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Peripheral and Central Aspects of Trigeminal Nociceptive Systems

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Peripheral and central aspects of trigeminal nociceptive systems

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Three aspects of trigeminal pain are considered: the peripheral mechanisms of pain from teeth and from the cornea, and the role of the trigeminal brainstem nuclei in pain.

Pain is probably the only sensation that can be evoked by stimulation of dentine or dental pulp in man. Five nerve-endings enter dentinal tubules from the pulp but do not extend into the outer dentine, which is nevertheless sensitive. In teeth of limited growth in experimental animals, the dental pulp is supplied by Aβ, Aδ and C fibres and these are associated with two categories of receptor: one responds to cooling and to other stimuli that cause displacement of the contents of the dentinal tubules such as probing and drying the dentine, and the other group responds most vigorously to heating. Some cold sensitive units have Aβ fibres and the evidence suggests that stimulation of these is capable of evoking both muscle reflexes and pain and, near threshold, 'pre-pain' sensations.

Thermal stimulation of the cornea produces sensations of pain and, with less intense stimuli, irritation, Mechanical stimulation also produces pain but it is not clear whether, below the pain threshold, such stimuli produce touch sensation or some other sensation related to pain. Histologically, the nerve-endings in the corneal epithelium consist of fine, bare processes closely associated with the surface of the epithelial cells. Recordings in experimental animals have shown that many of the receptors respond to several different forms of stimulus and their properties correlate well with those predicted from psychophysical experiments in man.

The results of trigeminal tractotomy in man and recordings from the trigeminal brainstem nuclei in anaesthetized animals, have generally indicated that nucleus caudalis is the main relay in the pain pathway from the face and associated structures. Recent observations have, however, shown that tractotomy does not produce complete analgesia of this region and responses to thermal stimulation of teeth and noxious stimulation of other oro-facial tissues have been recorded from the more rostral parts of the brainstem nuclear complex. The surgical procedures employed to set up an animal for stereotaxic recording may induce long-lasting depression in the excitability of neurons in these nuclei, which masks some of their properties. The mechanism of this depression has not been established.

1. Introduction

The basic mechanisms of pain originating in the tissues innervated by the trigeminal nerve are probably not different to those involved in pain from other regions of the body. There are, however, certain features of this area that are of special interest for research on pain and I shall consider some recent work in relation to three of them.

(*a*) Pain may be the only sensation that can be produced by stimulating nerves in teeth. Hence all the afferent nerves in teeth may be associated with pain.

(*b*) The same may also be true of the cornea.

(*c*) Trigeminal primary afferents project to a column of nuclei in the brain stem and there is evidence that their main relay in the pain pathway is confined to the most caudal of these nuclei.

2. The innervation of dentine and pulp

Some teeth, such as the incisors of rodents, grow continuously throughout the life of the animal whereas others, including human teeth, are of limited growth. Both types contain a connective tissue core, the dental pulp, which receives its innervation through the foramen at the apex of the root. The pulp of continuously growing incisors has a very sparse innervation, consisting almost entirely of non-myelinated fibres (Bishop 1981), whereas the pulp of teeth of limited growth contains a much denser supply of both myelinated and non-myelinated fibres (Beasley & Holland 1978). In teeth of limited growth, most of the nerves which enter the apical foramen pass up through the root pulp to the pulp of the crown, branching as they go (Fearnhead 1961), and an unknown proportion enter tubules in the dentine. These nerves have their cell bodies in the trigeminal ganglion. In the cat, their peripheral conduction velocities range from less than 1 m s^{-1} to almost 60 m s^{-1} with a large proportion in the Aβ range (Cadden *et al.* 1982, 1983; Nähri *et al.* 1982*c*).

(*a*) *Human studies*

The most obvious function associated with nerves in teeth in man is pain, although it has not been clearly established that this is their only sensory function (Cadden *et al.* 1982). There is no conclusive evidence that sensations of warmth, cold or touch can be evoked by stimuli that can excite only nerves within the teeth, although there is some evidence that subjects can differentiate between hot and cold stimuli (Grüsser *et al.* 1982). Electrical stimulation of intradental nerves at and just above sensory threshold is usually not painful, although the sensations produced are often difficult to describe and do not provide firm evidence for a separate functional role for the lowest threshold fibres.

The dental pulp of human permanent teeth is exquisitely sensitive and for this reason has not been subjected to extensive sensory testing. However, from clinical experience it is known that even very gentle mechanical stimulation of it produces severe pain.

Human dentine, when it is exposed by removing the overlying enamel in the crown of a permanent tooth, is sensitive to several different forms of stimulus, of all which produce pain (Anderson *et al.* 1970). Such stimuli include probing and drilling, heating and cooling, drying the dentine surface, applying solutions of high concentration (irrespective of their chemical composition), and large changes in hydrostatic pressure above or below atmospheric. Dentine appears to be sensitive throughout its thickness to these stimuli, and clinical experience indicates that it may be more sensitive just beneath the enamel and near the pulp than at intermediate levels. Topically applied local anaesthetics do not abolish its sensitivity and solutions such as bradykinin, which cause pain when applied to other tissues, are not painful when placed on dentine. Inflammation of the pulp is associated with increased sensitivity of the overlying dentine.

Enamel is insensitive, although thermal stimuli applied to the tooth surface will produce pain by their effect on the underlying dentine or pulp.

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(*b*) *Tooth pulp stimulation in the rat*

Nothing is known of the sensitivity of dentine and pulp in teeth of continuous growth, although electrical stimulation of intradental nerves in these teeth evokes a reflex response in the digastric muscle (Jiffry 1981). It is extremely difficult technically to excite intradental nerves in rat incisors without running the risk of also exciting nerves in the periodontal or gingival tissues by stimulus spread (Hayashi 1980; Jiffry 1981; Toda *et al.* 1981; Engstrand *et al.* 1983). For both of these reasons, investigating responses evoked in the central nervous system by electrical stimulation of rat incisors is not a satisfactory way of studying the mechanisms of pain.

(*c*) *Histology of dentine*

The receptors responsible for the sensitivity of dentine in teeth of limited growth have not been identified. Each dentinal tubule contains the process of an odontoblast, whose cell body lies at the opening of the tubule at the pulp surface. Some tubules also contain nerves: this was first demonstrated unequivocally by Fearnhead (1957, 1961) in light microscope studies of silver-stained sections of decalcified, human teeth. These nerve terminals were approximately 0.2 μm in diameter and had a beaded appearance. Because the tubules are not straight, he was unable to follow them in individual sections for more than 0.4 mm into the tubules. Nerves entered up to 8% of tubules in the crown (the highest density being over the pulpal cornua under the cusps), about 1% of tubules near the neck of the tooth, and only very rarely in root dentine. These results were obtained in fully formed teeth; no nerves were found in the tubules of coronal dentine before root formation was complete. It is not known what proportion of nerves present in these preparations would have been stained and resolved by the light microscope, but the distribution found does not correspond with clinical findings on the sensitivity of dentine to cavity preparation. Root dentine is sensitive to stimulation (Lilja 1980), and coronal dentine of permanent molars is sensitive before the roots are complete.

When it became possible to examine sections of dentine in the electron microscope, it was hoped that these problems would be resolved, but the position is still far from clear. As the nerve fibres approach the dentine from the pulp they lose their investing myelin sheaths and Schwann cells, and in dentine cannot be identified on the basis of ultrastructural criteria alone. At their pulpal ends, some dentinal tubules contain just a single odontoblast process while others contain a process of about the same size and structure but accompanied by one or more smaller processes, some of which have a structure similar to that of the odontoblast processes (Holland 1981). Such structures have been found in the inner dentine of teeth of limited growth from several different species, including man (e.g. Byers *et al.* 1982; Gunji 1982). Until recently, it has not been known which, if any, of these smaller processes were nerve fibres and which might be of other cells, possibly branches of odontoblast processes. Arwill *et al.* (1973) found no secondary processes in cat dentinal tubules 2–4 weeks after the teeth had been denervated, whereas in a similar study Frank *et al.* (1972) were able to find small processes 2 months after denervation, although by then there is likely to have been some reinnervation of the teeth (Robinson 1981). The interpretation of the results is further complicated by the fact that, in normal cat teeth, the proportion of tubules in the inner dentine that contain secondary processes varies between 0 and 45%; some of the variability being between teeth and some between areas within a tooth (Holland 1981). When such quantitive data were obtained for denervated teeth, it was found that 2 days after the nerve section,

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secondary processes were found only extremely rarely compared with contralateral controls, and after 12 weeks they had returned in 25–60% of tubules in the predentine over the pulp cornu (Holland & Robinson, unpublished observations). Also, at 12 weeks, responses could be recorded from some intradental nerves when dentine was stimulated (Matthews & Robinson 1983). It seems likely, therefore, that all the secondary processes in normal dentinal tubules are of nerves. In the canine teeth of adult cats, their incidence is highest over the pulp cornu, decreases down the side of the crown and is very low in the root (Holland 1981): results which are similar to those obtained by Fearnhead in human teeth (see above). It would be of interest to have quantitive data on the incidence of secondary processes in human dentinal tubules and to know how the incidence changes with age in man and cats.

Much less is known of the contents of the dentinal tubules further away from the pulp in these teeth. Transmission electronmicroscopy of dentine indicates that the outer one half to two thirds of each tubule contains no live cell processes (Holland 1975, 1976; Thomas 1979; Thomas & Payne 1984). However, with alternative techniques such as scanning electronmicroscopy (Kelly *et al.* 1981; Grossman & Austin, 1983; Crookes *et al.* 1983) and by examining the dentine after digestion of some of the extracellular matrix (Yamada *et al.* 1983; Gunji & Kobayashi 1983), evidence for processes extending out as far as the enamel/dentine junction has been obtained. These structures may, however, be merely cellular debris and cannot be identified as normal odonoblast processes without details of their internal structure.

Axonal transport techniques have also been used to investigate the extent to which dentined is innervated. Tritiated proline injected into the trigeminal ganglion of rats has been shown to be transported along nerves into the pulps of the teeth and into the inner dentine of the molars, which are teeth of limited growth, and similar results have been obtained in cats (Byers & Matthews 1981) where the distribution of the labelled material through the dentine was similar to the distribution of secondary processes in the tubules (Holland 1981).

(*d*) *Electrophysiological studies*

Electrophysiological experiments have also been carried out on cats and dogs to study the properties of the receptors in teeth of limited growth. Recordings have been made from the nerves which supply pulp and dentine by either isolating such fibres in filaments dissected from nerve trunks outside the teeth, or by using electrodes in contact with coronal dentine (e.g. Horiuchi & Matthews 1974). With this latter technique, multi-unit records can be obtained from just the larger diameter axons in the crown of a tooth. The success of the method seems to depend upon the electrical characteristics of dentine, which is essentially a series of parallel biological microelectrodes directed towards the pulp. The most favourable recording conditions would apply to any nerves which entered the tubules under an electrode. The usefulness of the technique is limited, however, since it cannot be used to follow satisfactorily the activity of single sensory units, does not resolve impulses in the smallest myelinated or non-myelinated fibres, and, because stimuli are applied close to the recording electrodes, is prone to artefacts which are sometimes difficult to differentiate from action potentials.

From experiments in the cat (Kollmann *et al.* 1982) it appears that there may be just two main categories of receptor in dentine and pulp: one group characterized by their short latency responses to cooling and the other by their greater sensitivity and longer latency response to heating. In addition to thermal stimuli, the cold-sensitive units usually responded to drying and mechanical stimulation of exposed dentine and sometimes to the application of high

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concentration solutions to the dentine and to pressure changes of 500 mmHg† above or below atmospheric. The hot-sensitive units were generally much less responsive to stimuli other than heating. The cold-sensitive units had conduction velocities over the upper part of the range of intradental nerves, including Aβ fibres, whereas the hot-sensitive units had conduction velocities at the lower end of the range. Since not all the units in each group were found to have the same properties, this classification may be an over-simplification. However, some if not all of the variability between units within a class could be accounted for by differences between the location of the receptors in relation to the site of application of the stimuli. Also, it has not been possible to test each unit with all of the stimuli under optimal conditions. Nähri *et al.* (1982*a*, *b*, *c*) have also described units with similar properties in the dog and cat.

A very significant step forward in our laboratory in carrying out such experiments was our finding that we blocked the exposed ends of dentinal tubules when we cut dentine with a very slowly running bur under Ringer solution, and that by etching the cut surface with acid we could remove these obstructions and produce a very large increase in the sensitivity of units to mechanical stimulation of the dentine. It was already known that high-speed drills left the tubules obstructed, but we wrongly assumed that our much more gentle treatment of the dentine would not have this effect. We were misled in this by finding that, in cats, dentine exposed by fracturing was not appreciably more sensitive to mechanical stimulation than that exposed by gentle drilling, and by previous experience, which indicated that human dentine exposed with a slow-running bur was sensitive to mechanical stimulation and the application of high concentration solutions.

The responses evoked in the cold-sensitive type of unit in the cat canine by mechanical stimulation of dentine are of particular interest. As indicated above, these units are not readily excited by mechanical stimulation of dentine that has been exposed to drilling. Etching the dentine with acid increases enormously the sensitivity of the units to mechanical stimulation, and even gentle contact with the fire-polished tip (diameter 0.5 mm) of a fine glass probe is sufficient to excite some units. By using such a probe to test, after etching, the circular area of dentine that is exposed by removing the terminal 1.0–1.5 mm from the tip of the crown of a canine tooth, it can be shown that units can be excited by stimulation of dentine at all levels from close to the enamel–dentine junction to near the pulp. We initially etched the dentine with a 30 s application of a 30% orthophosphoric acid, but have recently shown that a similar effect can be produced by much lower concentrations of acids applied for the same time. Partial sensitization can be produced by either 0.5% orthophosphoric acid or 2% citric acid and double these concentrations have essentially the same effect as 30% orthophosphoric acid. When the dentine was examined in the scanning electron microscope it was found that the treatments that caused partial sensitization also caused partial unblocking of the dental tubules (Matthews, Pamplin & Shellis, unpublished observations). The lack of response of units to mechanical stimulation of fractured dentine mentioned above appears to be due to the roughness of the hard dentine surface and a lack of intimate contract between it and the glass stimulating probe, since the sensitivity of the preparation can be increased by etching to a similar degree as that obtained with drilled dentine. The response evoked by mechanical stimulation of etched dentine is stable over many minutes, indicating that the acid has not damaged the receptors, and is not abolished by topical application of local anaesthetic (2% lignocaine) to the dentine surface for 2 min.

† 1 mmHg ≈ 133.3 Pa.

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(*e*) *Receptor mechanisms*

The observations described above suggest that the response of the receptors in cat teeth to mechanical stimuli is caused by displacement of the contents of the dentinal tubules. Each of the other stimuli that excite the cold-sensitive group of units would also be expected to cause some displacement of tubule contents and this is therefore a possible common mechanism of action of them all. It has been thought for many years that the sensitivity of human dentine might depend upon some form of 'hydrodynamic' mechanism (see Anderson *et al.* 1970). The receptors would, however, have to be very sensitive to respond to the very small disturbance set up by gently touching the dentine surface with a fine glass probe. The location of the receptors has not been determined. The histological evidence suggests that there are no nerves in the outer dentine, but if the receptors are excited indirectly by the movement of tubule contents they do not have to be present throughout the tissue to account for its sensitivity; they might be associated with the nerve endings at the pulpal ends of the dental tubules or possibly those near the odontoblast cell layer of the pulp. The properties of the heat-sensitive units suggest that their receptors may be similar to heat-sensitive nociceptors in skin and located either near the pulp surface or in the inner dentine.

There is no evidence at present to implicate the odontoblast directly in the receptor mechanism, although this possibility should not be overlooked, particularly if it is confirmed that odontoblast process extended into the outer dentine.

(*f*) *Discussion*

Intradental nerves with the properties described above could account for the observations that have been made on pain from dentine in man. They could provide the information required to distinguish between a hot and cold stimulus, particularly if the subject knew that a response was due to one or the other (Grüsser *et al.* 1982), but it seems unlikely that they could support sensations of cold, warmth or touch. An electrical stimulus of an intensity that will excite just the lowest threshold pulpal fibres (i.e. cold-sensitive units with Aβ conduction velocities), can evoke a jaw-opening reflex in the cat (Robinson 1981). In man, a stimulus of equivalent intensity evokes a similar reflex and and a sensation (Matthews *et al.* 1976; McGrath *et al.* 1981). The sensation at threshold is not usually described as painful but has a pulsing, throbbing or pricking quality. Pain is always produced when the stimulus is more intense. These observations suggest that, if the nerves in human teeth have properties similar to those in the cat, the fibres responsible for the 'pre-pain' sensation produced by an electrical stimulus near threshold have fast (Aβ) conduction velocities and are of the same type that produce pain when dentine is subjected to stimuli such as cooling, probing and drying. The synchronous nature of the discharge evoked by the electrical stimulus and the lack of spatial summation could account for the fact that the sensation produced is often of an unfamiliar type.

The accumulated evidence creates a picture of a very complex receptor mechanism in dentine, more complex than is usually associated with nociceptors that detect damaging or potentially damaging stimuli in other tissues. It would be surprising if some of the stimuli that excite intradental receptors were to cause any tissue damage, particularly mechanical stimulation with a fine glass probe. The advantage to an animal during evolution of having receptors that could respond to small movements of the contents of the dentinal tubules may

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have been in detecting tubules that had been opened at their outer ends as a result of rapid attrition or superficial fracture of a tooth. Under these conditions, the receptors would respond vigorously to mechanical stimulation of the dentine. The open tubules would allow bacteria and toxins to gain access to the pulp where they might produce infection and the loss of the tooth. The primary function of the receptors may thus have been to initiate a change in the animal's chewing pattern to reduce use of the affected tooth until reparative dentine was laid down to close the tubules and reduce the risk of infection becoming established. The finding that nerves in dentine are mainly found under the cusps is consistent with this explanation.

3. Pain and the innervation of the cornea

(*a*) *Sensations evoked by corneal stimulation*

The question of whether or not pain is the only sensation that can be evoked in man by stimulation of the cornea has been debated over many years and the evidence was reviewed in 1956 by Lele & Weddell. They concluded that touch, warmth and cold could be perceived as well as pain. More recent experiments with thermal stimuli have shown that both warming and cooling produce sensations of irritation or pain, but no sensations of warmth or cold, provided that the adjacent tissues are protected from the stimuli (Kenshalo 1960; Beuerman *et al.* 1977; Beuerman & Tanelian 1979). With rapid heating from an adapted temperature of 33 °C, the threshold for discomfort was approximately 37 °C and for cooling, 31 °C, (Beuerman *et al.* 1977; Beuerman & Tanelian 1979). Although cold stimuli down to 26 °C caused irritation, they were not described as painful. The pain threshold with heating was about 42 °C. Mechanical stimulation with fine filaments produces a non-painful sensation at stimulus intensities below the pain threshold and the sensory thresholds determined with filaments contacting but moving across the cornea were approximately 20–30 mg (Lele & Weddell 1956; Schirmer 1963; Millodot 1973). It is not clear whether this non-painful sensation is touch, or similar to the sense of irritation produced by thermal stimuli.

(*b*) *Histology*

Both myelinated and non-myelinated nerves enter the stroma of the cornea and end in non-myelinated terminals in the outer part of the stroma and in the epithelium (Zander & Weddell 1951; Whitear 1960) The terminals show little evidence of structural specialization and in the epithelium are not ensheathed by Schwann cells, but lie in grooves in the surface of the epithelial cells (Whitear 1960). Conduction velocity measurements indicate that some of the nerves supplying the cornea have non-myelinated, C-fibre axons and others have myelinated, Aδ axons.

(*c*) *Electrophysiology*

Electrophysiological studies in the cat by Lele & Weddell (1959) showed that the majority of the Aδ fibres from the cornea respond to both mechanical and thermal stimuli. Belmonte & Giraldez (1981) also recorded from Aδ fibres in the cat and they selected units for study that responded to mechanical stimulation of the corneal surface with a moist brush. All but a few of these units also responded to heating the cornea. They had thresholds between 38 and 46 °C and showed increasing responses up to 50 °C, the highest temperature tested. Some also responded to cooling or chemical stimulation. Their mechanical thresholds, tested with fine filaments, were between 11 and 100 mg. These units showed many of the properties of

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cutaneous polymodal nociceptors, including sensitization or depression of their response following repeated stimulation with noxious heat.

Tanelian & Beuerman (1984) caried out similar experiments on the rabbit cornea, except that units were selected by using both thermal and mechanical stimuli. The maximum temperature they applied was 43 °C and probably on account of this, and the consequent reduced thermal sensitization of units, they found that the majority of mechanosensitive units did not respond to thermal stimuli: only 13 out of 96 responded to both mechanical stimulation and warming. They also found some units that responded only to cooling, giving a vigorous response when the corneal temperature fell from 33 °C, but none that responded only to heating. The mechanosensitive units all had Aδ conduction velocities and the cold-sensitive units were all C fibres.

(*d*) *Discussion*

The data show that there is more than one class of receptor in the cornea. It remains to be established whether all these receptors are concerned only with pain and irritation. It may be that a low rate of discharge in the afferent fibres causes the feeling of irritation and that higher frequencies produce pain. It would be interesting to know what sensations are produced by electrical stimulation of the cornea and how these compare with those produced by electrical stimulation of dental pulp.

4. The role of the trigeminal brainstem nuclei in pain

Trigeminal primary afferent fibres project to a column of nuclei in the brainstem consisting of the trigeminal main sensory nucleus and the spinal nucleus, the latter being subdivided into three subnuclei: n. oralis, n. interpolaris and n. caudalis. There is a considerable body of evidence, based largely on the effects of lesions of the spinal trigeminal tract in man for the treatment of trigeminal neuralgia, that n. caudalis is the main site of relay in the pathways responsible for pain from the face and jaws. There have also been numerous reports of neurons in n. caudalis which respond to noxious stimulation of the face and mouth (Dubner *et al.* 1978). Our recent experiments suggest, however, that the more rostral parts of the complex may also make an important contribution to pain in this region, supporting the findings of Eisenman *et al.* (1963), Young (1982) and Azerad *et al.* (1982).

It is known from anatomical studies (Westrum *et al.* 1980; Arvidsson & Gobel 1981) and from the results of antidromic stimulation in the brainstem (Matthews & Lisney 1978) that the afferent fibres from tooth-pulp terminate at all levels of the ipsilateral trigeminal brainstem nuclei in the cat. It has also been shown frequently that responses can be evoked from neurons at all these levels by electrical stimulation of tooth pulp in the cat, although the representation in n. interpolaris seems to be rather small (see Clarke 1984). Of particular interest has been the projection of tooth-pulp afferents to the more rostral nuclei in view of the evidence that pain is the only sensation that can be evoked from tooth-pulp and that pain pathways project through n. caudalis. In an attempt to throw further light on the significance of this arrangement we set out to investigate how neurons at different levels of the brainstem nuclei responded to the application to dentine of a range of different stimuli, such as those we had used in studies on the primary afferent (see above). Previously responses had been recorded with only electrical stimulation of tooth-pulp.

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In preliminary experiments we found that stimuli that would produce a vigorous response in some primary afferents in a tooth were generally ineffective in eliciting responses from the brainstem neurons. The experiments were carried out in anaesthetized cats set up in the normal way for stereotaxic recording. At about the time that we were doing these experiments we were also standardizing the methods we used for applying electrical stimuli to tooth-pulp (Cadden *et al.* 1983) and we realized that the electrical stimuli we often had to apply to tooth-pulp to drive brainstem neurons would be exciting the majority of the myelinated fibres in a tooth, indicating that a large amount of summation was required to excite the central neurons. This explained why we had little success with non-electrical stimuli.

In other experiments taking place in our laboratory it was found that, by comparison with these results, much less intense stimuli were generally required to evoke a jaw-opening or digastric reflex by electrical stimulation of tooth-pulp in lightly anaesthetized cats that had been subject to a minimum of other procedures (Robinson 1981). Under those conditions it was clear that brainstem neurons involved in the reflex were being excited by activation of a much smaller proportion of tooth-pulp afferents than in the previous studies. We therefore carried out some experiments to determine if it was the period of anaesthesia or something else that was responsible for the apparent change in the excitability of the central neurons.

(*a*) *The effect of remote noxious stimulation on the threshold of the jaw-opening reflex evoked by tooth-pulp stimulation*

Cats were anaesthetized with a continuous intravenous infusion of the steriod anaesthetic alphaxalone–alphadolone (Saffan, Glaxo Laboratories). This was chosen because it had been used by Robinson and also because it permits rapid changes to be made in the depth of anaesthesia, by varying its rate of administration. In cats that had been subjected only to the minimum surgical preparation necessary for stimulation of the canine tooth and to record the e.m.g. from the digastric muscle, it was found that the jaw opening reflex threshold usually remained at a stable, low level throughout long periods of anaesthesia and was relatively little affected by changes in the level of anaesthesia (Clarke & Matthews 1984*b*). However, the application of high intensity electrical stimuli (6 mA, 1.0 ms, 10 s^{-1}) for 5 min to a foot-pad, or pinching it intensely, caused an increase in the threshold of the reflex, provided that the animal was not at a very light level of anaesthesia. The increase in threshold occurred if the depth of anaesthesia was such that the conditioning stimuli to the foot did not themselves evoke a flexion–withdrawal reflex. The threshold increase was maintained unless the depth of anaesthesia was reduced, when the threshold fell, and it returned to its previous level on deepening the anaesthesia unless the animal was first allowed to recover consciousness fully.

When the jaw-opening reflex threshold was monitored in cats, which were being set up for stereotaxic recording from the brainstem and which were deeply anaesthetized with alphaxalone–alphadolone, it was found that the threshold tended to increase progressively with each stage of the procedure that involved any noxious stimulation (Clarke & Matthews 1984*b*). Insertion of the ear-bars was a particularly strong stimulus in this respect. The cumulative effect of these procedures was that the threshold increased from what was usually a single stimulus of 0.1 ms duration and less than 100 μA intensity, to a stimulus of 1.0 ms duration and around 1 mA intensity. The increase was maintained for several hours after the preparations were complete. In subsequent experiments (Clarke & Matthews 1984*a*) it was shown that the abrupt increase in jaw-opening reflex threshold, which usually accompanied

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insertion of the ear-bars, was not associated with a change in arterial blood pressure, or a change in end-tidal CO_2 or blood gas concentrations. Also, the threshold of the jaw-opening reflex evoked by stimulation of the periodontal mechanoreceptors did not increase at the same time as that of the responses to electrical stimulation of the pulp of the same tooth. It has not been possible to reverse the increase in threshold to tooth-pulp stimulation with the opiate antagonist naloxone (1–5 mg kg^{-1} i.v.) or in any reliable way by making electrolytic lesions in the periaqueductal grey matter, raphe nuclei or brainstem reticular formation (Clarke, unpublished observations).

(*b*) *Responses of trigeminal brainstem neurons to stimulation of tooth-pulp and other oro-facial tissues*

In view of these findings, we investigated the thresholds of brainstem neurons to electrical stimulation of tooth-pulp in animals that had been set up for recording with precautions to minimize the stimulation of nociceptive inputs to the central nervous system during the immediately preceding period. Some cats were prepared a few days previously, when a chronic recording chamber with provision for head fixation was attached to the skull. These animals could subsequently be anaesthetized and recordings made with minimal additional trauma. Recordings were also made in some acute preparations in which surgery was kept to a minimum, local anaesthetic was applied to the skin where incisions had been made, and shortened ear-bars were used to fix the animal's head in the stereotaxic frame. Under both conditions the thresholds of units at all levels of the brainstem trigeminal nuclear complex were found to be lower than those obtained in animals prepared in the conventional manner (Clarke & Matthews 1983*a*).

These observations showed that noxious stimulation of anaesthetized cats was capable of causing a long-lasting and profound depression in the excitability of at least some of the brainstem neurons with an input from tooth-pulp, although we have so far been unable to identify the cause of this effect.

In recent experiments, we have investigated whether this effect may have caused some of the properties of brainstem trigeminal neurones to have been masked in earlier studies. Recordings have been made from these neurons in cats prepared acutely with the precautions outlined above. Under these conditions, it has been found that cells in n. caudalis, n. oralis and the main sensory nucleus respond to thermal stimulation of the terminal 3–4 mm of the crown of the ipsilateral canine teeth (Clarke & Matthews 1983*b*). The stimuli are applied by passing Ringer at 30, 50 and 10 °C for up to 5 s each through a cap attached to the tooth (Kollmann & Matthews 1982). Many of these neurons, at each level in the nuclear complex, also receive inputs from low threshold mechanoreceptors in adjacent areas of the mouth and the skin of the face. In addition, some respond vigorously to noxious mechanical stimulation of oral and facial tissues, and can often be excited by mechanical stimulation of the cornea and nasal mucous membrane. Some respond just to tooth-pulp stimulation and others to both tooth-pulp stimulation and the other stimuli that would be painful in man (Campbell & Clarke, this symposium).

These results suggest that parts of the trigeminal brainstem nuclear complex other than n. caudalis may play an important role in pain from the face and jaws. This conclusion is consistent with the results both of Azerad *et al.* (1982), who also recorded responses to noxious stimulation in n. oralis of lightly anaesthetized cats, and of Young *et al.* (1981) and Young

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1982) who showed that lesions in the descending spinal trigeminal tract did not abolish responses to electrical stimulation of tooth pulp in monkeys or responses to thermal stimulation of the teeth in human subjects.

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