

Experimental Manipulation of the Neural Control of Antler Growth

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Introduction

The permanent pedicles and growing velvet antlers of deer are richly supplied with nerves. Wislocki & Singer (1946) pointed out that antler nerves were collaterals of nerve fibers from the pedicles, which were destroyed and regrown annually. This means that regenerating nerve fibers grow at up to 1 cm/day in red deer stags during the most active period of antler development.

This review considers direct manipulation of the nerves supplying the pedicle and antler and indirect manipulation by geophysical and traumatic forces.

Innervation of the Antler

The parasympathetic supply is derived predominantly from two terminal branches of the trigeminal (5th cranial) nerve. The supraorbital branch emerges over the dorsal rim of the orbit about 2 cm (in adult deer) from the medial canthus of the eye. The branches of the nerve pass through the orbicularis oculi muscle to supply the frontal region of the head. Major branches run caudomedially to innervate the rostral and medial aspects of the pedicle and antler. The zygomaticotemporal nerve travels caudolaterally through the periorbita to emerge from the caudal margin of the zygomatic process of the frontal bone. The nerve then passes caudodorsally through the retroorbital fat and beneath the frontalis muscle, being positioned ventral to the frontal crest and dorsal to an accompanying artery and vein, before dividing into several branches which disperse on the lateral and caudal aspects of the pedicle (Adams 1979). In some deer nerve fibers from the zygomatic branch of the auriculopalpebral nerve, itself a branch of the facial (7th cranial) nerve, innervate the medial aspect of the pedicle and antler (Adams 1979; Suttie & Fennessy 1985). The sympathetic supply to the pedicle comes from the second cervical nerve (Adams 1979; Rayner & Ewen 1981). The antler normally lacks sympathetic innervation (Wika 1980), the exception being the long-term velvet-antlers grown by castrated male deer (Rayner & Ewen 1981).

Direct Manipulation and Measurement

Antler Nerve Stimulation

Lake et al. (1982) applied a cathodal direct current to antlers of mule deer during the entire antler growth phase. The current ranged from 0.06–3.0 μA , with a voltage of less than 1 volt. Treated antlers were smaller than control antlers, showed abnormal branching patterns, and grew in atypical directions. Further, in three of the four treated deer, premature antler branching occurred; i.e., the antlers did not grow sufficiently to attain the species specific form. The authors concluded that long-term constant low-level DC application caused a reduction in longitudinal growth and altered normal morphology and direction of antler bone growth. In contrast, Bubenik, G. et al. (1982b) considered that Lake et al. (1982) had overstimulated the antlers and that this has been responsible for the reduction in bone growth. Bubenik, G. et al. (1982b) carried out a series of three experiments each on one white-tailed buck; in Experiment 1 they stimulated the lateral, and in Experiment 2 the medial branch of the supraorbital branch of the trigeminal nerve with square wave pulses (0.9 V, 1 msec duration, 175 pulses/min) while in Experiment 3, they used a cardiac pacemaker (5.4 V, 0.7 msec duration, 75 pulses/min) to stimulate the antler periosteum. Electrical stimulation of the lateral branch of the trigeminal nerve stimulated longitudinal antler growth and increased the number of points compared to the control unstimulated antler. The specific gravity values of both antlers from the stimulated buck were reduced compared to unmanipulated control bucks (1.27 for the stimulated, 1.32 for the control, and 1.5 for normal bucks). The stimulated antler was the normal shape for the species. In contrast, the buck in which the medial branch of the nerve was stimulated grew a pair of antlers that were of abnormal shape for the species; that is, stimulation of the nerves to one antler resulted in effects to the contralateral side. Both antlers were about the same size and of similar specific gravity to controls. It may be speculated that stimulation of the medial branch of the nerve to the antler was carried to the contralateral antler either by branches of the nerve crossing the midline or by conduction of the stimulatory current through the skin. Stimulation of the periosteum of the pedicle resulted not only in an increase in antler weight, length, and number of points on the stimulated side, but a gross departure from the species-specific shape and delayed antler mineralization. The stimulated antler grew at right angles to the normal (i.e., outward and downward rather than upward and inward). Remarkably, in subsequent years, despite lack of further stimulation, both antlers (previously stimulated and previously unstimulated) grew abnormally with the antlers curved downward rather than upward, but whether this was an effect of the electrical stimulation is not known.

Taken together, these results show that moderate electrical stimulation of antler nerves results in an increase in antler size and departure from the normal species-specific antler shape. Instances where contralateral antlers have been stimulated indicate that either nervous communication exists between antlers or

the stimulation has jumped from one to the other. If long-term poststimulation effects do occur, as is suggested from Experiment 3, it is probable that they reflect large-scale damage to pedicle nerves by the electrical treatment.

Antler Denervation

In the above section it was noted that stimulation of nerves leading to the antler resulted in larger antlers and a disruption of normal antler shape. What, then, are the consequences of depriving the antler of parasympathetic innervation?

Wislocki & Singer (1946) unilaterally exposed and sectioned the supraorbital and zygomaticotemporal branches of the trigeminal nerve leading to the antler in two adult white-tailed deer bucks before antler growth had begun. The control antler regrew normally, but the denervated antler was stunted and deformed because the bucks repeatedly injured the denervated antlers by rubbing them against foliage. The authors concluded that the reduction in antler size and the deformation were consequences of the rubbing due to insensitivity rather than a direct growth-reducing result of antler denervation. The bucks cleaned both antlers at virtually the same time, and there were no significant differences in casting date. This study shows that the trigeminal nerve carries sensory fibers to the antler but does not shed any light on whether nerves actually stimulate antler development.

If the single-spike antlers grown by yearling red deer stags are removed while they are still in velvet, a branched antler similar to that of a 2-year-old stag will often regrow, during the same season, from the cut stump. To test to what extent an intact nerve supply was necessary for this effect, Suttie & Fennessy (1985) used six 9-month-old stag calves, penned indoors and fed to appetite. At intervals from October–December (Southern Hemisphere), according to stage of growth, with the stags under general anesthesia, the antlers were unilaterally denervated using a method similar to Wislocki & Singer (1946) and both velvet-antlers were removed. Success of denervation was demonstrated histologically. Recovery from anesthesia and subsequent healing were uneventful. All stags regenerated their antlers, and on the day after antler-cleaning the stags were castrated to induce premature antler casting. The cast antlers were weighed and measured and the specific gravity determined. After antler casting, exploratory surgery was performed under general anesthesia and pieces of tissue were removed for subsequent histological examination, to determine the extent of any nerve regeneration. At the same time the antlers on the denervated side were removed without local anesthesia; significantly, one of the six stags reacted to amputation despite recumbance under general anesthesia. Subsequent histological examination of pieces of tissue removed at surgery revealed successful denervation in all six stags. It is likely that the antlers of the stag which reacted were innervated by a branch of the facial nerve—a rare individual variation in red deer—and consequently his data were omitted from further analysis along with those from another stag which had severely damaged his denervated antler, presumably due to lack of sensitivity.



FIGURE 1. Hard antlers grown by a 1-year-old red deer stag after denervation of the left antler and amputation of both previous antlers. Branched regrowth has occurred from both antlers. Although the left antler, to the right of the figure, is shorter than the right control, the brow tine is longer.

No substantial regeneration of nerve tracts took place, though small cutaneous fibers were located on final examination of the stags. The results are summarized in Table 1, and show that the denervated antlers were significantly smaller but of similar specific gravity to the control side, in marked contrast to the predenervation antlers where length and weight were very similar. The shape of the denervated antlers was also altered: the denervated brow tines were relatively longer and the main beams relatively shorter than the controls; that is, the main beam was less dominant in the denervated antlers (Fig. 1). The denervated antlers had fewer tines (1.3 ± 0.22 mean \pm sem) compared to control antlers (1.6 ± 0.35), but this was not significant. Both antler-cleaning and casting were unaffected by

TABLE 1. Primary data and mean \pm standard error of the mean for the six pre-experimental amputated segments and four experiment antlers.^a

	Pre-experimental amputated segments of antler			Experimental antler		
	Control	Subsequently denervated	Difference	Control	Denervated	Difference
Antler length (cm)	$\bar{x} \pm \text{sem}$ 22.0 \pm 3.4	20.9 \pm 2.8	1.08 \pm 1.08	35.5 \pm 5.4	24.3 \pm 5.5	11.3 \pm 3.4*
Antler weight (g)	$\bar{x} \pm \text{sem}$ 105.7 \pm 19.6	94.6 \pm 20.1	11.1 \pm 10.6	196.5 \pm 34.0	126.5 \pm 42.2	70 \pm 24.3*
Antler specific gravity	$\bar{x} \pm \text{sem}$ 1.07 \pm 0.01	1.06 \pm 0.01	0.003 \pm 0.02	1.39 \pm 0.04	1.36 \pm 0.07	0.035 \pm 0.031

^aStatistical comparisons (two-tailed paired t test) are between the differences in the means.

All antlers were amputated to 5 cm above the head.

*P < 0.05, otherwise differences are not significant.

TABLE 2. Antler growth rate and electric potential. Retabulated from Lake, Davis and Solomon 1982.

	Early (Apr-May)	Mid (Jun-Jul)	Late (Aug-Sept)
Antler growth rate mm/day	3.4 ± 0.4 ^a	4.0 ± 0.3	0.9 ± 0.2
Electric potential mV	-4.9 ± 0.7	-8.2 ± 1.4	-0.1 ± 0.6

^aMean ± standard error of the mean.

antler denervation. This study showed that although parasympathetic nerves are not necessary for antler regeneration, cleaning, or casting, they nonetheless perform a role in determining antler size and species-specific shape.

Although the antler itself lacks sympathetic (adrenergic) innervation, fibers have been located histologically in the pedicle. The antler can be sympathetomized by removal of the superior cervical ganglion (SCGX). However, Wika & Krog (1980) found that neither unilateral SCGX nor preganglionic cutting of the cervical ramus ganglionarius affected antler growth in reindeer. Lincoln (1984) found that although bilateral SCGX delayed antler growth during the first season after surgery (as the pineal gland had been denervated), there were no obvious abnormalities of antler size or shape.

It thus appears that only the parasympathetic nervous system exercises any control over growth of the antler. Denervation results in shorter, lighter antlers of the same specific gravity, with fewer points and with an altered shape, both in terms of relative size of points and their orientation. This is consistent with the postulate of Goss (1965b), who pointed out that if the eventual function of a structure does not require nerves, then likewise regeneration may be expected to be independent of innervation.

Bioelectricity and Antler Growth

Bioelectric potentials have been measured from a variety of regenerating tissues. In particular, amputation of urodele limbs evokes a large positive peak of voltage of +30 mV. This is followed by a change to -30 mV over the next 5 days. Potentials remain negative until regeneration is complete after about 40 days (Becker 1961). As the antler is an actively regenerating organ, knowledge of the bioelectric potentials associated with growing antlers could be valuable. In this respect, Lake et al. (1978) and Lake et al. (1979) have measured the potential difference between a reference electrode placed on the medial aspect of the antler base and a recording electrode placed on the top in mature male deer (80 recordings on 15 animals) under anesthesia. Clearly, rapid antler growth is associated with significant negative bioelectric potentials (Table 2). In a further study in July, the electric potentials were measured in 14 mule deer between the antler base as a reference and points along the growing antler (Table 3). The antler became significantly more negative with progression toward the tip.

TABLE 3. Potentials found along the antler in July. Retabulated from Lake et al. 1982.

Potential mV	Position of recording electrode from reference electrode in relation to total antler length			
	1/4 distance	1/2 distance	3/4 distance	tip of antler
	+0.6	+0.3	-2.3	-11.1

Taken together, these results show that electronegativity increases as antler growth rate increases, and decreases as antler growth rate declines, while less differentiated antler tissues at the tips are more negative than more differentiated tissues at the base. It is highly likely that these biopotentials play a significant role in initiating regeneration. Such biopotentials in regenerating urodele limbs are skin-driven and occur in the absence of nerves (Borgens et al. 1977). While electric potentials have not been measured in the absence of innervation in antlers, neither have electrical potentials been measured around the time of antler casting, healing, and early regeneration. It is hypothesized that electric potentials will be independent of nerves in the growing antler and that depolarization will take place around antler casting similar to that which occurs in an amputated urodele limb. Another possibility, which leads to an alternative hypothesis, is that the casting of a dead antler may not affect electric potentials, but cleaning of the velvet from the antler at the time of its death during the previous autumn might well cause depolarization. For this hypothesis, the stimulatory effects of depolarization for regeneration would have to be suppressed until antler casting. As casting is under hormonal control, can we invoke an endocrine control of biopotentials?

Leg Amputation

There is anecdotal evidence that the amputation of portions of hindlegs of stags leads to permanent antler asymmetry with the antler contralateral to the amputated leg being smaller and of abnormal shape (Goss 1983). Marburger et al. (1972) amputated the left hindleg of a 4.5-year-old white-tailed buck, who was in hard antler, 7.6 cm below the metatarsal joint. Subsequently, two similar bucks who previously had been subjected to partial tibial neurectomy were amputated in the same way. A single control was retained untreated. All bucks whose legs were amputated grew antlers on the contralateral side which were small and abnormal in shape. The tibial nerve sectioning had in itself no effect on antler shape or size but exacerbated the effect of amputation.

Davis (1982) reported two cases concerning the influence of hindleg amputation on antler growth in a sambar stag and in an Indian muntjak. The sambar stag had his leg amputated at the metatarsus as a result of an accident before he began any antler growth, and although both antlers grew, cleaned, and were cast in synchrony, the right was shorter and lighter than the left and lacked the characteristic shape of the species. The average ratio of right/left over the 5 years of the

stag's life was 0.37 for length and 0.31 for weight. For comparison, Suttie & Fennessy (1985) found that the ratio between denervated and control antlers in red deer was 0.69 for length and 0.59 for weight; thus it would appear that leg amputation may have had a more serious effect than specific antler nerve ablation on antler size. In the Indian muntjak which had the right tibia removed 2 months after birth, not only were its antlers shorter but its pedicle was also shorter on the left (contralateral) side.

What then are the lessons (if any) for the study of antlers which can be drawn from the effects of leg amputation on antler size and shape? Some authors (e.g., Fowler 1894) have concluded that antler abnormalities and leg damage are both common in deer and that it is natural for some deer to carry both injuries. Others, such as Morrison-Scott (1960), suggest that a deer licking wounds leaves the contralateral growing antler exposed to danger. Neither of these conclusions explain why perfectly shaped, albeit smaller, antlers are produced by leg amputees; the antlers are not obviously broken or damaged. Although caution is necessary, the following hypothesis might explain how the effects of amputation are mediated. Ascending spinal nerve pathways carrying pain impulses from peripheral tissues synapse with neurons in the grey matter of spinal cord. These axons cross the grey matter to the opposite side of the spinal cord. This axon then travels to the thalamus and synapses with neurons in the brain, which go to the cortex. If these neurons carrying painful stimuli from an amputated hind limb in any way influenced the trigeminal nerve innervating the antler, they would certainly influence the antler contralateral to that in the injured side. The questions remain, however—could communication between a sensory spinal neuron from the hind limb and a cranial nerve (part of the autonomic nervous system) take place at all, and could this communication result in antler size and shape abnormalities?

Indirect Manipulation

Geophysical Forces

Although the left and right antlers grown by deer are normally grossly symmetrical, the reindeer and caribou represent a departure from this norm. The brow tine of this species is a prominent palmate projection which normally only develops on one antler, the other being a simple narrow tine. However, the larger tine does not consistently grow on the left or the right antler, either between individuals or within individuals between years; it does not appear to be a genetically transmitted condition (Goss 1980; Davis 1982). In the Northern Hemisphere, in a survey by Goss (1980), 53% of deer had the left brow tine enlarged, 30% the right side, 15% had both enlarged, and the remainder had neither enlarged. Whereas in reindeer introduced to South Georgia in the Southern Hemisphere, 27% had the left brow tine enlarged, 39% had the right brow tine enlarged, while 9% had both and 26% had neither brow tine enlarged. Thus, the left tine appears dominant in

the Northern Hemisphere and the right dominant in the Southern. These observations have been used as evidence that geophysical forces operate on the antler (Davis 1983).

Trauma

There have been few, if any, scientific studies, using an adequate number of animals, concerned with the effects of trauma to the pedicle or antler on subsequent antler development. In addition, it is very difficult to separate the effects of damage to pedicle nerves from damage to pedicle blood vessels, skin, and bone, on antler development. Much of the literature on this subject relies on anecdotal or one-time experiments, which tend to make interpretation of any results difficult.

The Pedicle

Although the pedicle is indispensable for normal antler growth, there is abundant evidence that antlers, albeit of abnormal size and shape, may grow in male or female deer lacking pedicles and hence pedicle nerves (Goss 1961; Robbins & Koger 1981; Bubenik, G. et al. 1982b). Nonetheless, pedicle nerve stimulation and ablation (as has been considered in previous sections) alter antler size, shape, and departure from the species-specific pattern. Thus, two facts seem clear: nerves do not play a role in antler regeneration but they do play a role in determination of size and shape. Although pedicle damage may result in abnormal growth that season and perhaps for several seasons to come, homeostatic mechanisms tend to return the antler size and shape to the species-specific norm as soon as possible.

Antler

In that antler nerves are regenerated annually, it seems unlikely that permanent antler damage can occur due to trauma to a single antler. However, there is a possibility that antler damage can have longer-term effects. Bubenik, A. & Pavlansky (1965) cut the dorsal portion of the antler tip off a red deer stag when the antler was about 40% of its expected final length. Although the injury did not arrest growth, a slight scar remained at the wound site. In the next two antler cycles (but not the third), an additional dorsally pointed tine grew from the site of the former injury but all other tines grew normally in the species-specific way. The authors concluded that this was evidence for a memory of antler injury. They hypothesized that the antler trauma had effected semipermanent changes to a center of antler stimulation that was sited in the central nervous system (CNS). Bubenik, G. et al. (1982b) have extended this hypothesis. They observed the consequences in successive antler cycles of an injury to a growing antler of a white-tailed buck who accidentally split his growing antlers into three parts against a metal grid. The originally split antler became massively hypertrophied, as did the



FIGURE 2. A stag showing regrowth of velvet-antler, about 40 days after velvet-antler removal. The velvet-antler was removed 65 days after antler casting. Three weeks after velvet-antler removal, the stump had healed and regrowth around the lateral edge took place.

uninjured antler. In subsequent antler cycles this gross hypertrophy was maintained. Experimental induction, under deep anesthesia, of injuries similar to those sustained by the buck above failed to reproduce the hypertrophy. The authors claim that the damage to the putative antler growth center in the CNS is proportional to the state of consciousness of the deer, i.e., the pain felt. They state that if the damage to the antler is so severe then the antler growth center (AGC) will remember the effect and perpetuate it. No mention is made of possible anatomical or physiological mechanisms for this effect. The hypothesis—an interesting one—must remain unproven. The case may be that white-tailed deer differ from red deer in their responses to velvet trauma, but any mechanism requires further study.

Each year thousands of stags in New Zealand have their antlers removed approximately 60–70 days after antler casting or when they are about two thirds grown. This is to satisfy an oriental market for the tissue's medicinal properties. Antler removal, which is carried out under the supervision of a veterinarian, is always done on stags whose antlers are rendered insensitive to pain with

anesthetics. A tourniquet is applied around each pedicle and a butcher's bone saw is used to cut off the growing antlers some 2 cm above the coronet. Some bleeding may occur when the tourniquets are removed. The antlers frequently regrow (Fig. 2), often only from the lateral edge, where the major arteries and nerves are located. Velveting, as the process is called, does not interfere with cleaning or casting. It might then be thought that such repeated antler ablations would ultimately result in adverse effects in antler size and shape. This does not appear, however, to be the case. The Chinese have carried out repeated velvet-antler removal for centuries, and although until recently no anesthetics were used, no deleterious effects on subsequent antler growth have been noted.

Although no thorough studies have been carried out on the long-term consequences of repeated velvet antler removal. Suttie & Fennessy (1985) found that hard antler weight and shape in 2-year-old deer was not affected by whether velvet-antler was removed when the stags were 1 year of age or not. Each stag had had velvet-antler removed from one side only as a yearling, yet the 2-year-old antler weight was 448 ± 53 ($\bar{x} \pm \text{sem}$) for the previously amputated side and 472 ± 90 g for the control side ($n=12$). This tends to confirm the hypothesis that antler removal leaves antler size and shape unaffected.

Lincoln (1984) removed the growing velvet-antlers from three castrated stags at the line between the pedicle and the antler at about 2–4 monthly intervals for over 2 years. Regeneration always occurred and antlers grew to approximately the same size each time.

The influence of damage to antler nerves on subsequent regeneration is inconclusive, but conservatively it is suggested that the nervous control of antler growth rests solely in the nerves of the pedicle or surrounding area.

Conclusions

The preceding sections have shown by their paucity of hard data that any conclusions must remain conservative and tentative pending further study. Much information is anecdotal; many anomalies are to be found—it would be rather unwise to firmly formulate a hypothesis on this basis. With that in mind, my conclusions are as follows. The annual cycle of regeneration, cleaning, and casting of the antler is independent of innervation. Nerves influence antler size and shape. Stimulation of antler nerves leads to larger antlers while nerve ablation results in smaller antlers. Either way, species-specific shape is altered. Bioelectric potentials exist in the antler; these may be relevant with respect to regeneration, but further study is warranted.

There is no conclusive evidence that trauma to the deciduous portions of the antler which may result in permanent changes in size or shape of antlers are due to changes in nerve function. In contrast, trauma to the pedicle results in long-term changes in antler size and shape. It is suggested that a reason for this could be that the parasympathetic nerve supply has been altered. Antler nerve axons are unique in that they must suffer an annual dieback with a subsequent burst of

regrowth from the pedicle. In amphibians, the nerves are known to branch to reinnervate cut limb stumps and thus increase the nerve supply. Were this the case for antlers, it might explain why traumatization of the pedicle stimulates antler growth, by increasing or altering the absolute amount or pattern of the nerve supply to the antler. Whether the parasympathetic nervous system communicates information to the brain about damage to the antler and how this information is stored is unknown. It is not possible to deny the body of evidence collected by Tony and George Bubenik that injury without anesthesia during early antler development has long-term consequences for antler shape. However, strong, ethical experiments are required to fully document and investigate this effect.

The antler must have a genetically determined size and shape. It can be concluded that nerves play a role in controlling size and shape. Whether they operate directly in response to genetic influences or whether they operate via a complex series of growth factors remains to be elucidated. However, the role of local nerves in the pedicle is considered to be of paramount importance.