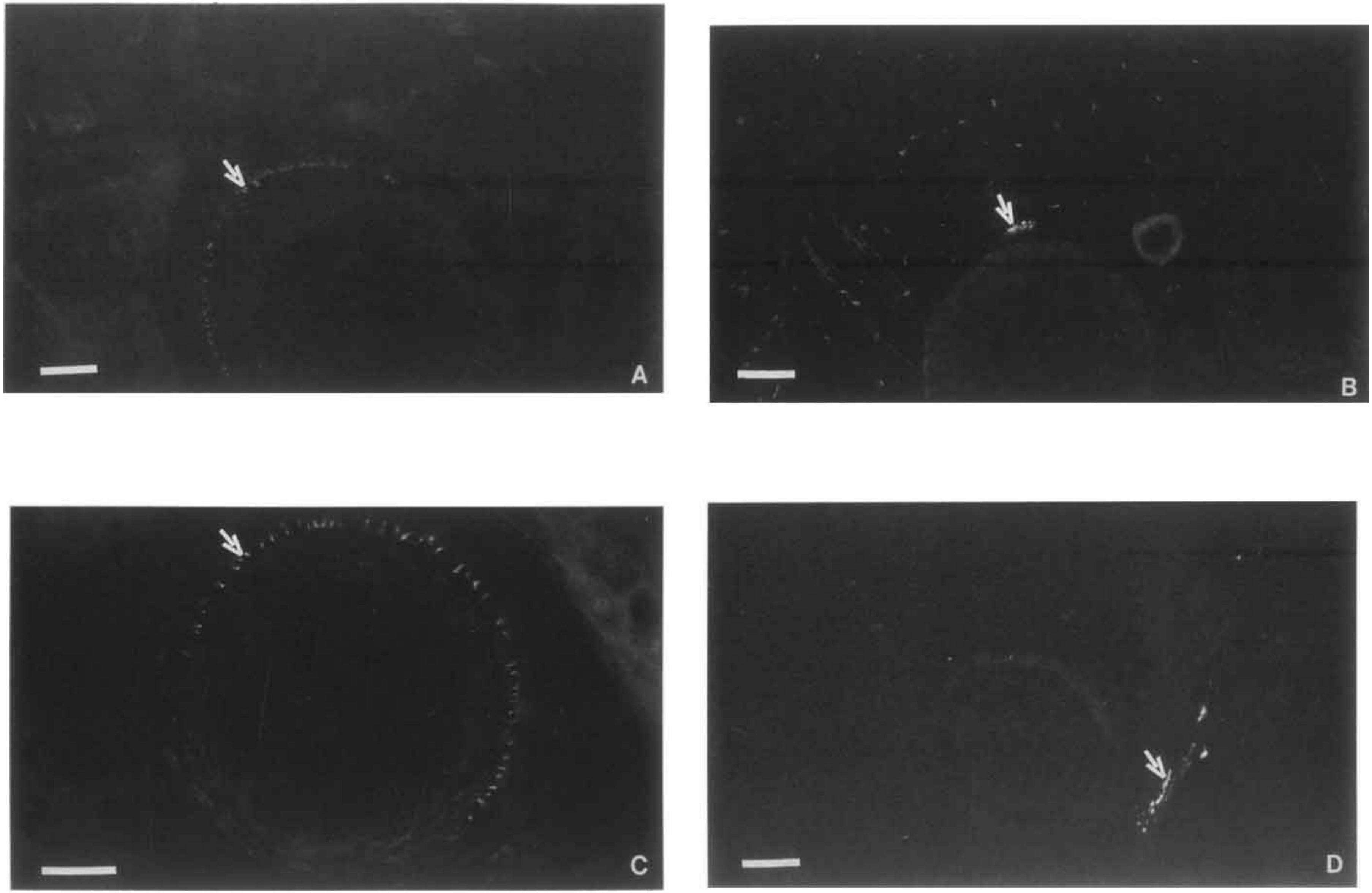

Fig. 2. Neurofilament surrounding the hair follicles in the pedicle integument. **A:** Left pedicle with all nerves intact. **B:** USX (sensory denervated pedicle). **C:** SGX (sympathectomised pedicle). **D:** SG/USX (sensory denervated and sympathectomised pedicle). Arrows indicate nerve bundles. Bar = 0.05 mm for panels A, B, and D and 0.03 for panel C.

vated pedicles lacked the large bundles of nerves close to the blood vessels, but in all sections a few fine fibres were present (Fig. 1). In the USX and SG/USX groups no fibres were observed around hair follicles or sebaceous glands, but in the SGX group such fibres were clearly present.

Nerves removed from all the operated sites had regenerated. Based on the histological examination, the extent of the nerve fibre regeneration was no more than 5% compared with the control tissues. In addition, no major tract of nerves regenerated. Only small fibres were encountered.

Effects of denervation on pedicle growth

Unilateral cranial sympathectomy either alone or with sensory nerve sectioning did not prevent pedicle growth, but nerve sectioning treatments altered some aspects of pedicle length and growth rate. The mean live weight at pedicle initiation for all stags was 56.1 kg, but among the groups live weight was significantly heavier in the SG/USX group compared with the SGX and USX groups, respectively, at this time; however, the weight at initiation of the two sides did not differ (Table 1). There were no significant differences either between the treatments or between the control and denervated pedicles in the timing of pedicle initiation or the duration of pedicle growth. In contrast there was a highly significant difference between the control and denervated pedicles in all groups, but not between the denervation treatments, in pedicle length at sampling and pedicle growth rate; denervated pedicles grew faster and were longer at sampling (Table 1). The difference in height between the control and denervated pedicles was 12, 17, and 22 mm (s.e.d. [standard error of the difference] = 3.5) for the USX, SGX and SG/USX groups, respectively, at the time of sampling. The pedicles from one typical deer from each group are illustrated in Figure 3. The typical USX stag had initiated antler growth from both pedicles at the time of sampling although the right (left side of figure) is larger. In contrast neither the typical SGX nor SG/USX stags had begun antler growth from the left (control) pedicle, but the advanced stage of development, in terms of antler initiation, defined as the emergence of velvet type hair, is clearly shown in the right denervated pedicle.

DISCUSSION

In the present study although sensory and sympathetic nerve removal appeared to be complete at surgery, a few fine nerve fibres were found in the denervated pedicles some 76 days later at the time of sampling. It is not known whether these fibres represent sprouting from other nerves, fibres remaining after surgery, or regeneration of nerves during pedicle growth. Sampling was planned to minimise the possibility of nerve regeneration but was arranged so that pedicle growth would be maximal. In a previous study Li et al. ('93a) found that pedicle growth in stags with no sensory nerves after sensory denervation did not differ significantly from those which had a few fine fibres and were consequently similar to the stags in the present study. It seems that substantial sensory nerve deprivation has similar effects to complete sensory nerve deprivation in terms of pedicle growth. It is unlikely that the sympathetic supply could regenerate because the superior cervical ganglion does not regenerate owing to removal of the cell bodies (Suttie et al., '84). Thus, although some fine fibres were present at sampling, the stags can be considered to have been functionally deprived of nerves to the pedicle at the time of initiation. Taken together the results reinforce the conclusion that nerves as a whole do not play a role in pedicle initiation and in fact seem to inhibit the rate of pedicle growth.

Bubenik ('82, '90b) considered that a functional neural link between the antlerogenic periosteum and a putative antler growth centre (AGC) situated in the central nervous system was a pre-requisite for pedicle initiation. However he acknowledged elsewhere in both above publications that the AGC and its neural link were highly speculative. In the present study, we set out to test whether a neural link was a pre-requisite and have shown that a substantial nerve reduction does not prevent pedicle growth. One possibility is that the neural link was established during pregnancy, when the foetal antlers form (Lincoln, '73) and subsequent removal of this link is without effect on pedicle growth. However, Bubenik ('82) states that a neural connection is required at the time of post-natal pedicle initiation and development. Thus, irrespective of whether a connection was present in the foetus we have shown in the present study that one is apparently not required at the time of post-natal pedicle initiation. Our data do not provide evidence for a fundamental neural link as a pre-requisite of pedicle growth.

In the present study the mean live weight at pedicle initiation, 56.1 kg, was close to the value of 56.3 kg reported for Invermay stags previously (Suttie et al., '91) but the precise live weight at pedicle initiation differed significantly with treat-

TABLE 1. Effect of denervation on pedicle growth parameters and live weight at pedicle initiation[1]

		Mean live weight at pedicle initiation (kg)		Timing of pedicle initiation (mean days from surgery)		Mean pedicle height at slaughter (mm)		Pedicle growth rate (mean mm/day)		Days of pedicle growth (mean days from initiation to sampling)	
	n	Control	Denervated	Control	Denervated	Control	Denervated	Control	Denervated	Control	Denervated
Sensory denervation (USX)	5	54	54	52	53	52	62	0.63	0.79	81	80
Sympathetic denervation (SGX)	4	57	57	63	62	45	61	0.62	0.86	72	73
Sensory and sympathetic (SG/USX)	3	58	59	52	57	32	54	0.45	0.77	77	73
s.e.d. (treatment)		1.0*		14.0		7.2		0.134		10.5	
s.e.d. (control/denervated side)		0.5		4.9		4.3**		0.074**		4.9	

[1]In all cases the control pedicles were on the left side and the denervated pedicles were on the right side. s.e.d. (treatment) refers to the standard error of the difference between denervation treatments and s.e.d. refers to the standard error of the difference between pedicles within treatments. *$P < 0.01$ and **$P < .001$, respectively; otherwise the differences were not significant. The treatment × control/denervated interaction was in no case significant.

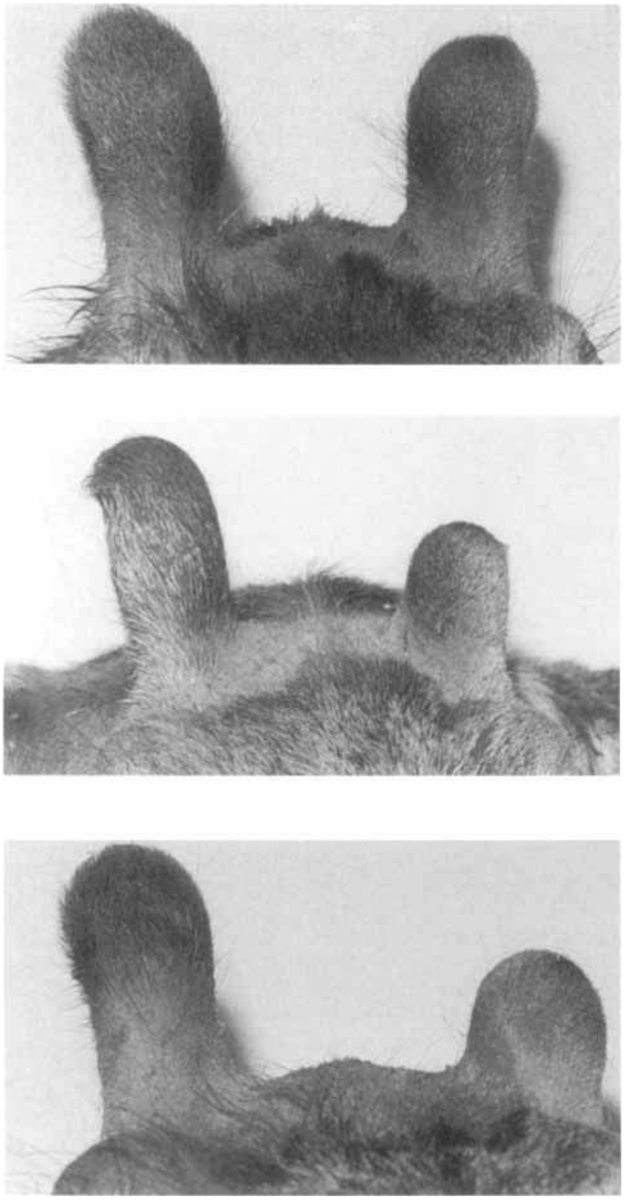

Fig. 3. Pedicles of typical stags from each group at the time of sampling. **Upper panel:** USX (sensory denervated pedicle). **Middle panel:** SGX (sympathectomised pedicle). **Lower panel:** SG/USX (sensory denervated and sympathectomised pedicle). The lack of hair on the frontal bones is due to shaving at the time of surgery. Note the lack of development of antler tissue on the left untreated pedicles as defined by a lack of velvet-like hair (to the right of each panel in the figure) compared with the right denervated pedicle.

ment. Generally stags which had had longer and more invasive surgery were heavier at pedicle initiation although the time from surgery to pedicle initiation did not differ significantly. There was a highly significant effect of denervation, USX, SGX, or SG/USX, on pedicle length at sampling and pedicle growth rate which was not observed by Li et al. ('93a) in a previous study. The present study differed in that the auriculo-palpebral branch of the facial nerve was also sectioned, the stags were housed outdoors on pasture rather than indoors on concentrates and hay, and a sophisticated pedicle detector (Li et al., '93b) was used to precisely indicate pedicle initiation. It is not thought that the sectioning of the auriculo-palpebral branch is responsible for the difference between studies because stags in the previous study which lacked sensory nerves, and presumably lacked a natural auriculo-palpebral supply to the pedicles, had similar length pedicles compared with stags which had some fine fibres. Likewise, the time taken to grow pedicles in the present study (76 days) was similar to the pedicle growth span in Li et al. ('93a) of 69–84 days. It is unlikely the improvement in precision of pedicle initiation detection could be responsible for the difference in pedicle size because both left and right pedicles were measured in the same way. However the growth rate of the denervated pedicles in the present study was 0.81 mm/day which is similar to the value of 0.75 mm/day presented previously by Li et al. ('93a) in pedicles following sensory deprivation. In the present study the control pedicles grew 0.58 mm/day. Thus, it appears that the control pedicles grew more slowly rather than that the denervated pedicles grew faster. In support of this, in Li et al. ('93a) the stags were kept indoors at a high plane of nutrition. In contrast, in the present study, they were kept outdoors in more standard husbandry conditions for deer, where the most rapid growth was not possible. As nutrition plays a role in pedicle and antler growth, it seems likely that a lower plane of nutrition could have led to slower pedicle growth. Any trophic effect of denervation could thereby have been unmasked by the reduced nutrition. It is possible that the mechanism of this trophic effect could be via stimulation of blood flow. Despite this enigmatic finding the main effect in the present study was that reduced innervation did not prevent pedicle growth.

Pedicle growth took place in the present study despite substantial sensory nerve reduction and sympathectomy. Suttie and Fennessy ('85) found that sensory nerve sectioning altered antler size, but no aspect of the timing of the antler cycle was affected. Li et al. ('93a) found that sensory nerve removal did not alter the timing of pedicle initiation or the emergence of the first antler from the pedicle. Taken together these findings imply that a continuous neural connection is not a pre-requisite for any aspect of the antler cycle but plays a role in the regulation of the size of the pedicle or antler or their respective growth rates. Hormonal systemic control systems, particularly androgens, are likely to exert a substantially more potent controlling influence on the timing of pedicle growth. Pre-pubertal castration prevents pedicle growth, but testosterone replacement causes a pedicle to develop (Jaczewski and Krzywinska, '74). Also plasma levels of testosterone are high during pedicle growth but decline during the growth of the first antler in intact stags (Suttie et al., '91). Although little is known about the mechanisms underlying the transformation of the pedicle to the first antler, the present study emphasises local control. Stags which had one pedicle in excess of 60 mm in length had visible antler, whereas the contra-lateral pedicle, if shorter, lacked visible antler tissue (Fig. 3). The identities of the local mechanisms are unknown. This also means that denervation did not alter the height of the pedicles at which the transformation from pedicle to antler occurs. We conclude that future hypotheses on the histogenesis of pedicles should centre around a systemic steroid control system with local modification but should not stress the importance of an intact neural connection with the central nervous system.

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