Journal of Physiology (1989), **411**, *pp.* 663–675
With 6 text-figures
Printed in Great Britain

AFFERENT C FIBRE INNERVATION OF CAT TOOTH PULP: CONFIRMATION BY ELECTROPHYSIOLOGICAL METHODS

BY E. JYVÄSJÄRVI* AND K.-D. KNIFFKI

From the Physiologisches Institut der Universität Würzburg, Röntgenring 9, D-8700 Würzburg, FRG

(*Received 26 July 1988*)

SUMMARY

1. The presence of afferent C fibres innervating the lower canine tooth was investigated in Nembutal-anaesthetized cats.

2. Twenty-five single fibres with conduction velocities (CV_p) of less than 2·5 m/s, as calculated from the shortest response latency using monopolar electrical stimulation of the tooth, were recorded from the inferior alveolar nerve. In addition, the extradental conduction velocity (CV_n) of the fibres was determined by using bipolar electrical stimulation of the trunk of the inferior alveolar nerve.

3. The mean CV_p was $1{\cdot}4 \pm 0{\cdot}4$ m/s ($n = 25$; range, 0·6–2·4 m/s); the mean CV_n was higher, $1{\cdot}7 \pm 0{\cdot}9$ m/s ($n = 25$; range, 0·6–4·0 m/s). For 20% of the fibres CV_n exceeded 2·5 m/s; these were slowly conducting Aδ fibres. For 80% of the fibres, however, the extradental conduction velocity was in the C fibre range.

4. The relationship between CV_p (y) and CV_n (x) was $y = 0{\cdot}66 + 0{\cdot}40x$, the correlation coefficient being $r = 0{\cdot}85$. According to the present results this implies that for a reliable classification of pulpal C fibres ($CV_n \leqslant 2{\cdot}5$ m/s) by monopolar tooth stimulation alone, CV_p should be less than 1·7 m/s.

5. For twenty-three of the twenty-five fibres, one to three discrete shortenings of the response latency occurred when the intensity of the tooth stimulation was increased. When the nerve trunk itself was stimulated, a constant response latency was measured at all stimulus intensities applied.

6. For twelve fibres tested, the mean rate of electrical stimulation of the tooth, which the response followed with a constant latency, was $4{\cdot}1 \pm 2{\cdot}3$ Hz (range, 1·5–10·0 Hz). With higher rates of stimulation the response latency increased until the fibres failed to follow each stimulus pulse.

7. Fifteen of the nineteen fibres tested responded to radiant heat stimulation of the tooth they were innervating. The mean temperature threshold was $41{\cdot}4 \pm 2{\cdot}7$ °C ($n = 11$; range, $37{\cdot}4 \pm 46{\cdot}4$ °C).

8. For eight heat-sensitive pulpal C fibres the receptive field was determined by mechanical stimulation of the exposed pulp tissue. Four C fibres developed a long-lasting on-going discharge after intense mechanical stimulation of the receptive field.

* Present address: Department of Physiology, University of Helsinki, Siltavuorenpenger 20 J, SF-00170 Helsinki, Finland.

9. The discharge evoked by heat and mechanical stimulation of the tooth occluded the response evoked by simultaneously applied electrical current pulses to the nerve trunk, indicating that the same fibres were activated by both tooth and nerve stimulation.

10. The findings confirm the presence of afferent C fibres in the dental pulp of the cat. The response behaviour of these C fibres suggests that they might be involved in nociception.

INTRODUCTION

Histological studies have revealed an extensive population of unmyelinated nerve fibres in the dental pulp of various species (Reader & Foreman, 1981; for a review, see Byers, 1984). Some of these fibres are postganglionic sympathetic efferent fibres (Arwill, Edwall, Olgart & Svensson, 1973; Fehér, Csányi & Vajda, 1977; Noga & Holland, 1983); some might be terminal branches of larger, myelinated sensory fibres (cf. Byers, 1984). The number of unmyelinated pulpal axons outside the tooth apex is unknown because fibre spectrum analyses from the nerve at its place of entry into the tooth have not been made so far. However, fibre counts of the inferior alveolar nerve (Holland, 1978; Hoffmeister & Schendel, 1986) or of the sensory root of the trigeminal nerve (Kerr, 1966; Young & King, 1972; Young, 1977) have shown that in man and cat about 50% of the axons in these nerves are unmyelinated.

The range of latencies of compound action potential volleys or of single-fibre responses to electrical stimulation of the lower canine tooth in the cat and the dog, recorded from the inferior alveolar nerve, also supports the presence of tooth pulp C fibres (Bessou, Gauthier & Pagés, 1970; Anderson & Pearl, 1974, 1975; Närhi, Jyväsjärvi, Hirvonen & Huopaniemi, 1982; Närhi, Virtanen, Huopaniemi & Hirvonen, 1982; Cadden, Lisney & Matthews, 1983). However, this has not yet been verified by measurements of the conduction velocities of pulpal nerve fibres by directly stimulating the axons outside the tooth.

When compared to fast-conducting intradental fibres, fibres with long-latency responses seem to be distinguished not only by their response characteristics to monopolar electrical stimulation of the tooth (Närhi *et al.* 1982*b*) but also by their response to natural stimulation of the tooth (Närhi *et al.* 1982*a*; Närhi, 1985). These findings again suggest that there exists a distinct group of slowly conducting afferent fibres in the pulp, which might be C fibres.

The purpose of the present study was to obtain electrophysiological evidence of afferent C fibre innervation of the cat's canine tooth pulp. This was done by measuring the axonal conduction velocity of single intradental fibres outside the tooth and comparing these values to those obtained by stimulating the tooth itself, as well as by using heat stimulation of the tooth and mechanical stimulation of the pulp to non-electrically activate the identified fibres.

METHODS

General procedures. The experiments were carried out on fifteen adult cats, 2·0–4·4 kg in weight, with intact and fully developed permanent teeth. The cats were premedicated with a S.C. injection of atropine sulphate (1 ml) to prevent salivation during manipulation in the mouth in the course of the experiments. Deep anaesthesia was induced with an I.P. injection of sodium pentobarbitone

(40 mg kg^{-1}) and maintained after catheterization of the right femoral artery and vein with a constant I.V. infusion (3 mg kg^{-1} h^{-1}) of the same anaesthetic. For undisturbed spontaneous ventilation, the animals were tracheotomized but not artificially ventilated. The mandible was fixed to the maxilla with the mouth in an open position using dental acrylic bite blocks cemented between the upper and lower molar teeth. The animals were placed on their backs on a feed-back-controlled heating pad. The head was fixed to the recording frame with a metal bar across the palate. Throughout the experiments body core temperature and mean arterial blood pressure were monitored and kept within normal physiological limits.

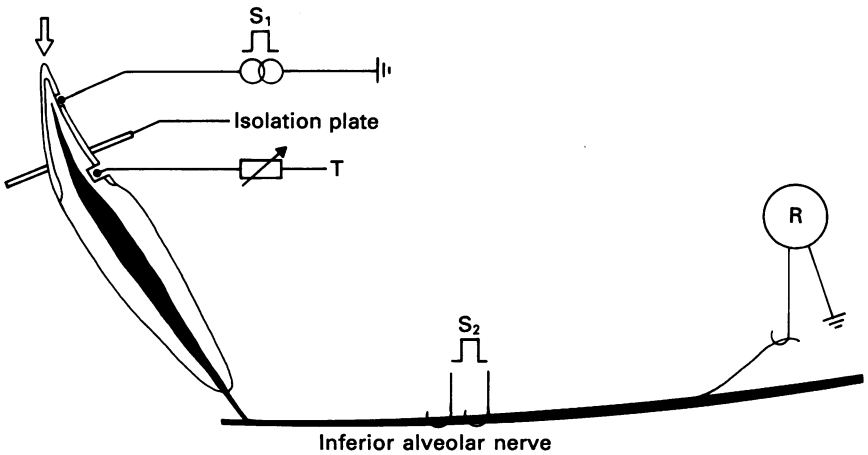

Fig. 1. Schematic drawing showing the experimental arrangement for identifying single intradental C fibres of the inferior alveolar nerve supplying the cat's lower canine tooth. R symbolizes the recording devices. For identifying intradental C fibres the tooth was stimulated monopolarly (S_1); in addition, the trunk of the inferior alveolar nerve was stimulated bipolarly (S_2) with platinum hook electrodes using a constant-current stimulator. The arrow represents the applied non-electrical stimuli, either radiant heat stimulation of the tooth crown or mechanical probing of the exposed pulp tissue. The coronal third of the tooth was isolated from surrounding tissues by a Teflon isolation plate. In experiments using heat stimulation of the tooth the temperature near the pulp was recorded by a thermistor (T).

Tooth and nerve preparation. The left lower canine tooth was cleaned, dried and its coronal third was isolated from the gingiva with a 1 mm thick Teflon plate, which was cemented around the neck of the tooth (Fig. 1). For electrical stimulation of the tooth, a shallow cavity was drilled just through the enamel near the tip of the tooth at the level of the pulp horn; a platinum wire electrode (S_1, diameter 0·4 mm) with a piece of cotton wool soaked in Tyrode's solution was pressed into the cavity to make a firm contact. The indifferent electrode was attached to the lower lip. The impedance between the electrodes was controlled during the experiments; it varied in different experiments between 30 and 120 kΩ. The impedance via the dried enamel surface and the isolation plate exceeded 10 MΩ. In experiments in which radiant heat was applied to the tooth, a thermistor probe (T) was sealed into a deep cavity proximal to the Teflon plate (Fig. 1).

The lower margin of the mandible ipsilateral to the prepared tooth was removed between the mental and mandibular foramina to expose the inferior alveolar nerve in the mandibular canal. The nerve was carefully freed from connective tissue with minimal disturbance of the blood circulation and cut as far proximally as possible. A pair of platinum hook electrodes (S_2) was carefully placed under the nerve trunk (Fig. 1). A pool for microdissection of the nerve and for recording was made out of the skin flaps and filled with paraffin oil at body temperature. The temperature of the pool was measured and kept above 34 °C to avoid changes in conduction velocity due to cooling of the axons (cf. Franz & Iggo, 1968). This level of temperature was based on measurements of the tissue temperature (34–35·5 °C) in the mandibular canal immediately after exposure of the nerve. Fine

filaments were split from the cut end of the nerve under a binocular preparation microscope and placed on a platinum recording electrode. The indifferent electrode was attached to the adjacent connective tissue. The splitting of filaments was continued until the responses of functional single fibres could be recorded. The neuronal activity was amplified, filtered (bandpass, 100 Hz–4 kHz), audiomonitored, displayed on an oscilloscope screen and stored as original records on a magnetic tape.

Identification of the intradental fibres. As a search stimulus and also to identify intradental nerve fibres, single monopolar cathodal square-wave current pulses were delivered through the tooth electrode using a constant-current stimulator. Electrical thresholds of all-or-none action potentials were determined by using stimulus pulses of 1 ms duration with intensities of less than 200 μA. The response latencies were measured at different stimulus intensities. Fibres that had a conduction velocity of less than 2·5 m/s (presumably C fibres), as calculated from the shortest response latency and the conduction distance, were then additionally stimulated by the pair of electrodes placed under the nerve trunk, the cathode being the proximal electrode. Again, response latencies and conduction velocities were determined. Occlusion was tested if, as judged by the shape of the action potential, the fibre under study was apparently activated by both tooth and nerve stimulation. For this purpose radiant heat stimuli (symbolized by the arrow in Fig. 1) were applied in twelve experiments, from a controlled heat stimulator to the isolated coronal third of the tooth, whilst the nerve trunk was simultaneously electrically stimulated at a suprathreshold intensity. After two or three heat stimuli the pulp was exposed, mechanically stimulated to determine the receptive fields of the fibres and the occlusion experiment repeated.

RESULTS

In the present series of experiments twenty-five single intradental fibres were recorded, which had a conduction velocity (CV_p) of less than 2·5 m/s, as calculated from the shortest latency of the response evoked by a single monopolar stimulus pulse applied to the tooth and the conduction distance between the stimulating cathode and the recording electrode. With the tooth intact, none of the fibres showed any on-going activity in the absence of intentional stimulation. The mean CV_p ($\pm$S.D.) was $1{\cdot}4 \pm 0{\cdot}4$ m/s ($n = 25$, range 0·6–2·4 m/s). The mean electrical threshold using 1 ms cathodal rectangular current pulses was $115{\cdot}4 \pm 51{\cdot}0$ μA (range, 36–200 μA).

In twenty-three of the twenty-five recordings, one to three discrete shortenings of the response latency were observed when the stimulus intensity was gradually increased above threshold. An example of this response behaviour is shown in Fig. 2*A*. The upper sweep shows the latency at threshold stimulation intensity (1·0T). When the stimulus strength was increased, this latency remained constant until at 1·4T the latency shortened. This new latency was again stable when increasing the stimulus strength further, but at 2·1T another shortening of the response latency was observed. A third shortening occurred at 4·2T, but no further reduction in the latency was observed when the stimulus strength was again increased. In this particular recording the differences between the successive shorter latencies were 6·3 ms, 2·1 ms and 4·2 ms. In no case was a longer latency observed even when increasing the intensity of a single stimulus pulse up to 50T.

In twelve of the twenty-five fibres the response to repetitive electrical stimulation of the tooth was studied. For individual fibres a particular maximal repetition rate of stimulation was found at which they still responded with a constant latency to a suprathreshold stimulus. Averaged over the twelve fibres the maximal stimulation frequency evoking a response with a constant latency (f_c) was $4{\cdot}1 \pm 2{\cdot}3$ Hz (range,

1·5–10·0 Hz). When the stimulus repetition rate was increased above f_c for an individual fibre, the latency to consecutive stimulus pulses increased in almost constant steps until a failure occurred. An example of such behaviour is shown in Fig. 2*B*. This particular fibre was stimulated at 2·0*T* using a repetition rate of 5 Hz. Note that in the second sweep (consecutive sweeps displayed from top to bottom) the

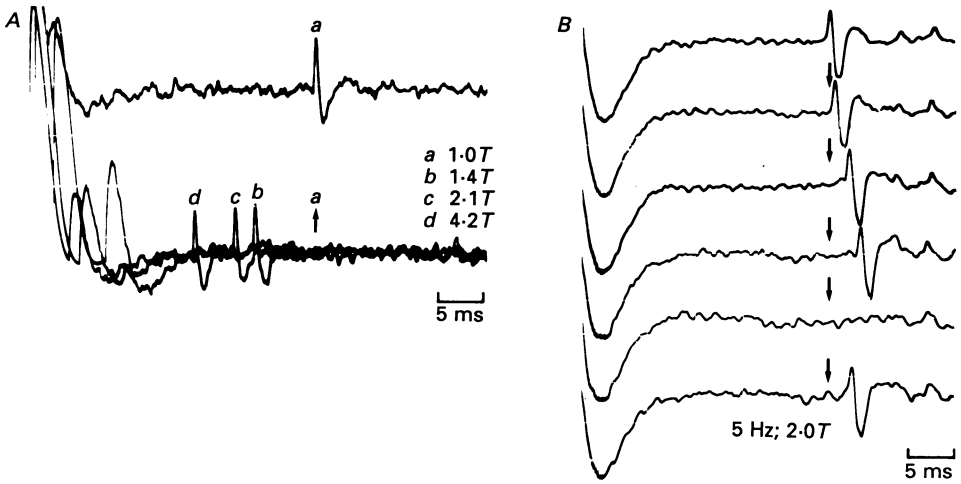

Fig. 2. Responses of intradental C fibres to cathodal current pulses applied monopolarly to the tooth crown of the lower canine tooth in the cat. In the upper trace of *A* the response to a stimulus of threshold intensity (1·0*T*) is shown. The lower trace is a superposition of three sweeps with different stimulus intensities indicated by the numbers of multiples of the threshold stimulus intensity. Note that the three different stimulus intensities (1·4*T*, 2·1*T*, 4·2*T*) each resulted in a shorter response latency. The conduction velocity of this particular fibre, calculated from the shortest latency and the distance between the cathode at the tooth crown and the recording electrode, was 1·9 m/s. The arrow (*a*) in the lower trace indicates the location of the evoked action potential at threshold stimulation intensity. In *B*, for another C fibre, the response latencies to repetitive electrical stimulation at 5/s with an intensity of 2·0*T* is shown. Note that in the four consecutive (top to bottom) upper sweeps the latency is increased. At the fifth sweep a response failure can be seen. From the sixth sweep onwards the action potential occurred randomly at varying latencies (not shown). The conduction velocity calculated from the shortest latency using a single stimulus pulse at 2·0*T* was 1·2 m/s. The arrows indicate where the constant-latency response would have been situated.

latency was already slightly increased. For this fibre the increments in the latency were almost constant during the first four sweeps, but in the fifth sweep the stimulus pulse failed to evoke a response. After that, the response occurred randomly at varying latencies. Decreasing the stimulus repetition rate below f_c led to a gradual return of the response latency to the original value. Using this kind of repetitive stimulation of the tooth, no obvious correlation between the conduction velocity of the twelve slowly conducting fibres and f_c was found.

In addition, the axonal conduction velocity of the twenty-five fibres identified by monopolar stimulation of the tooth was determined by using bipolar stimulation of the trunk of the inferior alveolar nerve. An example is given in Fig. 3. The shortest constant-latency response to monopolar stimulation of the tooth at 2·3*T*, which

corresponds to a conduction velocity of 1·7 m/s, is shown in the upper trace of Fig. 3*A*. A section of a similar sweep at an expanded time scale is shown in the lower trace of Fig. 3*A* to compare the shape of the action potential with that evoked by bipolar stimulation of the nerve trunk itself (Fig. 3*B*). In this case the form appeared to be identical, i.e. the same fibre was activated both by monopolar tooth and bipolar

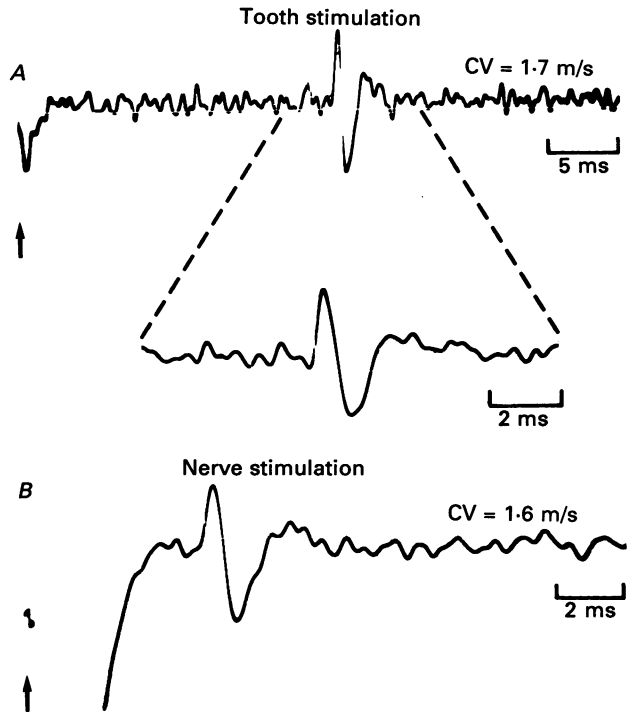

Fig. 3. Identification of an intradental C fibre by monopolar tooth (*A*) and bipolar nerve trunk (*B*) stimulation. Conduction velocity calculated from the shortest latency to tooth stimulation at 2·3*T* was 1·7 m/s and from the nerve trunk 1·6 m/s. The spike form was compared by expanding the tooth stimulation sweep (second trace in *A*; in this case not for the same stimulus pulse) to the same time scale as the nerve stimulation sweep. The dashed oblique lines in *A* indicate the section of the tooth stimulation sweep that was displayed with the faster sweep velocity. The onset of the cathodal stimulus pulses are indicated by the arrows.

nerve stimulation. The axonal conduction velocity determined by nerve stimulation (CV_n) was in this case 1·6 m/s. The mean CV_n was $1{\cdot}7 \pm 0{\cdot}9$ m/s ($n = 25$, range 0·6–4·0 m/s); it did not significantly differ from the CV_p. For five of the fibres (20%), however, the CV_n exceeded 2·5 m/s; they were in fact slowly conducting Aδ fibres (see below).

With bipolar stimulation of the inferior alveolar nerve trunk, shortenings of the response latencies were never observed when increasing the stimulation intensity above threshold, as was the case when stimulating the tooth (see above). Only a minute deviation in the latency was observed when the threshold was determined several times. The effect of repetitive stimulation of the nerve trunk was studied for six C fibres. The values for f_c did not statistically differ from those obtained by monopolar stimulation of the tooth.

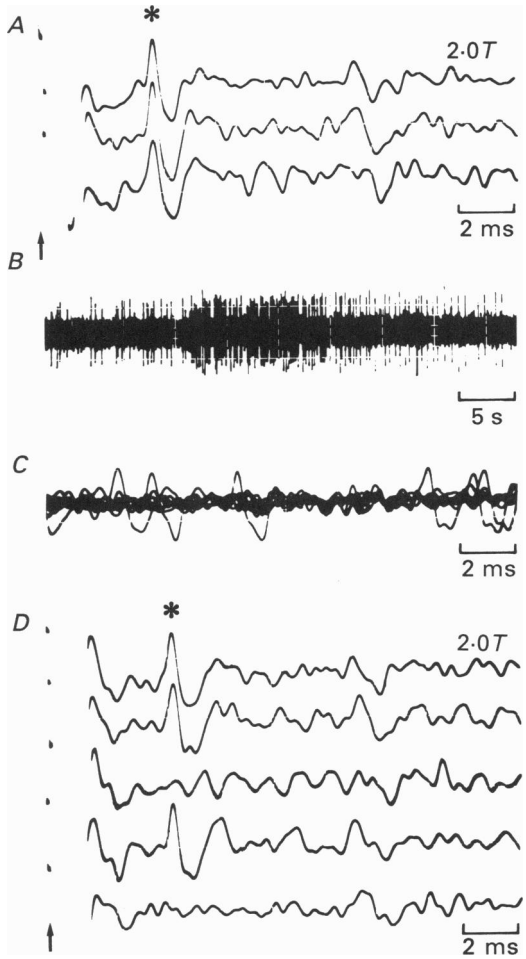

Fig. 4. Responses of an intradental C fibre (indicated by the asterisk) to bipolar stimulation of the nerve trunk during radiant heat stimulation of the lower canine tooth crown. *A*, prior to the heat stimulus, electrical nerve stimulation at a suprathreshold intensity (2·0*T*) using a repetition rate of 1/s resulted in a constant-latency response. Three consecutive sweeps (top to bottom) are shown. *B* and *C*, response of the fibre to radiant heat stimulation of the tooth crown. Time scale of *B* expanded in *C* to correspond with that of *A*. *D* , repetitive electrical nerve stimulation (2·0*T*, 1/s) during heat-evoked discharges of the fibre. Note that in the third and fifth sweeps the generation of the action potential at the latency marked by the asterisk was occluded.

Of the twenty-five fibres identified as C fibres using the criterion $CV_p \leqslant 2{\cdot}5$ m/s, nineteen were tested by radiant heat stimulation of the tooth. Fifteen fibres were responsive. Eleven of them had their CV_n equal to or below 2·5 m/s and four of them above 2·5 m/s. An example of the heat-induced activity of an intradental C fibre is shown in Fig. 4*B*. This particular fibre had a temperature threshold of 42·2 °C. During the rising phase of the temperature near the pulp (not shown in Fig. 4*B*) the discharge rate increased and some bursting occurred. The temperature threshold was recorded for eleven of the fifteen heat-sensitive fibres; the mean threshold was $41{\cdot}4 \pm 2{\cdot}7$ °C (range, 37·4–46·4 °C). As a rule, the radiant heat-evoked neuronal

activity ceased rapidly when the temperature gradient became zero or negative. The response behaviour of the fibres, classified by CV_p as C fibres, to heat stimulation of the tooth was generally rather uniform and similar to that described by Närhi *et al.* (1982*a*) using contact heat stimuli.

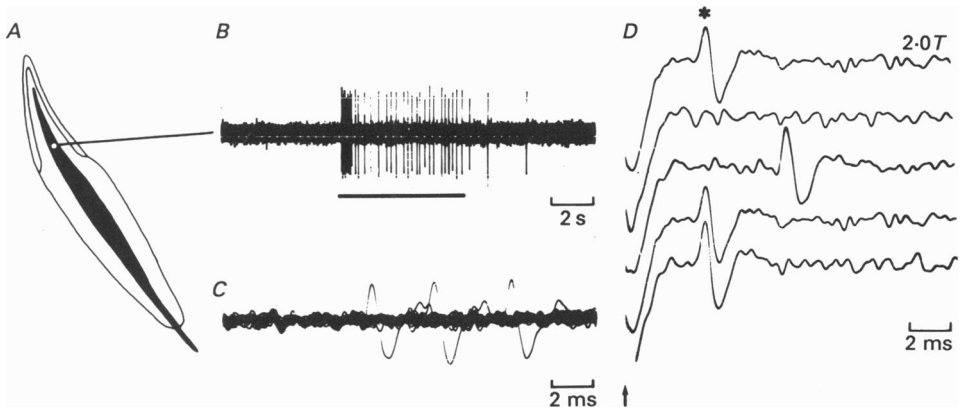

Fig. 5. Responses of an intradental C fibre to bipolar stimulation of the nerve trunk during mechanical stimulation of the exposed pulp tissue of the lower canine tooth of the cat. *A*, location of the mechanically determined receptive field of the C fibre. The fibre was previously identified by monopolar stimulation of the tooth. *B* and *C*, response to moderate pressure by a dental probe at the indicated location in *A*. The continuous line beneath *B* marks the duration of the stimulation. The trace of *B* is expanded to a time scale in *C* corresponding to that of *D*. *D*, repetitive electrical nerve stimulation (2·0*T*, 1/s) during mechanical stimulation of the pulp at the indicated location (*A* and *B*). Note that in the second and third of the consecutive sweeps (top to bottom) the generation of the action potential at the latency marked by an asterisk was occluded.

For eight heat-sensitive C fibres a receptive field was determined by mechanical stimulation of the pulp tissue after exposure of the pulp. None of the fibres responded when the overlying dentine was removed with a turbine burr without otherwise stimulating the dentine surface. An example of the response of a C fibre to mechanical stimulation of the receptive field is shown in Fig. 5*B*. The receptive field is indicated in Fig. 5*A*. The eight C fibres responded similarly to intense mechanical stimulation. After an initial high-frequency response, the discharge rate rapidly decreased. For four fibres, however, the firing lasted for several minutes. The conduction velocity of these eight fibres was also determined by electrically stimulating the receptive field; its mean value was $0{\cdot}9 \pm 0{\cdot}3$ m/s (range, 0·6–1·5 m/s).

Examples of the responses of intradental C fibres, classified by the criterion $CV_n \leqslant 2{\cdot}5$ m/s, to electrical nerve stimulation during simultaneous radiant heat stimulation of the tooth crown or mechanical stimulation of the pulp are indicated in Figs 4 and 5. After a fibre had been identified by electrical tooth and nerve stimulation, nerve stimulation was used during the non-electrical stimulation of the tooth. As a rule, an occlusion of the electrically evoked response was observed (cf. Fig. 4*D*, third and fifth traces; Fig. 5*D*, second and third traces). This indicated that the recorded action potential was from the same fibre that was stimulated non-electrically within the tooth and electrically in the nerve trunk.

In Fig. 6 the conduction velocity to nerve stimulation (CV_n) of the twenty-five intradental slowly conducting fibres is plotted against the conduction velocity to tooth stimulation (CV_p). The dashed line indicates $CV_n = CV_p$. For all but five fibres CV_n was equal to or greater than CV_p. Using a linear least-squares fit procedure the relationship between CV_p and CV_n was $y = 0{\cdot}66 + 0{\cdot}40x$, the correlation coefficient

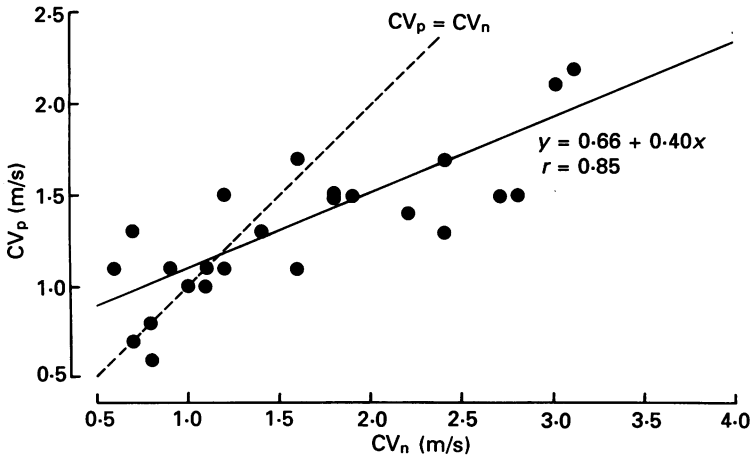

Fig. 6. Axonal conduction velocity (CV_n) determined by bipolar stimulation of the inferior alveolar nerve of twenty-five intradental slowly conducting fibres, plotted against their conduction velocity calculated from the shortest response latency to monopolar tooth stimulation (CV_p).

being $r = 0{\cdot}85$. Note that $CV_p \leqslant 2{\cdot}5$ m/s was initially the criterion used to classify C fibres. As can be seen in Fig. 6, for five fibres classified according to CV_p as C fibres, the CV_n exceeded 2·5 m/s; these fibres were actually slowly conducting Aδ fibres. If monopolar tooth stimulation alone is used to identify intradental C fibres, according to the above calculated equation CV_p (y) should not exceed a value of 1·67 m/s in order to permit the assumption that CV_n (x) remains below 2·5 m/s.

DISCUSSION

In the present study electrophysiological evidence of afferent C fibre innervation of the cat dental pulp has been obtained. For each fibre recorded the conduction velocity was determined in two ways: by using monopolar tooth stimulation and bipolar nerve stimulation. It was observed that the conduction velocity calculated using monopolar stimulation of the tooth was, in all but five cases, and in every case when the receptive field was directly electrically stimulated, lower than the measured extradental conduction velocity. This could be explained by the slowing down of conduction at the terminal parts of the peripheral nerve axon. The present results are in agreement with the observation for myelinated axons that the conduction velocity of pulpal fibres of the cat (Horiuchi, 1965; Lisney, 1978; Cadden *et al.* 1983) and the rat (Jiffry, 1981) is lower within the pulp. This has led to the suggestion that many of the histologically verified unmyelinated axon profiles in the pulp are in fact terminal branches of myelinated axons of the alveolar nerve (cf. Byers, 1984) and

that the recorded C fibre latencies result from this slowing down of the conduction velocity. In the present study, 20% of the recorded fibres were found to be in fact slowly conducting Aδ fibres, which by using monopolar tooth stimulation alone would have been classified as C fibres. The remaining 80% of the fibres, however, had their conduction velocity in the C fibre range. As indicated in Fig. 6, the linear relationship between CV_p and CV_n ($y = 0{\cdot}66 + 0{\cdot}40x$) demonstrates that the C fibres also have a lower conduction velocity within the pulp. According to the present results this means that in experiments in which monopolar stimulation of the tooth alone is used to identify pulpal C fibres, the upper limit of the CV_p to accept fibres as C fibres should be set to $CV_p = 1{\cdot}67$ m/s provided that $CV_n \leqslant 2{\cdot}5$ m/s is a reliable criterion to classify C fibres.

It has also been suggested that the long-latency responses evoked in the fibres of the alveolar nerve by electrical tooth stimulation and interpreted as C fibre responses are ephaptically conducted responses from other fibres, which are 'coupled' to the recorded fibres within the pulp (Matthews & Holland, 1975). The discrete shortenings of the latency with increasing stimulus intensity observed in the present and other studies (Matthews, 1977; Lisney, 1978; Närhi *et al.* 1982*a*, *b*; Cadden *et al.* 1983; Virtanen, Närhi, Huopaniemi & Hirvonen, 1983) could imply the activation of other pulpal fibres, which are ephaptically connected to the recorded fibre. Such connections could explain why the extradental conduction velocity, for five fibres of the present study, was lower than the conduction velocity determined by monopolar stimulation of the tooth.

A further possibility is that the shortenings of the latency may reflect that different terminal branches of a single axon were excited at different stimulus intensities (cf. McMahon & Wall, 1987).

On the other hand, when monopolar stimulation of the tooth is used, the effective stimulation site might be at different locations within the pulp depending on the stimulation strength. In the case of a high stimulus intensity the current density in the root canal may increase enough to excite the axons at the apex (cf. Mumford & Newton, 1969). The present findings that many C fibres had more than one response latency could most simply be explained by the different locations of the actual cathode within the pulp. The difference between the threshold latency and the shortest latency observed in these experiments fits with the relative proportion of the intradental conduction distance to the whole conduction distance. In the present experiments about 40% of the conduction (measured from the tooth stimulation electrode) occurred within the tooth.

The reason only a small number of pulpal C fibres were recorded in some studies might lie in the method of search stimulation. When the tooth is stimulated bipolarly with two electrodes attached to the crown, the current flow may be restricted to the coronal pulp (Mumford, 1959) and those fibres that are located deep in the apical root pulp may not be activated at all. Using monopolar tooth stimulation, the current flow through the pulp and out of the apical foramen of the tooth to the indifferent electrode can also excite the fibres located deep in the pulp. In a study in which monopolar and bipolar stimulation of the tooth were compared, it was observed that the activation thresholds were almost two times higher with bipolar than monopolar stimulation and that 29% of the fibres classified as C fibres did not respond at all to bipolar stimulation (Virtanen, 1985).

The present results also clearly showed that afferent C fibres exist in the cat dental pulp. Most of the examined fibres were activated by radiant heat stimulation of the tooth. The response properties were similar to those recorded from slowly conducting cat intradental fibres using contact heat (Närhi *et al.* 1982*a*). In addition every heat-sensitive C fibre (and one Aδ fibre) tested could also be activated by mechanical stimulation of the pulp tissue, indicating that radiant heat stimulation of the tooth had excited intradental fibres.

Responses to bipolar nerve stimulation were occluded during the naturally evoked activity of intradental C fibres. In these experiments the frequency of bipolar nerve stimulation was adjusted to be so low that response failures or shifts to longer latencies did not occur. Thus, occlusion of the responses to bipolar nerve stimulation confirmed that the C fibres identified by electrical and natural tooth stimulation were the same as those that were identified by nerve stimulation. The four fibres identified by monopolar stimulation of the tooth that did not respond either to heat or to mechanical stimuli could have been pulpal sympathetic efferent fibres, which have been shown to exist in the inferior alveolar nerve (Matthews & Robinson, 1980). On the other hand, it cannot be excluded that they might have originated from the periodontal tissue, because it has been shown that periodontal fibres could be activated by monopolar stimulation of the tooth (Greenwood, Horiuchi & Matthews, 1972; cf. Matthews & Searle, 1976).

The intensities of the heat and mechanical stimuli required to activate the recorded afferent pulpal C fibres suggest that these fibres are involved in nociception. In addition, it has been shown (Jyväsjärvi & Kniffki, 1988) that heat- and mechano-sensitive pulpal C fibres of the cat also respond to cold stimulation of the tooth and to stimulation of the pulp with KCl and bradykinin. It thus seems that at least some pulpal C fibres supply polymodal nociceptors. The finding that some of the recorded fibres developed a long-lasting on-going discharge after mechanically induced trauma to the pulp suggests that that on-going activity of pulpal C fibres might be involved in the aching dental pain often present during pathological processes of the pulp.

The authors are greatly indebted to Mrs Petra Haumann and Mrs Christa Erhard for their skilful technical assistance, and to both of them, as well as Mrs Margit Schulze, for their patience in preparing the figures and the manuscript. We are grateful to Drs W. Kollmann, R. W. A. Linden and Christiane Vahle-Hinz for their critical comments on the manuscript. This study has been supported by the Wilhelm Sander-Stiftung (grant No. 83.018.1+2).

REFERENCES

ANDERSON, K. V. & PEARL, G. S. (1974). Conduction velocities in afferent fibers from feline tooth pulp. *Experimental Neurology* **43**, 281–283.

ANDERSON, K. V. & PEARL, G. S. (1975). C-fiber activity in feline tooth pulp afferents. *Experimental Neurology* **47**, 357–359.

ARWILL, T., EDWALL, L., OLGART, L. & SVENSSON, S.-E. (1973). Ultrastructure of nerves in the dentinal-pulp border zone after sensory and autonomic nerve transection in the cat. *Acta odontologica scandinavica* **31**, 273–281.

BESSOU, P., GAUTHIER, J. & PAGÉS, B. (1970). Mise en évidence de fibres afférentes du groupe C innervant la pulpe de la canine chez le Chat. *Comptes rendus des séances de la Société de biologie et de ses filiales* **164**, 1845–1850.

BYERS, M. R. (1984). Dental sensory receptors. *International Review of Neurobiology* **25**, 39–94.

CADDEN, S. W., LISNEY, S. J. W. & MATTHEWS, B. (1983). Thresholds to electrical stimulation of nerves in cat canine tooth-pulp with Aβ-, Aδ- and C-fibre conduction velocities. *Brain Research* **261**, 31–41.

FEHÉR, E., CSÁNYI, K. & VAJDA, J. (1977). Ultrastructure and degeneration analysis of the nerve fibres of the tooth pulp in the cat. *Archives of Oral Biology* **22**, 699–704.

FRANZ, D. N. & IGGO, A. (1968). Conduction failure in myelinated and non-myelinated axons at low temperatures. *Journal of Physiology* **199**, 319–345.

GREENWOOD, F., HORIUCHI, H. & MATTHEWS, B. (1972). Electrophysiological evidence on the types of nerve fibres excited by electrical stimulation of teeth pulp with a pulp tester. *Archives of Oral Biology* **17**, 701–709.

HOFFMEISTER, B. & SCHENDEL, K. (1986). Analyse markhaltiger und markloser Axone an den Zähnen des Unterkiefers der Katze. *Deutsche Zahnärztliche Zeitschrift* **41**, 863–867.

HOLLAND, G. R. (1978). Fibre numbers and sizes in the inferior alveolar nerve of the cat. *Journal of Anatomy* **127**, 343–352.

HORIUCHI, H. (1965). A study of pulp-nerve excitation through a silver-wire electrode. *Journal of Dental Research* **44**, 1257–1263.

JIFFRY, M. T. M. (1981). Afferent innervation of the rat incisor pulp. *Experimental Neurology* **73**, 209–218.

JYVÄSJÄRVI, E. & KNIFFKI, K.-D. (1988). Functional properties of afferent C-fibres in the cat's dental pulp. In *Current Trends in Pain Research and Therapy*, vol. 3, ed. SHARMA, K. N., NAYAR, U. & BHATTACHARYA, N. Delhi: Indian society for Pain Research and Therapy (in the Press).

KERR, F. W. L. (1966). The ultrastructure of the spinal tract of the trigeminal nerve and the substantia gelatinosa. *Experimental Neurology* **16**, 359–376.

LISNEY, S. J. W. (1978). Some anatomical and electrophysiological properties of tooth-pulp afferents in the cat. *Journal of Physiology* **284**, 19–36.

MCMAHON, S. B. & WALL, P. D. (1987). Evidence for and possible functional significance of multiple branches of afferent C fibres in peripheral nerve. In *Fine Afferent Nerve Fibers and Pain*, ed. SCHMIDT, R. F., SCHAIBLE, H.-G. & VAHLE-HINZ, C., pp. 95–104. Weinheim: VCH Verlagsgesellschaft.

MATTHEWS, B. (1977). Responses of intradental nerves to electrical and thermal stimulation of teeth in dogs. *Journal of Physiology* **264**, 641–664.

MATTHEWS, B. & HOLLAND, G. R. (1975). Coupling between nerves in teeth. *Brain Research* **98**, 354–358.

MATTHEWS, B. & ROBINSON, P. P. (1980). The course of post-ganglionic sympathetic fibres distributed with the trigeminal nerve in the cat. *Journal of Physiology* **303**, 391–401.

MATTHEWS, B. & SEARLE, B. N. (1976). Electrical stimulation of teeth. *Pain* **2**, 245–251.

MUMFORD, J. M. (1959). Path of direct current in electric pulptesting – using two coronal electrodes. *British Dental Journal* **106**, 243–245.

MUMFORD, J. M. & NEWTON, A. V. (1969). Zone of excitation when electrically stimulating human teeth. *Archives of Oral Biology* **14**, 1383–1388.

NÄRHI, M. V. O. (1985). The characteristics of intradental sensory units and their responses to stimulation. *Journal of Dental Research* **64**, special issue, 564–571.

NÄRHI, M., JYVÄSJÄRVI, E., HIRVONEN, T. & HUOPANIEMI, T. (1982*a*). Activation of heat-sensitive nerve fibres in the dental pulp of the cat. *Pain* **14**, 317–326.

NÄRHI, M., VIRTANEN, A., HUOPANIEMI, T. & HIRVONEN, T. (1982*b*). Conduction velocities of single pulp nerve fibre units in the cat. *Acta physiologica scandinavica* **116**, 209–213.

NOGA, B. R. & HOLLAND, G. R. (1983). Sympathetic innervation at the apex of the cat's canine tooth – a quantitative analysis. *Anatomischer Anzeiger* **153**, 137–148.

READER, A. & FOREMAN, D. W. (1981). An ultrastructural quantitative investigation of human intradental innervation. *Journal of Endodontics* **7**, 493–499.

VIRTANEN, A. S. J. (1985). Electrical stimulation of pulp nerves – comparison of monopolar and bipolar electrode coupling. *Pain* **23**, 279–288.

VIRTANEN, A., NÄRHI, M., HUOPANIEMI, T. & HIRVONEN, T. (1983). Thresholds of intradental A- and C-nerve fibres in the cat to electrical current pulses of different duration. *Acta physiologica scandinavica* **119**, 393–398.

YOUNG, R. F. (1977). Fiber spectrum of the trigeminal sensory root of frog, cat and man determined by electronmicroscopy. In *Pain in the Trigeminal Region*, ed. ANDERSON, D. J. & MATTHEWS, B., pp. 137–147. Amsterdam: Elsevier/North Holland Biomedical Press.

YOUNG, R. F. & KING, R. B. (1972). Fiber spectrum of the trigeminal sensory root of the baboon determined by electron microscopy. *Journal of Neurosurgery* **38**, 65–72.