Functional properties of tooth pulp neurons responding to thermal stimulation

Dong K. Ahn[a*], Elena A. Doutova[b], Kirk McNaughton[c], Alan R. Light[d], Matti Narhi[e] and William Maixner[b*]

[a]Department of Oral Physiology, School of Dentistry, Kyungpook National University, Daegu, Korea; [b]Center for Neurosensory Disorders and [c]Department of Cell and Molecular Physiology, University of North Carolina, Chapel Hill, NC, 27516, USA; [d]Department of Anesthesiology and Neurobiology, University of Utah, Salt Lake City, UT 84131, USA; Institute of Biomedicine, Physiology, University of Eastern Finland, Finland

*Correspondence:
Dong-Kuk, Ahn
Department of Oral Physiology, School of Dentistry, Kyungpook National University, 188-1 Sam Deok 2ga, Chung-gu, Daegu (700-412), Korea
Tel: 82-53-660-6840
Email: dkahn@knu.ac.kr

William Maixner
Center for Neurosensory Disorders, University of North Carolina, Chapel Hill, NC, 27599, USA
Tel: 919 966 0684
Email: maixnerw@dentistry.unc.edu

Abbreviated title: Thermal response of tooth pulp neurons

Number of words in Abstract: 176
Total number of total words (from Introduction to Discussion): 2524
Number of tables and figures: 3
Number of references: 28
Supplemental manuscript: 590
Supplemental figures: 2
Supplemental table: 1

Abstract

The response properties of tooth pulp neurons that respond to noxious thermal stimulation of the dental pulp have been not well studied. The present study was designed to characterize the response properties of tooth pulp neurons to noxious thermal stimulation of the dental pulp. Experiments were conducted on 25 male ferrets and heat stimulation was applied by a computer-controlled thermode. Only 15% of tooth pulp neurons (n=39) responded to noxious thermal stimulation of tooth. Tooth pulp neurons were found in both the superficial and deep nuclear regions of the subnucleus caudalis (Vc) and in the interface between nucleus caudalis and interpolaris (Vc/Vi). Thirty-seven neurons had cutaneous receptive fields and were classified as either NS (16) or WDR (21) neurons. Repeated heat stimulation of the dental pulp sensitized and increased the number of electrically evoked potentials of tooth pulp neurons. These results provide evidence that both the Vc and Vc/Vi region contain neurons that respond to noxious thermal stimulation of the dental pulp and that these cells may contribute to the sensitization process associated with symptomatic pulpitis.

Key words: Tooth pulp neuron; Trigeminal; Sensitization; Thermal stimulation; Dental pain, Tooth

Introduction

The trigeminal brainstem sensory nuclear complex relays somatosensory afferent information from the face including the oral cavity. Several previous studies demonstrated that trigeminal subnucleus caudalis relays orofacial nociceptive information, including input from the tooth pulp. Transganglionic tracers applied to the tooth pulp have revealed afferent projections to various rostro-caudal subdivisions of the trigeminal complex (Arvidsson and Gobel, 1981; Marfurt and Turner, 1984). Electrophysiological studies have also demonstrated that a large number of neurons in the medullary dorsal horn responded to electrical stimulation of the tooth pulp (Nord and Young, 1975; Yokota, 1975; Nord, 1976). However, most electrophysiological studies, which have examined tooth pulp input to trigeminal brain stem neurons, have been performed with non-natural electrical stimulation.

Very little is known about the response properties of tooth pulp neurons that respond to natural stimulation of the tooth pulp which evoke dental pain, although a few studies reported that tooth pulp-driven neurons respond to thermal stimulation of the tooth pulp (Hu and Sessle, 1984). Therefore, the present study was designed to characterize the response properties of tooth pulp neurons that respond only to natural stimulation of the tooth pulp in ferrets. The natural stimulus we utilized was noxious heat stimulation by the computer-controlled device and cold stimulation by Endoice® application. We also investigated the sensitization of tooth pulp neurons evoked by repeated noxious heat stimulation.

Methods

Animal preparation

All procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill. Experiments were carried out on 25 adult male ferrets (*Mustela putorius furo*) weighing between 0.9-1.4 kg. The animals were anesthetized with 3% halothane with oxygen for induction, followed by a mixture of chloral hydrate (110 mg/kg) and pentobarbital sodium (20 mg/kg) intraperitoneally. Anesthesia was maintained with periodic intravenous injections of a mixture of chloral hydrate (22 mg/kg) and pentobarbital sodium (4 mg/kg). Arterial blood pressure and body temperature were maintained at 90 ~ 120 mmHg and 37^{o}C ~ 38^{o}C, respectively. The neck muscles and the dura were removed to expose the medulla. The exposed surface of the medulla was covered with warm saline. During the recording sessions, animals were immobilized with vecuronium bromide (0.4 mg/kg) and artificially ventilated. End tidal CO_2 level was kept at 3.5 ~ 4.5%.

Tooth preparation for stimulation

The right upper and lower canine teeth were prepared for electrical and heat stimulation. The teeth were isolated by placing a dental rubber dam around the tooth, to prevent unintentional stimulation of other intra-oral tissues. In a recent study, we introduced a stimulator system that allows both electrical and thermal stimulation of the intact tooth using a computer-controlled device (Ahn et al., 2011). The computer-controlled device system delivers precisely controlled heat stimulus for thermal stimulation of the canines in ferrets. A custom-designed, computer-controlled probe was placed over the exposed tooth. This device was able to deliver either constant electrical current or noxious heat stimuli through the same probe. The cathode electrode was connected to the

canines and the anode electrode was positioned on the animal's neck muscle. The stimulating probe was secured to each canine with colloidal silver liquid (No. 16031, TED PELLA Inc.).

Extracellular recording of tooth pulp neurons

Extracellular recordings were conducted with glass electrodes with a resistance of 2-10 MΩ. In general, the first penetration was made 1.8 - 2.3 mm lateral to the midline at the level of the obex. In ferrets, this area shows dense c-*Fos* expression following noxious thermal stimulation of the maxillary and mandibular canines (Chattipakorn et al., 1999). Recording of single unit activity and measuring latency followed the conventional method which described in supplemental methods. Minimum conduction velocity was determined by post-stimulus histogram constructed from successive 50 consecutive electrical pulses delivered to the tooth pulp with standard techniques (Price et al., 1976; Hu et al., 1981; Bossut and Maixner, 1996).

Electrical and thermal stimulation of tooth

Constant current square wave pulses (2 ms duration) were delivered to the tooth once every second, as a search stimulus. Delivered current was monitored by assessing the voltage drop across a 100 Ω resistance in series with the preparation. In order to prevent stimulation of adjacent tissues, the intensity of the applied current did not exceed 150 -200 μA (Matthews and Searle, 1976). A computer-controlled probe also delivered heat stimuli applied either in a staircase or single pulse manner as described previously (Ahn et al., 2011). Endoice® (The Hygenic Co) was applied to the labial surface of the tooth. The cold stimulation was applied by soaked cotton pellets attached to a non-conducting material such as a gutta-percha rod for 10 sec. So, this stimulation did not produce electrical interferences during the recording of tooth pulp neurons, as described previously (Jyväsjärvi and Kniffki, 1987). In an *in vitro* experiment with the extracted canines, the rapid

cooling of the tooth decreased the pulp temperature to between 5-10°C, as described previously (Jyväsjärvi and Kniffki, 1987; Hu and Sessle, 1984).

Stimulation of the skin and oral mucosa

Only the tooth pulp neurons responding to thermal stimulation of tooth were further characterized by testing their responses to both non-noxious tactile and noxious stimuli, to assess convergent connections from the overlying facial skin and surrounding oral mucosa. Tooth pulp neurons with receptive fields in surrounding tissues were classified as low-threshold mechano-receptive neurons (LTM), wide dynamic range neurons (WDR), or nociceptive specific neurons (NS), as previously described (Hu and Sessle, 1984; Bossut and Maixner, 1996).

Statistical analysis

Sensitization of neuronal activities by repeated thermal stimulation were presented as mean ± SE for arithmetic averaging and compared statistically using the Student's t-test. In all statistical comparisons, $p<0.05$ was used as the criterion for statistical significance.

Results

Only thirty-nine tooth pulp neurons activated by noxious heat and/or cold applied to the canine teeth were recorded in the present study. Responding tooth pulp neurons were found in both the superficial and deep nuclear regions of the subnucleus caudalis (Vc) and in the interface between nucleus caudalis and interpolaris (Vc/Vi). Seven responding neurons showed spontaneous background activity.

Responses to natural noxious stimulation of the tooth

Fig. 1 shows the typical response of a tooth pulp neuron activated by stimulation of the right upper canine. This tooth pulp neuron had a cutaneous receptive field in the face and was located in lamina II (Fig. 1A). Electrical stimulation of the tooth with 50 successive pulses evoked responses with 10 - 15 msec delay, consistent with the Aδ fiber input (Fig. 1B). The tooth pulp neuron responded to both noxious (pinch) and non-noxious (brush) stimulation of the skin and was classified as a WDR neuron. The neuron was excited by both noxious heat and cold stimulation of the canine (Fig. 1C).

Responses of tooth pulp neurons to cutaneous stimulation

Almost all the tooth pulp neurons (37/39) activated by noxious heat and/or cold stimulation of tooth had a cutaneous receptive field located in the facial skin or in the oral cavity (Supplement Table 1). Two neurons, however, did not have identifiable receptive fields. Both these neurons were activated by both noxious heat and cold stimulation of the mandibular canine. Sixteen tooth pulp neurons that responded only to noxious stimulation of the cutaneous receptive fields were classified as NS neurons, whereas 21 tooth pulp neurons that responded to both noxious and non-noxious stimulation of the cutaneous receptive fields were classified as WDR neurons.

Tooth pulp neurons were also classified by their noxious heat and/or cold stimulation of the tooth. Three tooth pulp neurons were excited only by noxious heat stimulation of the tooth pulp. Fourteen neurons were excited only by noxious cold stimulation. Twenty neurons were excited by both types of stimuli to the tooth pulp. A total of 19 tooth pulp neurons were located in the superficial lamina and 18 in the deep lamina. Sixteen tooth pulp neurons were activated by stimulation of the maxillary canines and 18 by stimulation of the mandibular canines. Three neurons responded to electrical stimulation of both maxillary and mandibular teeth.

Response latencies of tooth pulp neurons to electrical stimulation

Minimum conduction velocity was determined to identify the latency distribution of tooth pulp neurons that responded to thermal stimulation. Almost all the tooth pulp neurons (36/39) received only Aδ-fiber input and three neurons received C-fiber input as well. The latency distribution of afferent input to the individual tooth pulp neurons is illustrated in Supplemental Fig. 1.

Sensitization of tooth pulp neurons by repeated noxious heat stimuli

Fig. 2 illustrates a typical example of sensitization by repeated noxious heat stimuli to the tooth pulp. This tooth pulp neuron was activated by noxious heat applied to the mandibular canine and was classified as a WDR neuron. The neuron was located in the lamina V and afferent input from the tooth pulp was in the Aδ range. Heat stimuli were applied to the canine with 1 min inter-trial intervals. During the first heat trial, the neuron responded at 60°C. The impulses evoked by heat stimulus were increased and the threshold temperature was lowered during the second heat trial. This tooth pulp neuron had spontaneous activity which was enhanced by repeated heat stimuli. Four (3WDR and 1NS) of the seven examined neurons (4WDR and 3NS) were sensitized by repeated noxious heat stimulation. In the four sensitized neurons, the second noxious heat stimulus

significantly increased the number of evoked impulses of tooth pulp neurons by 190%, compared to their response to the first noxious heat stimulus ($P<0.05$).

We also examined the effects of noxious heat conditioning on responses to electrical stimulation of the tooth in 7 tooth pulp neurons. Fig. 3 shows that noxious heat conditioning increased evoked responses produced by electrical tooth pulp stimulation. Ten consecutive electrical pulses were applied to the canine before and after a 2 min period of noxious heat conditioning. After heat stimulation, the responses evoked by electrical tooth pulp stimulation increased from 4.3 ± 0.2 to 6.6 ± 0.4 action potentials per test pulse ($p<0.01$).

Discussion

The present study demonstrated the response properties of tooth pulp neurons that responded to noxious thermal stimulation of the dental pulp. Although a few previous studies reported that tooth pulp neurons respond to thermal stimulation (Hu et al., 1981; Hu and Sessle, 1984), their response properties in noxious thermoreception of teeth have not been extensively investigated. The present study demonstrated the characterization of the response properties of trigeminal neurons that responded to noxious heat and/or cold stimuli applied to the canines, using a computer-controlled stimulator system (Ahn et al., 2011, in press) that allows both electrical and thermal stimulation. Only 15% of the neurons that responded to electrical stimulation of the tooth pulp also responded to noxious thermal stimulation of the tooth. These findings are consistent with results of previous studies which reported that less than 20 % of the neurons were excited by natural tooth pulp stimulation (Hu and Sessle, 1984). They also reported that heat stimulation was more effective than cold stimulation in evoking neuronal responses. However, our findings showed that a large proportion of tooth pulp neuron neurons responded to both noxious heat and cold stimulation of the tooth pulp. These contrasting findings may have resulted from variations in stimulation modalities and procedures. Moreover, our computer-controlled stimulator system, which accurately controls the target temperature, provides experimental conditions for identifying tooth pulp neurons that can respond to noxious heat.

In the present study, similar to the trigeminal neurons with cutaneous receptive fields (Beitel and Dubner, 1976), tooth pulp neurons with thermal input were sensitized by repeated heat stimuli applied to the tooth. Previous studies have also shown that repeated heat stimulation of the dental pulp causes sensitization of pulpal primary afferents (Matthhews, 1977; Ahlberg, 1978). In contrast, the finding of an apparent lack of sensitization of the tooth pulp neurons in a previous study (Hu and Sessle, 1984) is not in agreement with our findings. The present study demonstrated that the

second trial of thermal stimulation evoked tooth pulp neurons with low threshold of thermal stimuli and increased the thermally evoked responses, compared to that of the first trial of thermal stimulation. Several previous studies on central sensitization of trigeminal nociceptive neurons in the Vc demonstrated the involvement of P2X7 receptors (Itoh et al., 2011), NMDA receptors subunits and opioid-related inhibitory mechanisms (Kaneko et al., 2011), astroglial glutamate-glutamine shuttle (Tsuboi et al., 2011), and glia (Xie et al., 2007).

We recorded 39 tooth pulp neurons that responded to local noxious heat and/or cold stimulation of the dentition and they were found in both the Vc and the Vc/Vi interface. Similar to previous studies using electrical stimulation (Nord and Young, 1975; Yokota, 1975), neurons that responded to electrical tooth pulp stimulation fell into two groups. One group was located in the superficial layers and the other group in the deeper layers of both the Vc and the Vc/Vi interface. These results are consistent with our previous findings that these regions contain neurons that express the immediate early gene *c-Fos* in response to noxious heat stimulation of the canine teeth (Chattipakorn et al., 1999). The largest cluster of *Fos* positive neurons were found in the superficial laminae and deeper regions of the medullary dorsal horn near the level of the obex.

The present study demonstrated the tooth pulp neurons that received Aδ input from the tooth but very few neurons with C-fiber input. Only 3 tooth pulp neurons that responded to thermal stimulation received C-fiber input. In contrast to our findings, previous histological studies of the dental pulp have shown C-fibers to constitute a considerable proportion of the pulpal afferent nerve fibers in anatomical studies (Fried and Hildebrand, 1981; Holland and Robinson, 1983) and electrophysiological studies (Jyväsjärvi and Kniffki, 1987, 1989). Several previous studies demonstrated that conduction velocity was slower in the pulp compared to that outside of the tooth pulp (Cadden et al., 1983; Dong et al., 1985; Jyväsjärvi and Kniffki, 1989). These findings might partially explain why electrophysiological studies did not reveal a large number of central

trigeminal cells with C-fiber input. It is also possible that C-fiber input to trigeminal neurons is suppressed by electrical stimuli that cause a concomitant activation of myelinated A-β fibers, which may inhibit synaptic transmission via C-fibers.

It has been established that the central trigeminal neurons receive convergent inputs from the dentition and the nearby skin and mucosa (Robinson, 1979; Sessle et al., 1986). The extensive convergence of afferent input onto central trigeminal neurons is probably one of the central mechanisms responsible for the poor localization of pulpal pain. Therefore, patients often have difficulties in localizing an inflamed painful tooth (Friend and Glenwright et al., 1968). In the present study, almost all the tooth pulp neurons that responded to thermal stimuli received convergent input from the nearby skin or mucosa. Thus, the tooth pulp neurons were classified as either NS or WDR neurons based on their response to stimuli applied to their cutaneous or mucosal receptive fields.

In conclusion, the present study was designed to characterize the response properties of tooth pulp neurons that respond to noxious thermal stimulation of the dental pulp. The present results provide evidence that both the Vc and Vc/Vi interface contain neurons that respond to natural noxious stimulation of the dental pulp and that these cells may contribute to the sensitization process associated with symptomatic pulpitis.

Acknowledgements

This study was supported by DE 11661 from NIDCR to Dr. W. Maixner. The authors have no conflicts of interests to declare related to this study.

UU IR Author Manuscript

References

Ahlberg KF (1978). Influence of local noxious heat stimulation on sensory nerve activity in the feline dental pulp. *Acta Physiol Scand* 103:71-80.

Ahn DK, Monbureau O, Narhi M, Maixner W (2011). A novel computerized system for thermal stimulation of tooth in ferrets. *Journal of Neuroscience Methods* 203:305-310.

Arvidsson J, Gobel S (1981). An HRP study of central projections of primary trigeminal neurons which innervate tooth pulps in the cat. *Brain Res* 210:1-16.

Beitel RE, Dubner R (1976). Response of unmyelinated (C) polymodal nociceptors to thermal stimuli applied to monkey's face. *J Neurophysiol* 39:1160-1175

Bossut DF, Maixner W (1996). Effects of cardiac vagal afferent electrostimulation on the responses of trigeminal and trigeminothalamic neurons to noxious orofacial stimulation. *Pain* 65:101-109.

Cadden SW, Lisney SJ, Matthews B (1983). Thresholds to electrical stimulation of nerves in cat canine tooth-pulp with A beta-, A delta- and C-fibre conduction velocities. *Brain Res* 261:31-41.

Chattipakorn SC, Light AR, Willcockson HH, Närhi M, Maixner W (1999). The effect of fentanyl on c-fos expression in the trigeminal brainstem complex produced by pulpal heat stimulation in the ferret. *Pain* 82:207-215

Dong WK, Chudler EH, Martin RF (1985). Physiological properties of intradental mechanoreceptors. *Brain Res* 334:389-395.

Fried K, Hildebrand C (1981). Pulpal axons in developing, mature, and aging feline permanent incisors. A study by electron microscopy. *J Comp Neurol* 203:23-36.

Friend LA, Glenwright HD (1968). An experimental investigation into the localization of pain from the dental pulp. *Oral Surg Oral Med Oral Pathol* 25:765-774.

Holland GR, Robinson PP (1983). The number and size of axons at the apex of the cat's canine tooth. *Anat Rec* 205:215-222.

Hu JW, Dostrovsky JO, Sessle BJ (1981). Functional properties of neurons in cat trigeminal subnucleus caudalis (medullary dorsal horn). I. Responses to oral-facial noxious and nonnoxious stimuli and projections to thalamus and subnucleus oralis. *J Neurophysiol* 45:173-192.

Hu JW, Sessle BJ (1984). Comparison of responses of cutaneous nociceptive and nonnociceptive brain stem neurons in trigeminal subnucleus caudalis (medullary dorsal horn) and subnucleus oralis to natural and electrical stimulation of tooth pulp. *J Neurophysiol* 52:39-53.

Itoh K, Chiang CY, Li Z, Lee JC, Dostrovsky JO, Sessle BJ (2011). Central sensitization of nociceptive neurons in rat medullary dorsal horn involves purinergic P2X7 receptors. *Neuroscience* 192:721-731.

Jyväsjärvi E, Kniffki KD (1987). Cold stimulation of teeth: a comparison between the responses of cat intradental A delta and C fibres and human sensation. *J Physiol* 1987;391:193-207.

Jyväsjärvi E, Kniffki KD (1989). Afferent C fibre innervation of cat tooth pulp: confirmation by electrophysiological methods. *J Physiol* 411:663-675.

Kaneko M, Kaneko T, Kaneko R, Chokechanachaisakul U, Kawamura J, Sunakawa M, Okiji T, Suda H (2011). The role of N-methyl-D-aspartate receptor subunits in the rat thalamic mediodorsal nucleus during central sensitization. *Brain Res* 1371:16-22.

Marfurt CF, Turner DF. The central projections of tooth pulp afferent neurons in the rat as determined by the transganglionic transport of horseradish peroxidase. J Comp Neurol 223:535-547.

Matthews B (1977). Responses of intradental nerves to electrical and thermal stimulation of teeth in dogs. *J Physiol* 264:641-664.

Matthews B, Searle BN (1976). Electrical stimulation of teeth. *Pain* 2:245-51.

Nord SG (1976). Electrical stimulation of the tooth pulp in the study of pain. *Brain Res Bull* 1:251- 254.

Nord SG, Young RF (1975). Projection of tooth pulp afferents to the cat trigeminal nucleus caudalis. *Brain Res* 90:195-204.

Price DD, Dubner R, Hu JW (1976). Trigeminothalamic neurons in nucleus caudalis responsive to tactile, thermal, and nociceptive stimulation of monkey's face. *J Neurophysiol* 39:936-53.

Robinson PP (1979). The course, relations and distribution of the inferior alveolar nerve and its branches in the cat. *Anat Rec* 195:265-271.

Sessle BJ, Hu JW, Amano N, Zhong G (1986). Convergence of cutaneous, tooth pulp, visceral, neck and muscle afferents onto nociceptive and non-nociceptive neurons in trigeminal subnucleus caudalis (medullary dorsal horn) and its implications for referred pain. *Pain* 27:219-235.

Tsuboi Y, Iwata K, Dostrovsky JO, Chiang CY, Sessle BJ, Hu JW (2011). Modulation of astroglial glutamine synthetase activity affects nociceptive behaviour and central sensitization of medullary dorsal horn nociceptive neurons in a rat model of chronic pulpitis. *Eur J Neurosci* 34:292-302.

Xie YF, Zhang S, Chiang CY, Hu JW, Dostrovsky JO, Sessle BJ (2007). Involvement of glia in central sensitization in trigeminal subnucleus caudalis (medullary dorsal horn). Brain Behav Immun 21:634-641.

Yokota T (1975). Excitation of units in marginal rim of trigeminal subnucleus caudalis elicited by tooth pulp stimulation. *Brain Res* 95:154-158.

Figure Legend

Fig. 1. A representative example of a tooth pulp neuron that responded to noxious heat and cold stimulation. A: The cutaneous receptive field and camera lucida drawing of the cell's location within the trigeminal nucleus. The receptive field was located in the right facial area and the upper lip. The cell was classified as a WDR neuron and was located in lamina II. B: Peristimulus histogram to electrical stimulation of the tooth. The neuron received Aδ afferent input. C: Responses to noxious cold and heat stimulation of the upper canine. A computer-controlled probe delivered heat stimuli which were applied in a staircase manner, from an adapting temperature of 40^{o}C, increasing upto 60^{o}C in 5^{o}C increments every 25 seconds, or as a single pulse for 60 seconds. This particular neuron was excited by both noxious heat and cold stimulation of the tooth.

Fig. 2. Response of a tooth pulp neuron to repeated noxious heat stimulation. A: Location of the cutaneous receptive field and camera lucida drawings of the cell's location. The receptive field was located in the surrounding gingiva and right lower lip. The cell was classified as a WDR neuron and was located in lamina V. B: Peristimulus histogram to electrical stimulation of the tooth (50 presentations) revealed that this cell received Aδ afferent input. C: Responses of the cell to repeated noxious heat stimulation of the lower canine. The computer-controlled probe delivered repeated heat stimuli which were gradually applied from an adapting temperature of 40^{o}C, increasing upto 60^{o}C in 5^{o}C increments every 25 seconds. The second noxious heat stimuli significantly increased the firing rate of the tooth pulp neurons.

Fig. 3. Evoked responses produced by electrical stimulation before and after noxious heat stimulation of the ooth pulp neurons, performed to evaluate sensitization of the tooth pulp neurons. The evoked action potentials in 7 neurons that responded to 10 electrical stimuli applied to the dental pulp are presented. The responses after heat conditioning were significantly increased, compared to those before heating (* $p < 0.05$). Results are mean ± SEM at each time point tested.

Supplemental Methods

Extracellular recording of tooth pulp neurons

The single unit activity was amplified, fed to a window discriminator and audio monitor, and displayed on an oscilloscope. Additionally, the discriminated spikes were sent to a digital interface (CED 1401, Cambridge, UK) and computer for on-line and off-line analysis and storage. Latency values were corrected for a 0.5 msec synaptic delay and conduction distances were estimated by measuring the length of a thread positioned along the primary afferent route. Neuronal responses with estimated conduction velocities greater than 2 m/sec were assumed to receive A-fiber afferent input, whereas those with estimated conduction velocities less than 2m/sec were assumed to receive C-fiber afferent input.

Histological procedure

After the completion of the recording, animals were deeply anesthetized with an overdose of pentobarbital sodium. Cardiac perfusion with 0.01 M phosphate-buffered saline (PBS) followed by a 10% formaldehyde fixative solution was performed. After the perfusion, the brainstem was collected, postfixed in 10% formaldehyde for approximately 24 hours, and placed in 30% sucrose in 0.01 M PBS for 2-3 days. The 60 μm sections were processed. The histological sections were mounted on slides and stained with cresyl violet to confirm the recording sites.

Supplemental Results

Response latencies of the tooth pulp neurons to electrical stimulation

Supplemental Fig. 1 illustrates the latency distributions of individual tooth pulp neurons activated by electrical stimulation. The results indicate that almost all the tooth pulp neurons (36/39) received only Aδ-fiber input and three neurons received C-fiber input as well. Neurons were denoted to receive C-fiber input if time-locked neuronal discharge appeared at a latency of 50 msec or greater, which is consistent with an afferent conduction velocity of less than 2 m/sec. A neuron was denoted to

receive Aδ input if the afferent conduction velocity was between 2 m/sec and 40 m/sec corresponding to latency between 2.5 msec and 50 msec. The type of afferent input from the tooth pulp did not vary according to the cutaneous receptive field properties or the nature of the stimulus-evoked neuronal responses.

Responses of a tooth pulp neuron with a cutaneous receptive field and was classified as a NS neuron

Supplemental Fig. 2 shows the typical response of a tooth pulp neuron activated by stimulation of the right lower canine. This tooth pulp neuron had a cutaneous receptive field in the mandibular area and was located in lamina II (Suppl Fig. 2A). Electrical stimulation of the tooth with 50 successive pulses evoked responses with 12 - 15 msec delay, consistent with the Aδ fiber input (Suppl Fig. 2B). This tooth pulp neuron responded only to noxious stimulation of the skin and was classified as a NS neuron. The neuron was excited by noxious heat stimulation of the canine (Suppl Fig. 2C).

Supplemental Summary

In summary, the present study was designed to characterize the response properties of tooth pulp neurons that respond to noxious thermal stimulation of the dental pulp. The 39 recorded tooth pulp neurons were found in both the superficial and deep nuclear regions of the Vc and the Vc/V interface. Thirty-seven of the 39 examined tooth pulp neurons were classified as either NS (16) or WDR (21). Repeated noxious heat stimulation of the dental pulp increased the mean firing rate of the tooth pulp neurons and the number of potentials evoked by electrical stimulation of the tooth. These results provide evidence that both the Vc and Vc/Vi interface contain neurons that respond to natural noxious stimulation of the dental pulp and that these cells may contribute to the sensitization process associated with symptomatic pulpitis.

UU IR Author Manuscript

Supplemental Fig. 1. The latency distributions of afferent input to the individual tooth pulp neurons. Thirty-seven tooth pulp neurons were recorded. All the tooth pulp neurons responded to noxious cold and/or heat stimulation of the dental pulp and had cutaneous receptive fields.

Supplemental Fig. 2. A typical example of a tooth pulp neuron with a cutaneous receptive field and was classified as a NS neuron. A: The cutaneous receptive field and camera lucida drawings of the cell's location within the trigeminal nucleus. The receptive field was located n the right lower jaw area. The cutaneous receptor cell was classified as a NS neuron and was located in lamina II. B: Peristimulus histogram to electrical stimulation of the tooth. The neuron received Aδ afferent input. C: Responses to noxious heat stimulation of the lower canine. A computer-controlled probe delivered heat stimuli which were applied in a staircase manner, from an adapting temperature of 40^{o}C, increasing upto 60^{o}C in 5^{o}C increments every 25 seconds. This particular neuron was excited by both noxious heat stimulation of the tooth. V26 or V 60: application of 26 g or 60 g of Von Frey.

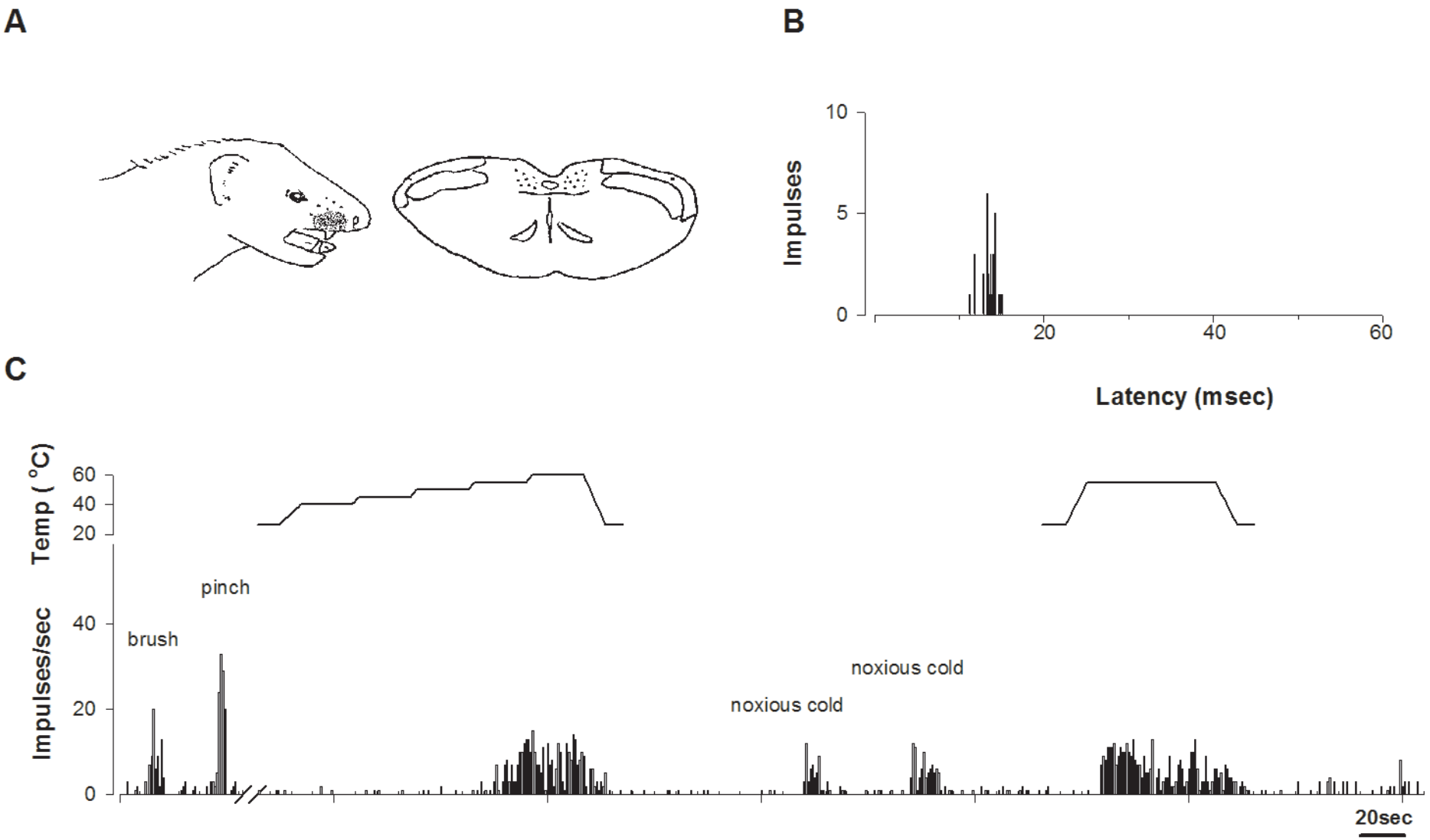
UU IR Author Manuscript

Supplemental Table 1. The number of individual tooth pulp cells in brainstem that responded to noxious stimulation

	Receptor types		Position		Location of tooth		
	WDR	**NS**	**Sup**	**Deep**	**Max**	**Man**	**Both**
Heat only	**2**	**1**	**1**	**2**	**2**	**1**	**0**
Cold only	**6**	**8**	**7**	**7**	**4**	**9**	**1**
Heat & Cold	**13**	**7**	**11**	**9**	**10**	**8**	**2**

Two tooth pulp neurons located in deep lamina did not have cutaneous receptive fields. Both were stimulated from the lower canine and were excited by noxious heat and cold stimuli.

UU IR Author Manuscript


A
B
10
5
0
Impulses
20
40
60
Latency (msec)
C
Temp (°C)
60
40
20
Impulses/sec
40
20
0
brush
pinch
noxious cold
noxious cold
20sec