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BRANCHED AFFERENT NERVES SUPPLYING TOOTH-PULP IN THE CAT

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SUMMARY

1. Recordings have been made from nerve terminals in canine and third incisor teeth of cats.

2. Ninety-three tooth-pulp units in ten cats could be excited either by mechanical or electrical stimulation of adjacent mucous membrane or by electrical stimulation of the pulp of a nearby tooth.

3. Section of the trigeminal nerve centrally did not abolish these responses.

4. Results for twelve out of thirteen units tested with collision techniques indicated that the nerves were branched.

INTRODUCTION

A number of incidental observations made during experiments in which recordings were made from nerves in the canine teeth of cats showed that some pulpal nerve fibres could be excited by stimulation of adjacent tissues. For example, a brief burst of impulses was sometimes recorded from a canine tooth when the crown of a neighbouring incisor was fractured off to gain better access to the canine, or occasionally when the lip was pinched or a suture needle passed through it. The nature of these stimuli usually precluded repeated testing except on a few occasions when responses were consistently obtained by pinching the lip with toothed forceps. There are several possible explanations for these observations: the potentials recorded may have represented reflex activity evoked in efferent nerves to the tooth pulp; they might have been antidromically conducted impulses in afferent nerves due to a trigeminal tract reflex (Darian-Smith, 1965); they could have been due to direct stimulation of axons which were taking circuitous routes to the canine tooth pulp; or alternatively the afferent nerves could have been branched with terminals in more than one tooth, or in one tooth and adjacent mucous membrane or skin. The experiments to be described were carried out to investigate these possibilities. A preliminary report has been published previously (Lisney & Matthews, 1976).

METHODS

Ten cats (2·5–3·5 kg) were anaesthetized with sodium pentobarbitone and electrodes inserted into the canine and incisor teeth for stimulation and recording. The method used for constructing Ag/AgCl electrodes for the canines was similar to that described previously (Horiuchi & Matthews, 1974) except that in some animals dentine was exposed by fracturing off, under Ringer, 1·5 mm from the tip of the tooth, instead of by drilling. For the incisor teeth, electrodes were placed in two 0·3 mm diameter cavities which were cut into the dentine with a hand-held twist drill under Ringer. One electrode was placed near the incisive edge of the tooth and the other close to the cervical margin so that the interelectrode distance was 2 mm. The end of a piece of silver wire (diam. 0·125 mm) was placed in each cavity and the cavity packed with Ag/AgCl mixture (Horiuchi & Matthews, 1974). In the first experiment, electrodes were inserted into almost all the teeth but subsequently the procedure was simplified by placing them in only the four canines and the four third incisors.

Mechanical stimuli were applied to the gingival mucous membrane by hand-held, blunt probes or by a standard probe with a smooth, circular, flat tip of diameter 1·2 mm which was mounted on a strain gauge. The lip was pinched with toothed forceps. Electrical stimulation of the mucous membrane was carried out with either a pair of steel pins mounted 1 mm apart on an insulated holder or with a concentric electrode made from a piece of etched tungsten wire (diam. 0·2 mm) sealed with epoxy resin into a piece of stainless-steel tube (diam. 1·0 mm) with its pointed tip extending 0·2 mm beyond the end of the tube. Hot and cold stimuli were applied to the mucous membrane with a copper probe heated to 60 °C in a water-bath or a small pledget of cotton wool soaked in ethyl chloride. Arterial blood pressure and end-tidal CO_2 % were monitored throughout all the experiments and data collected only when the mean blood pressure was greater than 75 mmHg and the end-tidal CO_2 was within the range 3·5–5·0 %.

Collision experiments were performed on four of the cats. These cats were decerebrated at the midcollicular level, paralysed with pancuronium bromide and ventilated artificially with room air. An I.V. infusion of 6 % Dextran in Ringer solution was given to replace lost blood and to maintain mean blood pressure above 75 mmHg. The cerebrum rostral to the decerebration was removed to expose the trigeminal ganglia. Each ganglion was stimulated with a flat ended, concentric electrode (0·125 mm diam. silver wire sealed into a 1 mm diam. steel tube) placed in contact with the surface of the ganglion.

RESULTS

Thirty-three units were found which responded to electrical stimulation of a nearby tooth and sixty which responded to mechanical and/or electrical stimulation of nearby gingiva. These units were obtained from teeth in which dentine had been exposed by drilling or by fracturing. None responded to pinching the lip, to thermal stimuli, or to movement of the tooth in its socket.

Responses to stimulation of the pulp of an adjacent tooth

In the thirty-six canine teeth from which recordings were made, sixteen units were found which could be excited by electrical stimulation of an ipsilateral incisor in the same jaw. Two of these, obtained in the first experiment, were excited by stimulation of the first incisor; the others responded to stimulation of the third incisor. In several canines, more than one unit responded to stimulation of one incisor. For six of the sixteen units (including one from a first incisor), a response of very similar latency could also be recorded from the incisor when an electrical stimulus was applied to the canine (Fig. 1). Responses of a further eleven units could be recorded from an incisor (one from a first incisor) when the canine was stimulated although in these no response of similar latency could be detected the other way round. The finding that in some instances a response could be recorded from the canine with stimulation of the incisor but not with the circuits reversed, or vice versa, does not necessarily mean that impulses were only conducted in one direction. It is possible that with these units the nerve was not positioned favourably for satisfactory recording in both teeth. The latencies of the responses recorded from a canine to incisor stimulation, or vice versa, at once a second with a stimulus twice threshold ranged from 1·0 to 9·0 msec (mean 2·77 msec, S.D. 1·89, $n = 33$). In previous experiments, long latency responses to electrical stimulation in some pulpal units have been attributed to coupling between the terminals of several nerves (Matthews, 1977). Complex all-or-none wave forms recorded from teeth have also been explained on the basis of such a mechanism and the latter parts of these wave forms fail with stimulation at 10/sec (Horiuchi & Matthews, 1974; Matthews & Holland, 1975). For these reasons, in the present experiments, failure of a response to follow stimulation at 10/sec was taken as an indication that the response was probably indirect and involved coupled nerves. Twenty-seven of the thirty-three units were tested

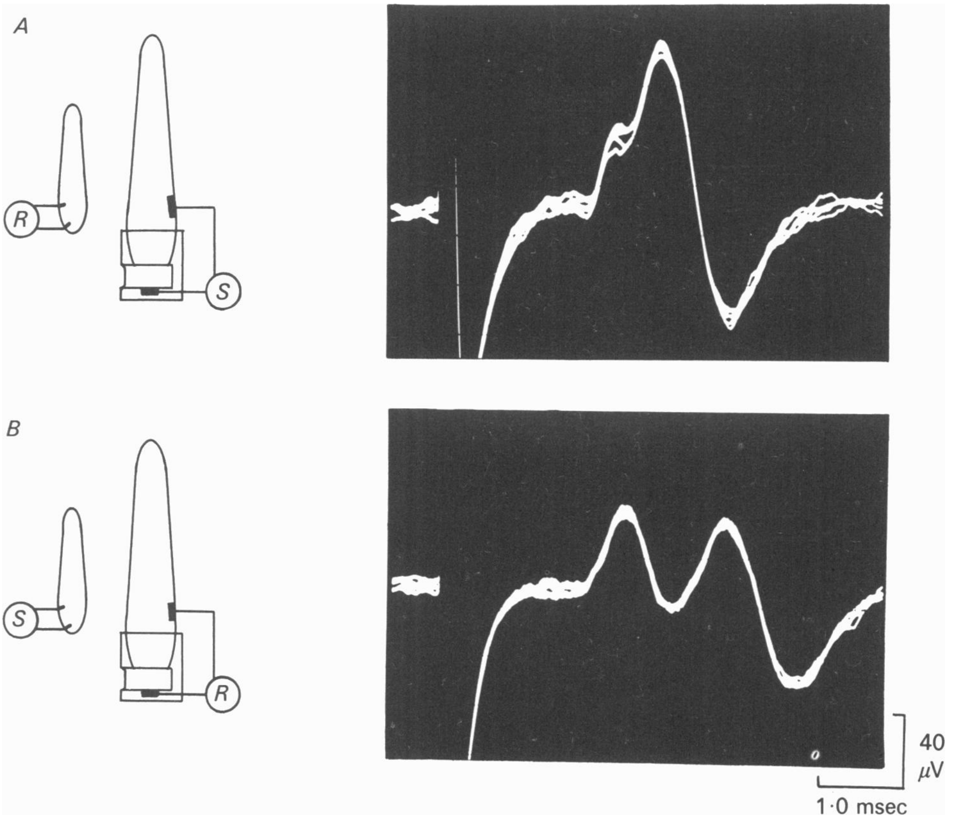

Fig. 1. All-or-none responses recorded from one tooth during electrical stimulation of an adjacent tooth. Five traces have been superimposed in each record. *A*, responses recorded from an upper third incisor during stimulation of the adjacent canine. *B*, responses obtained from the same teeth as in *A*, but with the stimulating and recording circuits reversed.

with a stimulation rate of 10/sec for 10 sec and of these twenty-three showed n significant change in latency. The latencies of the twenty-three units ranged from 1·7 to 4·7 msec (mean 2·26 msec, S.D. 0·81).

In two instances, sodium chloride solution (2·5 mole/l.) was applied to the exposed dentine at the tip of a canine to evoke a maintained discharge from its intradenta nerves (Horiuchi & Matthews, 1974). Simultaneous recordings were made from the canine stimulated and the adjacent third incisor. In one instance it was possible to identify a wave form in the incisor record which had a one-to-one relationship with the firing of a particular unit in the canine record (Fig. 2). In the other experiment, two incisor units behaved in this way.

Responses to mucous membrane stimulation

Fifteen units in canine teeth and twelve in incisors responded to mechanical stimulation of adjacent gingiva (Fig. 3). None of these responded to the hot or cold stimuli or to displacement of the tooth in its socket in a manner which would excite

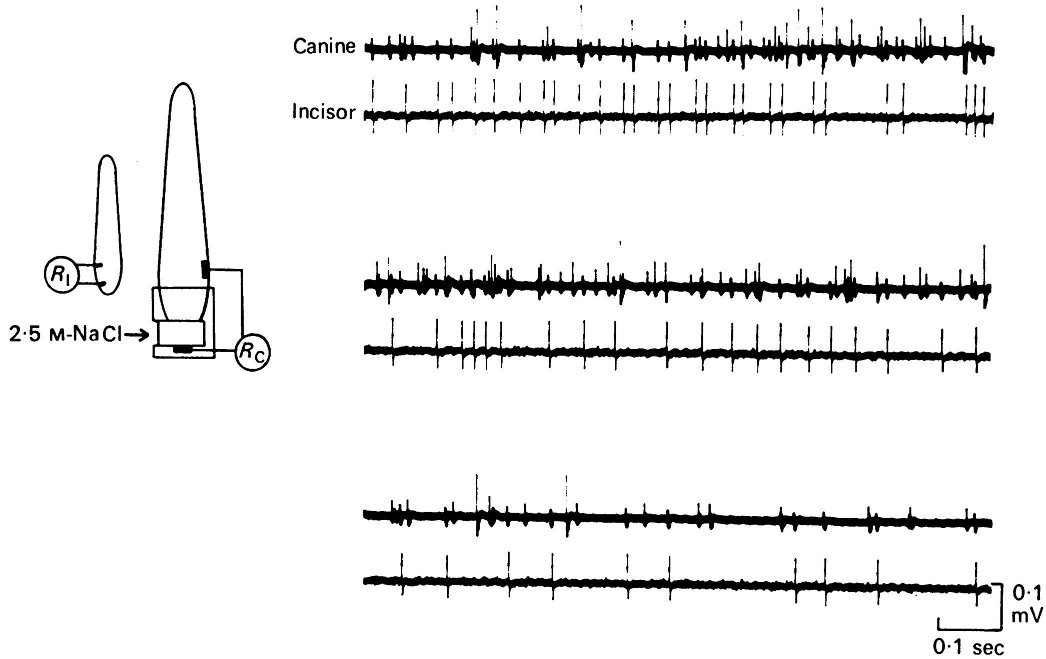

Fig. 2. Simultaneous recordings from the same canine and third incisor from which the results shown in Fig. 1 were obtained. A chemical stimulus (2·5 mole/l. NaCl) was applied to exposed dentine at the tip of the canine. The pairs of records are continuous from the end of one line to the beginning of the next.

periodontal mechanoreceptors. The receptor fields were about 1 mm^2 in area and within 3 mm of the free gingival margin, usually close to the crest of the alveolar bone. The receptor fields of twenty-three units were over the root of the tooth from which recordings were made, while 4 units had receptor fields over the adjacent tooth or just beyond. For most units, repeated stimulation produced diminishing responses, especially those with high thresholds. The mechanical thresholds ranged from 5 to 140 g/mm^2 (mean 51·56 g/mm^2, S.D. 42·58, $n = 27$). Forces above 50 g/mm^2 produced blanching and pitting of the gingiva. For many of these units, action potentials identical in shape were also evoked by electrical stimulation of the receptor field, although some units were obtained which responded to electrical stimulation of a small area of gum but not to mechanical stimulation of the same area. A total of thirty-three units, twenty-three in canines and ten in incisors, were obtained which could be excited by electrical stimulation of gingiva. The latencies of their responses ranged from 1·5 to 19·5 msec (mean 6·33 msec, S.D. 5·12). Twenty-eight were tested at a stimulation rate of 10/sec for 10 sec with a stimulus of twice threshold, and seventeen showed no significant change in latency. The latencies of these seventeen units ranged from 1·5 to 5·8 msec (mean 3·31 msec, S.D. 1·48).

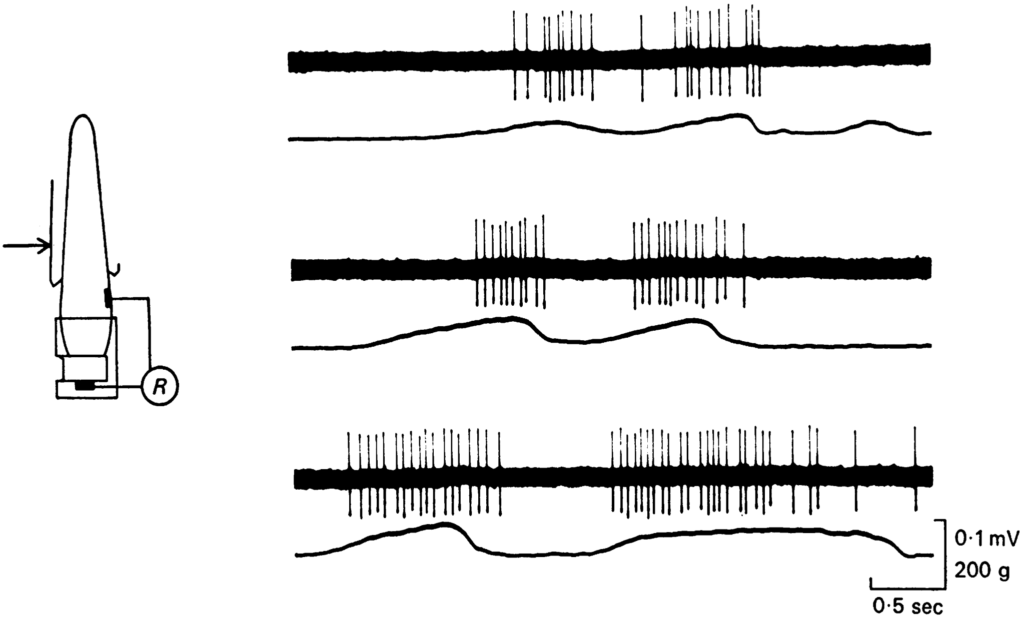

Fig. 3. Record of the response of a unit in an upper canine to mechanical stimulation of adjacent labial gingiva.

Evidence for branching

In one experiment the sensory and motor roots of the trigeminal nerve were sectioned on both sides close to the pons and in another both maxillary nerves were cut in the floor of the orbit. In both of these experiments the responses of units which could be excited by stimulation of an adjacent tooth or gingiva were not affected by the nerve section.

Collision techniques (Horrobin, 1966) were used to differentiate between the remaining possible explanations: that the nerves were branched, that they were taking circuitous routes to the tooth pulp or, in the case of responses to electrical stimulation over the root of a tooth, that the nerves were being excited in the root pulp by stimulus spread. By careful positioning of a concentric stimulating electrode on the surface of the ganglion, different groups of pulpal fibres could be excited at low threshold and their antidromic responses recorded from the tooth. A stimulation site was found at which one of the impulses could be made to collide with an orthodromic impulse initiated by electrical stimulation of the adjacent tooth or gingiva. An example of such a collision experiment is shown in Fig. 4. From recordings of this type the following measurements were made for a unit: its latency to ganglion stimulation, its latency to stimulation of the adjacent tooth or gingiva, its refractory period to double stimulation of this site, and the minimum interval between a ganglion stimulus and a subsequent stimulus to the adjacent tooth or gingiva at which

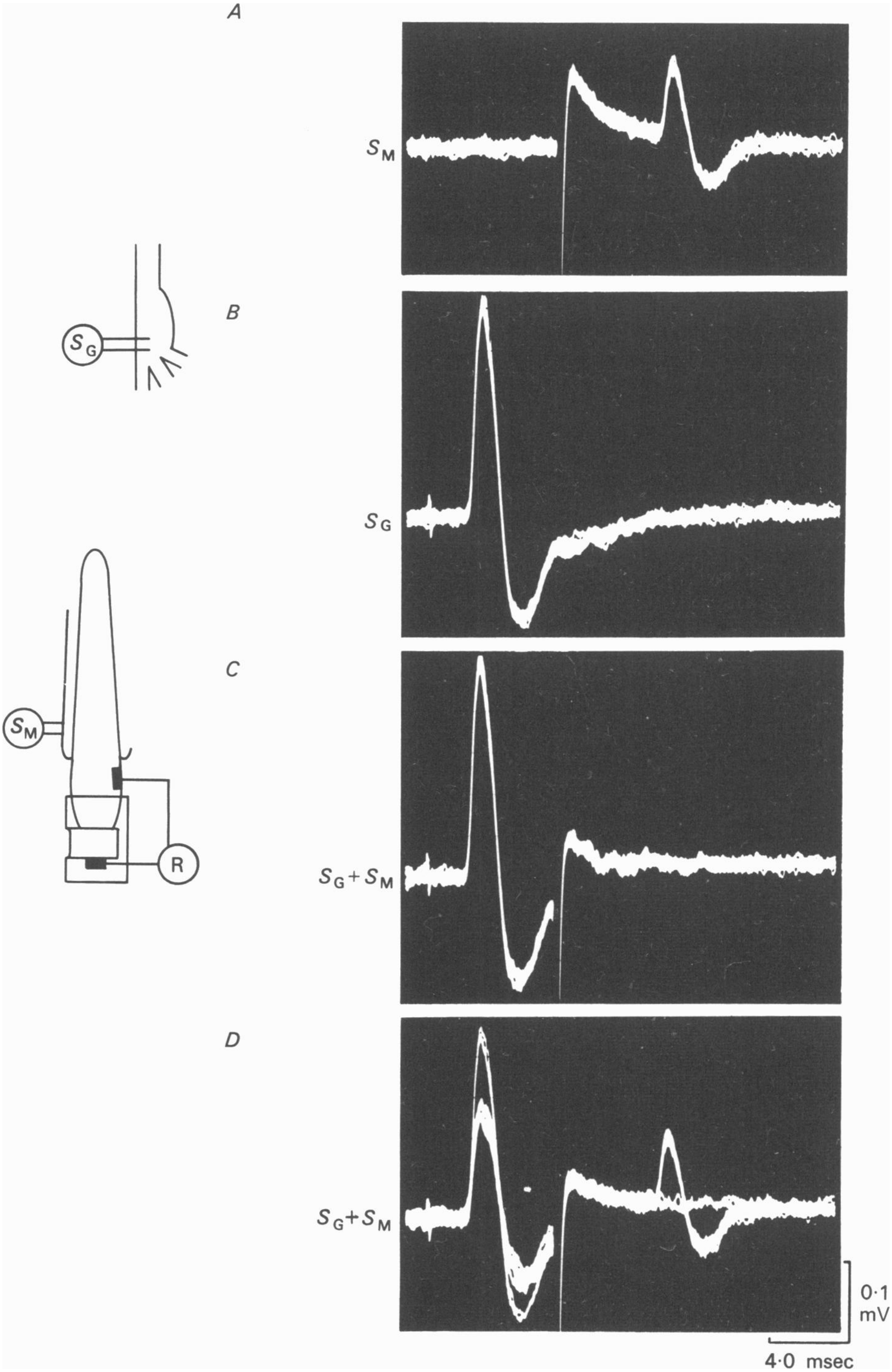

Fig. 4. For legend see facing page.

responses could be obtained to both stimuli. These data were obtained for eight units which responded to stimulation of gingiva and five which responded to stimulation of another tooth. They all responded to electrical stimulation at 10/sec without significant increase in latency. None of the units gave results which were consistent with their fibres taking an unbranched circuitous route to the tooth pulp or being excited in the root pulp by stimulus spread from overlying gingiva. For twelve of the units, the results could be explained by simple branching of the fibres (e.g. Fig. 5). Their conduction velocities measured between coronal pulp and the ganglion ranged from 17·1 to 39·4 m/sec (mean 26·68 m/sec, S.D. 6·66). To account for the results

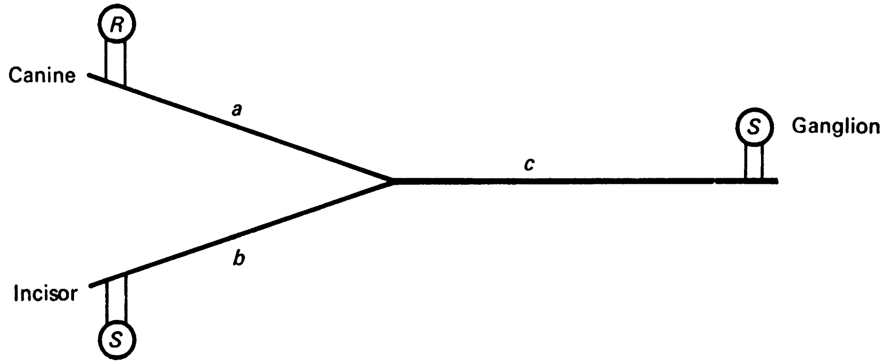

Fig. 5. Diagram of a branched axon supplying the pulps of a canine and an incisor. The conduction times in the three parts of the axon are represented by a, b and c. The results from one unit, in which recordings were made from the canine, were:

latency of response to ganglion stimulus $(a+c)$ = 3·0 msec.
latency of response to incisor stimulus $(b+a)$ = 4·7 msec.
refractory period with double stimulation of incisor (RP_i) = 1·6 msec.
minimum interval between a ganglion stimulus and a subsequent incisor stimulus which gave responses to both stimuli $(c+b+RP_i)$ = 6·1 msec.

From these results it was calculated that a = 1·6 msec, b = 3·1 msec and c = 1·4 msec. Utilization times at the sites of stimulation would have been small (< 50 μsec) compared with these values and were ignored in the calculation.

Fig. 4. Responses recorded from the same canine from which the results shown in Fig. 3 were obtained. In each record, fifteen successive sweeps have been superimposed.

A, response of a unit to electrical stimulation of the labial gingiva. This unit has the same wave form as that illustrated in Fig. 3 and the electrical stimulus was applied to the centre of the receptor field of that unit. The stimulus was delayed 7·5 msec after the sweep was triggered.

B, response of a small group of units to electrical stimulation of the surface of the ipsilateral trigeminal ganglion with a concentric electrode. The stimulus was delayed by 1·0 msec from the start of the sweep.

C, responses obtained when both the stimuli employed in *A* and *B* were delivered in the same sweep.

D, as *C*, but with the intensity of the ganglion stimulus reduced to threshold for one component of the first response. Whenever this component failed, the unit responded to the subsequent gingival stimulus.

of the other unit a more complex organization of the nerves, such as coupling between the terminals of several fibres in either the pulp (Matthews, 1977) or gingiva, would be required.

The sites of branching of the twelve units were estimated from these data. Some pulpal nerves tend to have slower conduction velocities within the pulp than outside (Matthews, 1977) but for the purpose of these calculations it was assumed that the fibres had a constant conduction velocity throughout their peripheral course. The estimated site of branching ranged from 10·2 to 49·2 mm (mean 31·7 mm, S.D. 10·7 mm) from the cervical electrode on the tooth from which recordings were made. If a nerve had a slower conduction velocity inside the tooth than outside, the estimated branching point would have been too far from the tooth. The distance from the cervical electrode to the apex of the canines was 10–13 mm and to the apex of the third incisors was 2–4 mm. The distance from the cervical electrode to the centre of the ganglion was approximately 58 mm for upper canines, 65 mm for upper third incisors and, for the corresponding lower teeth, the distance was 70 mm. For the branch going to gingiva or a second tooth, the estimated conduction velocity was within the range for small myelinated nerve fibres.

DISCUSSION

These experiments demonstrate that some nerves, presumably afferent, which supply the pulp of one tooth branch and also supply either the pulp of another tooth or the adjacent gingiva, periosteum or bone. The results indicate that the branching point of the nerves was between the apex of the tooth and the trigeminal ganglion and, in the case of the lower teeth, was within the inferior dental canal. Previous observations indicate that some nerves may also branch to innervate the lip but units of this type were not encountered in the present experiments.

The responses to mechanical stimulation of gingiva were similar in some respects to those of high threshold cutaneous mechanoreceptors having myelinated axons (Burgess & Perl, 1967) in that they were usually obtained only with intense stimulation of a small area of tissue, tended to fatigue, and did not respond to thermal stimuli. The exact location of the structures stimulated was not established and might have been in periosteum, gingiva or alveolar bone. The possibility that the responses were due to compression of axons rather than excitation of specialized receptor-endings cannot be excluded.

Mechanical stimulation of our own gingiva with the stimulator used for measuring the thresholds of units in the cat, produced pain as well as blanching and pitting when the force exceeded 50 g/mm^2. Stimulation of tooth pulp in man can cause pain and it is therefore possible that some of the fibres studied terminated as nociceptors in both gingiva and tooth pulp.

Pulpal nerves which branch and supply more than one tooth or one tooth and adjacent mucous membrane or skin could account for some of the convergence observed in recordings from second or higher order trigeminal afferent neurones (Sessle & Greenwood, 1976; Yokota, 1976; Young & Nord, 1975). The possibility that some primary afferents are branched should also be taken into account in interpreting the results of conditioning-testing procedures involving stimulation of tooth pulp and other oral tissues. Branched primary afferents could be a factor in poor localization and referral of dental pain.

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