Topical Review. Dental Pain and Odontoblasts: Facts and Hypotheses

Henry Magloire, DDS, PhD
Professor
University of Lyon; and
Ecole Normale Supérieure de Lyon

Jean Christophe Maurin, DDS, PhD
Professor
University of Lyon; and
Ecole Normale Supérieure de Lyon; and
Faculté d'Odontologie
University of Reims Champagne-Ardennes

Marie Lise Couble, MS
Researcher
University of Lyon; and
Ecole Normale Supérieure de Lyon

Yoshiyuki Shibukawa, DDS, PhD
Senior Assistant Professor
Oral Health Science Center; and
Department of Physiology
Tokyo Dental College, Chiba, Japan

Maki Tsumura, MS
Research Associate
Oral Health Science Center; and
Department of Physiology, Tokyo Dental College, Chiba, Japan; and
Department of Clinical Pharmacy
Toho University, Funabashi, Japan

Béatrice Thivichon-Prince, DDS, PhD
Senior Lecturer
University of Lyon; and
Ecole Normale Supérieure de Lyon

Françoise Bleicher, PhD
Professor
University of Lyon; and
Ecole Normale Supérieure de Lyon

Correspondence to:
Henry Magloire
Institut de Génomique Fonctionnelle de Lyon, Equipe Odontoblaste et régénération des tissus dentaires"
UMR CNRS 5242, Ecole Normale Supérieure
46, allée d'Italie
69364 Lyon Cedex 08, France
Email: magloire@univ-lyon1.fr

Dental pain arises from exposed dentin following bacterial, chemical, or mechanical erosion of enamel and/or recession of gingiva. Thus, dentin tissue and more specifically patent dentinal tubules represent the first structure involved in dentin sensitivity. Interestingly, the architecture of dentin could allow for the transfer of information to the underlying dental pulp via odontoblasts (dentin-forming cells), via their apical extension bathed in the dentinal fluid running in the tubules, or via a dense network of trigeminal sensory axons intimately related to odontoblasts. Therefore, external stimuli causing dentinal fluid movements and odontoblasts and/or nerve complex responses may represent a unique mechanosensory system bringing a new role for odontoblasts as sensor cells. How cells sense signals and how the latter are transmitted to axons represent the main questions to be resolved. However, several lines of evidence have demonstrated that odontoblasts express mechano- and/or thermosensitive transient receptor potential ion channels (TRPV1, TRPV2, TRPV3, TRPV4, TRPM3, K_{Ca}, TREK-1) that are likely to sense heat and/or cold or movements of dentinal fluid within tubules. Added to this, voltage-gated sodium channels confer excitable properties of odontoblasts in vitro in response to injection of depolarizing currents. In vivo, sodium channels co-localize with nerve terminals at the apical pole of odontoblasts and correlate with the spatial distribution of stretch-activated K_{Ca} channels. This highlights the terminal web as the pivotal zone of the pulp/dentin complex for sensing external stimuli. Crosstalk between odontoblasts and axons may take place by the release of mediators in the gap space between odontoblasts and axons in view of evidence for nociception-transducing receptors on trigeminal afferent fibers and expression of putative effectors by odontoblasts. Finally, how axons are guided to the target cells and which kind of signaling molecules are involved is extensively discussed in this review. J OROFAC PAIN 2010;24:335–349

Key words: dental pain, mechano/thermosensitivity, nerves, odontoblast, teeth

Dentinal sensitivity is a clinical condition daily encountered by practitioners. It results in pain arising in response to thermal, tactile, osmotic, or chemical stimuli and constitutes the symptoms of dentinal hypersensitivity, a common dental pain affecting between 4% to 74% of the population.[1] However, the management of this pathology is not always effective due to the lack of knowledge particularly concerning the means by which dental nociceptive signals are transduced. Dentin sensitivity results in the stimulation of exposed patent dentinal tubules currently encountered in cervical abrasion or erosion, regardless of their location.

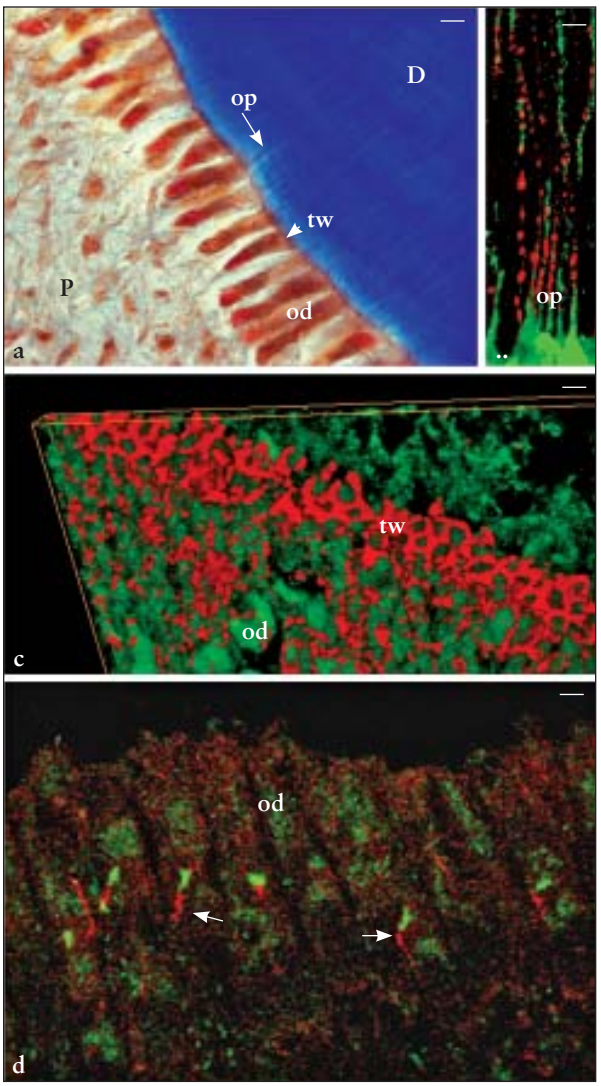

Fig 1 Spatial organization of odontoblasts and nerve fibers in the pulpal-dentinal border.

(a) Longitudinal section of a healthy human third molar germ (from 14- to 16-year old) extracted for orthodontic reasons. The odontoblast layer (od) is organized as a single layer at the interface between pulp tissue (P) and dentin (D) with cell processes (op) running in the dentinal tubules. At the site of the issue of the odontoblast processes corresponding to the borderline between cell processes and bodies are zonular tight junctions (terminal web, TW) (Masson's trichrome staining). Bar: 20 µm.

(b) Confocal laser microscopy and three-dimensional reconstruction (confocal image stacks were treated by 3D-visualization software: Amira version 3.1; Mercury) of the close relationship between nerve varicosities identified with antibodies against acetylated α tubulin *(red)* (clone 6-11B1, Sigma) and odontoblast processes running in dentinal tubules *(green)* identified with antibodies against β tubulin (H-235, Santa Cruz Biotech). Bar: 6 µm.

(c) Confocal laser microscopy and 3D reconstruction of the TW identified with specific antibodies against ZO-1 (*red*, Zymed Lab), between odontoblasts (*green*, β tubulin). Bar: 6 µm.

(d) Double staining of acetylated α tubulin *(red)* identifying the cilium axoneme and rootletin *(green)*, a protein constituting the rootlet of the cilium.[36] Bar: 5 µm.

However, the mechanisms underlying dentin sensitivity still remain unclear, probably due to the structural and functional complexity of the players that include odontoblasts, nerve endings, and dentinal fluid running in the dentinal tubules.

Various theories have been raised for more than a century and, among them, the hydrodynamic hypothesis first proposed in the 1960s remains the most widely accepted theory to date. This theory developed by Brannström and Astrom[2] is based on the specific architecture of dentin and movements of the dentinal tubules liquid content in response to various stimuli. Effluxes of dentinal fluid within tubules at exposed dentin have been carefully analyzed in vivo and in vitro.[3–8] Alteration of the flow and the pressure changes of the dentinal fluid could then distort the pulp nerve fibers, and their stimulation is believed to cause pain. Nevertheless, growing evidence suggests that odontoblast could be a key cell in the transduction process.[9–13] Odontoblasts, the dentin-producing cells, form a continuous cell layer at the junction between dentin and pulp (Fig 1a).[14,15] This unique spatial situation is enhanced by the localization of sensory axons, particularly dense near the pulp horn tips. Most of them terminate in the odontoblast and/or predentin layer and do not extend beyond the inner part of the dentin.[16] Beaded nerve endings situated in the dentinal tubules are thinner than the odontoblast processes they coil around (Fig 1b). Sensory afferents from trigeminal ganglia are classified into fast conducting A-β fibers (myelinated, large diameter), A-δ fibers (lightly myelinated, medium diameter) that terminate as free-nerve endings thought to be responsible for the "prepain" sensation, and C fibers (unmyelinated, small diameter), implicated in dull aching sensations.[17,18] The nerves

form a highly plastic system of nociceptive fibers, participating in tooth preservation by preventing damage and limiting the extent and duration of initial pulp injuries.[19] Pulpal nerve fibers are also involved in the repair processes by facilitating healing mechanisms and controlling the inflammatory and immune responses.[20] Collectively, these data suggest that odontoblasts are potentially best placed to sense both external stimuli and transient changes in pulp microcirculation.

This review will therefore be focused on the role of odontoblasts in the perception of dental pain with special attention to the molecular structures of identified effectors (ion channels, primary cilium), to the potential crosstalk between odontoblasts and nerve endings, and to molecules involved in the guidance of trigeminal axons to targeted odontoblasts. Finally, the possible role of the latter in dental pain during inflammatory processes will be also discussed.

Odontoblast: A Mechanosensory Cell

Dentin forms the bulk of the tooth, and the underlying soft pulp tissue provides dentinogenesis, nutritive, sensory, and defensive functions. Surrounding the pulp and separating it from the dentin are the odontoblasts that are organized as a single layer of palisade cells. They originate from neural crest-derived mesenchymal cells. Their terminal differentiation is characterized by the cessation of the mitotic activity, elongation, and cytological polarization, the nucleus occupying the proximal part of the cell body.[21,22] One of the architectural features of dentin is the presence of small parallel tubules corresponding to the means by which the pulp communicates with enamel and cementum. Each tubule contains the apical extension of one odontoblast that probably does not extend beyond the inner dentin in humans.[23] Added to this, the odontoblast process is filled with cytoskeletal elements (including vimentin, tubulin, actin)[24,25] and is bathed in the dentinal fluid containing an elevated concentration of potassium and lower values for sodium or calcium compared with serum.[26] These unique morphological features of odontoblasts are enhanced by the expression of specific functional markers including the dentin sialophosphoprotein (DSPP), dentin matrix protein -1 (DMP-1), neural molecules such as MAP-1B, MAP2, Tau (microtubules-associated protein), and nestin (intermediate filament).[27–33] Odontoblastic cell bodies are embedded in a dense network of trigeminal sensory axons intimately related to their cell membranes but without any known synapse-like structures.[34–37] At the apical pole of odontoblasts, ie, the zone connecting the cell bodies and their extensions, numerous junctional complexes, the so-called "terminal web" (desmosome-like, tight junctions) represent a selective barrier.[38–40] The latter could control the relationship between dentin and pulp and vice versa under physiological and pathological conditions (Fig 1c). Added to this, odontoblasts are able to communicate electrically and metabolically with each other and with the underlying subodontoblastic cells (Höhl's cells) via gap junctions and their intercellular channels, Cx43.[38,39,41,42] This provides the pulp/dentin complex with a major role in the local response to stress. Indeed, a wide range of external stimuli to teeth (high pressure, osmotic, chemical, thermal shocks) can elicit within dentinal tubules a fluid flow (acting as hydraulic links) all the way down to the odontoblasts that could conceivably translate tooth shock-induced signals into cell responses. This mechanotransduction process probably controls the dynamics of dentin architecture and plays a possible role in dental pain. Obviously, these processes involve mediators of signal transduction,[43] including at least extracellular matrix (ECM) components and their relationships with cell surface, cell-cell adhesion processes, cytoskeletal filaments, and specialized membrane structures (cilia, ion channels). In this context, the authors' investigations have been focused on odontoblast mechanosensitive ion channels and the primary cilium that could participate in signal transduction, molecular responses, and biological effects.

Primary Cilium of Odontoblasts: A Key Organelle for Sensing Microenvironment

Until recently, and for nearly a century, the primary cilium was considered as an orphan organelle and vestigial structure in cell biology.[44] This situation has changed dramatically in the past few years, and the primary cilium is now strongly recognized as a fundamental sensory organelle for detection and transmission of mechanical and/or chemical information from the cell microenvironment.[45–47] Cilia are present on almost all mammalian cells, and this structure projects into the extracellular space as an antenna. Mutations in genes that encode cilium components generate major human genetic diseases and syndromes, including polycystic kidney disease (PKD), retinal degenerative disease, and complex developmental defects in Bardet-Biedl syndrome (BBS) or oral-facial digital syndrome 1 (OFD1). Thus, it is becoming clear that the primary cilium plays a critical role by controlling important aspects of cellular physiology and development.[48–51]

The primary cilium of odontoblasts has been described close to the centriole and in the vicinity of the Golgi apparatus.[14,52,53] This typical structure emerges out of the cells and exhibits nine peripheral microtubule doublets forming the axoneme revealed by detyrosinated α tubulin antibody. A membrane-bound cylinder that is part of the plasma membrane surrounds this tubular backbone.[54] In the odontoblast layer, cilia are aligned parallel to the dentin walls, the top part oriented towards the pulp core, ie, following the cells pathway throughout the life of the tooth (Fig 1d). This suggests a possible role in cellular orientation or directed secretion of molecules from the Golgi apparatus, as described in cartilage.[55] In addition, proteins of intraflagellar transport machinery, ciliary rootlet, and basal body are also detected both in vivo and in vitro in odontoblast-like cells, thus demonstrating that odontoblasts express the major proteins of primary cilia.[56] Furthermore, close relationships between nerve fibers and cilia have been clearly documented as well as the expression of polycystins (PC1 and PC2), mechanosensor/Ca^{2+}-permeable cation channel heterodimeric complexes, localized at the base of the cilium and activated by a wide range of stimuli.[57–59] Interestingly, in kidney cells, the primary cilium is a fluid flow-sensor. Its bending opens Ca^{2+} channels through which Ca^{2+} flows into the cell and initiates signal transduction events. Thus, it is tempting to speculate that the odontoblast cilium might do the same thing in the sensing of the microenvironment, eg, if it bends over under dentin strain and fluid flow within dentinal tubules. Added to this, there may be a role in signal transduction for the establishment of cellular orientation related to dentin morphogenesis.[60] This hypothesis is supported by huge defects, observed on molars from Ofd1 knockout mice, showing the absence of odontoblast differentiation and disorganization of the tooth structure.[56]

Mechano/Thermosensitive Ion Channels at Strategic Sites

Analyses of effluxes of dentinal fluid flow within tubules have clearly demonstrated a relationship between dentin permeability and sensitivity that supports the hydrodynamic theory.[2,4,6–8] Thus, dentinal fluid shifts across dentin in response to the application of painful stimuli could cause sufficient shear forces to stimulate the odontoblast cell membrane. Cell membrane properties have been described in in vitro cultures of pulp cells, in freshly isolated odontoblasts from pulp cells, and in surviving odontoblasts from thick slices of pulp preparations. Thus, voltage-gated Na^+, K^+, and Cl^- selective channels have been described in the odontoblast membrane.[34,61–64] In addition, several lines of evidence indicate that calcium channels ($Ca_v1.2$) have a central role in odontoblast behavior, both at the physiological and pathological level.[65–68] In this context, the presence of K_{Ca} channels (high conductance Ca-activated K^+ channels) displaying mechanosensitivity (activation in response to membrane stretch) and their concentration at the apical pole (terminal web) of odontoblasts could have relevance in the sensory transduction process in teeth.[63] Additionally, stretch-activated TREK-1 K^+ channels whose expression in odontoblasts is closely related to the distribution of nerves in dental pulp[69] are assumed to be involved in polymodal pain perception.[70,71] Briefly, TREK-1 belongs to the family of K^+ channel subunits (15 members) with two pore domains and four transmembrane segments named K_{2P} channels.[72] They are opened at resting membrane potentials in physiological conditions and gated by a variety of chemical and physical stimuli, including stretch, cell swelling, intracellular acidosis, heat, polyunsaturated fatty acids, and volatile general anesthetic.[73] In addition, mechanosensitive Ca^{2+} channels of the N type ($Ca_v2.2$) clustered at the base of the cilium of odontoblasts could also contribute to odontoblastic transduction processes. Considering the general concept that the coordinated response to mechanical stress is based on increased intracellular free calcium,[74] it can be postulated that, in response to mechanical stimuli, the combination of increased intracellular Ca^{2+} plus membrane stretch could cause ion channels to open in odontoblasts, as previously shown by Shibukawa and Suzuki.[75] This could explain why K^+-containing agents placed in deep dentinal cavities induce a brief burst of high-frequency activity in the intradental nerves.[76,77] Very recently, mechanosensitive transient receptor potential (TRP) channels, a family of nonselective cation permeable channels such as TRPV4 and TRPM3, were identified in odontoblasts at the gene expression and functional level.[78]

In addition to this possible role of odontoblasts in the mechanotransduction of fluid movements within dentinal tubules into electrical signals, special attention should be paid to temperature changes perceived as pain by teeth and the putative role of temperature-sensing TRP channels (TRPV1, TRPV2, TRPV3).[79–82] The latter, also expressed by odontoblasts,[78,83] are directly related to sensory signals eliciting pain, particularly under thermal stimuli.[84–86] In this regard, TRPV1 (vanilloid receptor 1), sometimes referred to as the capsaicin receptor, can be activated by moderate heat (> 43°C); TRPV2, by heat stimulation above 52°C; and TRPV3, by nociceptive

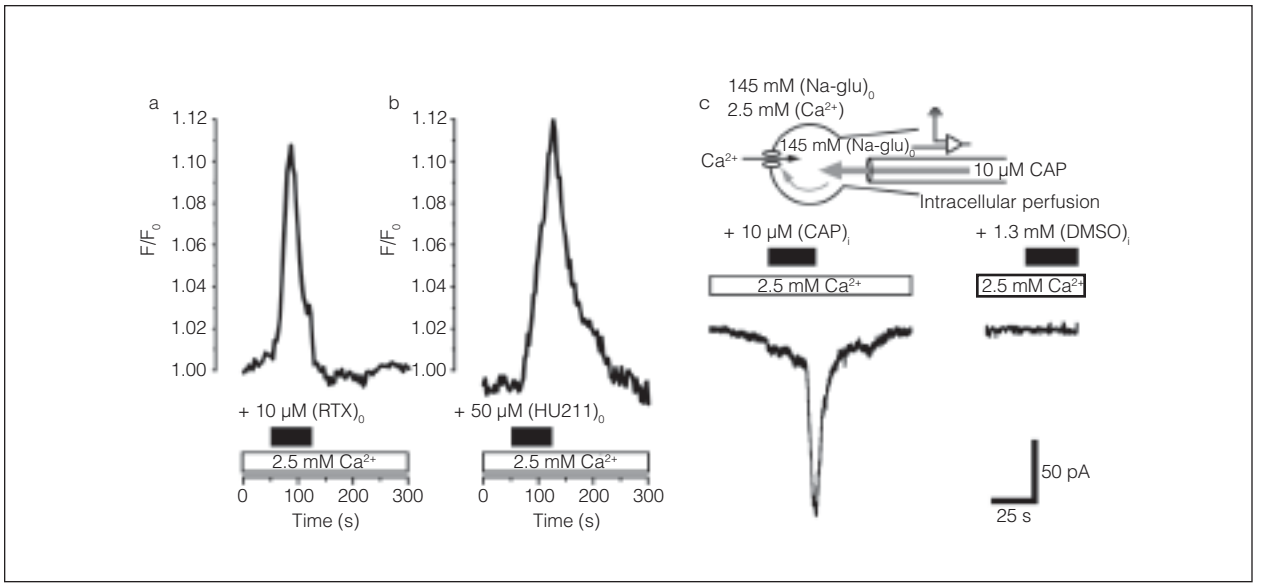

Fig 2 TRPV1 and TRPV2 channel activity recorded in rats' isolated odontoblasts.

Ca^{2+} entry by application of TRPV1 agonist resiniferatoxin (RTX, Alomone) *(a)*, and TRPV2 agonist HU211 (Tocris Cookson, UK) *(b)* was measured by fura-2 fluorescence. Odontoblasts were incubated in extracellular solution containing 10 μM fura-2 acetoxymethyl ester (AM) (Dojindo) and rinsed with fresh solution. Fura-2 fluorescent emission was measured at 510 nm in response to altering excitation wavelengths of 334 and 380 nm.[48,87,111,154] $[Ca^{2+}]i$ was expressed as fluorescence ratio (RF334/F380) at 380 nm and 334 nm. Response of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) was expressed as F/F0 units, with RF340/F380 values (F) normalized to resting value (F0). Both RTX and HU211 evoked a transient increase in $[Ca^{2+}]i$ (with extracellular 2.5 mM Ca^{2+} concentration), indicating expression of TRPV1 and TRPV2 in rat odontoblasts. Extracellular solution for the fura-2 experiments consisted of (in mM): 136 NaCl, 5 KCl, 2.5 CaCl2, 0.5 MgCl2, 10 Hepes, 12 NaHCO3, and 10 glucose (pH 7.4) in the presence or absence of agonist.

(c) Currents were evoked by TRPV1 channel agonist capsaicin (CAP, Sigma Chemical). Under continuous voltage-clamp conditions with a holding potential of –60 mV (with 2.5 mM extracellular Ca^{2+} concentration), CAP-induced inward current was recorded by intracellular application of 10 μM CAP ([CAP]i) by intracellular perfusion techniques (inset). However, intracellular application of vehicle (1.3 mM DMSO) could not activate ionic currents. In a conventional whole-cell voltage-clamp recording for odontoblasts, membrane current was obtained by intracellular solution (in mM; 145 K-glutamate, 5.0 MgATP, 5.0 EGTA, and 20 HEPES; [pH 7.2] in the presence or absence of capsaicin) and extracellular solution (in mM; 145 Na-glutamate, 2.5 $CaCl^2$, 1.0 $MgCl^2$, 10 HEPES, and 10 sucrose; [pH 7.4]). Whole-cell current was measured using a patch-clamp amplifier (L/M-EPC-7+, Heka Elektronik). Single living odontoblasts were prepared from dental pulp slices from newborn Wistar rats[64,68,75,83,87,88] and primarily cultured with alpha-minimum essential medium with 10% fetal bovine serum.

transduction mechanisms (32–39°C), and so these might play a major role in temperature-sensing and pain transmission in teeth (Fig 2).[48,64,69,75,83,87,88,111,154] Recent papers have demonstrated for the first time that excitatory TRP channels can work in association with K_{2P} channels (TRAAK and TREK-1, also known as a molecular sensor for temperature) to modulate the responsiveness to warm or cold of sensory neurons in the dorsal root ganglion.[70,89] Similarly in odontoblasts, the sensing of variations in temperature could correspond at least to a balance between TRP and TREK-1 channel activity.

The Odontoblast: An Excitable Cell

The view that odontoblasts could detect and transduce noxious stimuli into electrical signals raises the question whether these cells display excitable properties and possess voltage-gated Na^+ channels. The latter have been previously shown on dental pulp cells in vitro.[90] Recently, patch clamp recordings in cultured human odontoblasts and in vivo gene transcript analyses have demonstrated that odontoblasts express functional Na^+ channels composed of neural isoforms of α and associated β2 subunits.

Moreover, odontoblasts in vitro are able to produce action potentials when electrically stimulated.[34] The pore-forming α2 subunits are preferentially colocalized with β2 and nerve fibers at the terminal web of the odontoblast layer. Added to this, they correlate with the spatial distribution of mechanosensitive K^+ channels and L-type Ca^{2+} channels. Consequently, this unique concentration and network of key ions channels in the immediate environment where movements of dentinal fluid are first sensed suggests a pivotal role in signal transduction for the terminal web of the odontoblast layer. However, in vivo functional data are still needed to support this view.

Odontoblasts/Axons: A Possible Crosstalk?

Thus, it is tempting to speculate that odontoblasts might be able to integrate diverse somatosensory signals known to elicite nociceptive responses in the pulp (drilling, probing, fluid flow, heat, and cold) and initiate bursts of regenerative voltage responses. If true, this hypothesis raises the question of how the firing of odontoblasts is transmitted to neighboring nerve endings. Gap junctions (Cx43) have been identified in the odontoblast cell membrane and in trigeminal primary afferent cell bodies,[42,91–93] but no specific "junction-like structures" have been detected even when nerve fibers and odontoblasts are in close proximity.[12] In addition, no active intercellular communications between them has been demonstrated in vivo as well as in coculture of trigeminal ganglion cells with odontoblasts.[34,39] This is probably related to the fact that most connexin hemichannels open only under unphysiological conditions (extremely low extracellular calcium).[94,95] Thus, the release in the gap space between odontoblasts and nerve fibers of mediators from stimulated odontoblasts has to be seriously considered. In this context, neurotensin that has previously been detected in rat odontoblasts could modulate activity of the nociceptive fibers in the underlying nerve plexus.[96] Identification of nitric oxide synthases (NOS-I and NOS-III) in odontoblasts raises the possibility of a role for nitric oxide (NO) as a mediator when released by K^+ stimulation.[97,98] Added to this, extracellular ATP can also function as a signaling molecule, such as in taste bud cells, by activating P2X receptors expressed on sensory afferent nerves.[19,99–102] Indeed, $P2X_3$ receptor-expressing neurons have been previously identified in trigeminal ganglion cell bodies[103,104] and in the rat and human tooth pulp.[105,106] This purinergic receptor is a nonselective cation channel activated by ATP that may be released into the extracellular space as a result of tissue injury and inflammation. In teeth, $P2X_3$ are expressed on afferent nerve terminals, particularly in the odontoblastic layer (Fig 3). ATP is known to be a potent regulatory molecule, but the mechanism by which cells release ATP is still enigmatic. However, there is plausible evidence for a vesicle transport or exocytotic release through ATP-permeable hemichannels (pannexins, Panx1–3) depending on the type of cells. Interestingly, Panx3 is specifically expressed in skin and osteoblasts[107–111] and represents a second family of gap junction proteins in vertebrate stretch-activated channels. It would be of interest to focus attention on the role of this newly discovered channel in odontoblast physiology since it can be detected on the plasma membrane of odontoblast cell bodies (Fig 3). Thus, ATP released from stimulated odontoblasts could conceivably activate P2X receptors on nearby nerves and generate nociceptive signals. More recently, microarray analyses focused on gene expression profiles of human native and cultured odontoblasts have revealed a set of neuronal genes including tyrosine phosphatase receptor type Z1 (PTPRZ1) and galanin (GAL), both known to be involved in sensory signal transduction.[112–114] The identification of the GAL receptor 1 confined to unmyelinated axons (C-fibers) or thin Aδ fibers of the dental pulp near the odontoblasts also suggests a possible involvement of this peptide as a neuromediator.[20,115]

Collectively, these findings raise the question of whether a specific stimulus could activate a given mediator, thus interacting with target transducing-receptors expressed by dental axons (without considering the location of the stimulus applied to teeth). Indeed, trigeminal primary afferent neurons are chemically heterogeneous and appear to use various neuromediators for signal transmission.[116] Several lines of evidence have demonstrated that the first sharp pain induced in teeth (probing, drilling, osmotic and electric shock, heat, cold) may be related to stimulation of A fibers (Aβ and Aδ).[18] These fast-conducting fibers are particularly concentrated near the pulp horn tips where dental sensitivity is the greatest.[19,20] Here, mechanosensitive ion channels clustered at the apical pole of odontoblasts, close to the terminal web and in contact with axons, could be probably the most concerned in the induction of nociceptive signals. On the other hand, C fibers, which express peripherin filaments,[117] are located in the core of the pulp and extend to the subodontoblastic layer related to the basal pole of mature odontoblasts.[34] They need strong stimuli to be activated and are particularly sensitive to inflammatory mediators.[18] In this regard, ATP could be a good candidate as a mediator since $P2X_3$ receptors are predominantly expressed in C fibers.[104,116]

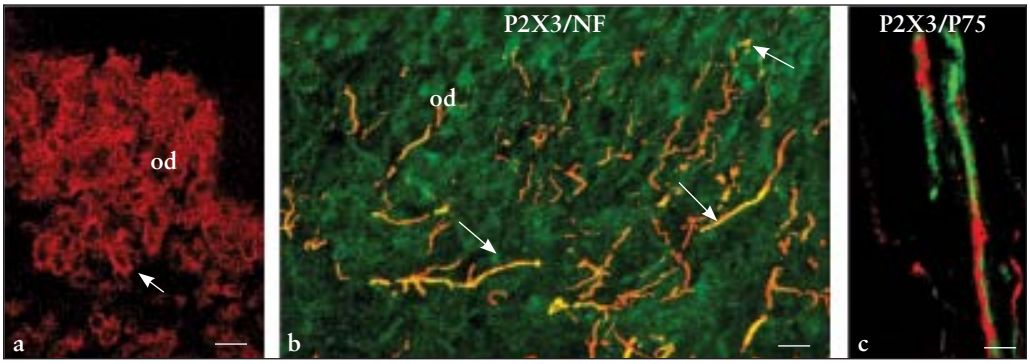

Fig 3 Localization of ATP-permeable hemichannels (Panx3) and $P2X_3$ receptors in human dental pulp.

(a) Panx3 antibodies (anti-Panx3, K16, Santa Cruz Biotechnology) produce marked fluorescence of odontoblast membrane *(arrow)*. Bar: 20 µm.

(b) Confocal laser microscopy clearly showing a similar localization of ATP receptors ($P2X_3$; *green*) and neurofilaments (*red*; antineurofilament H, Chemicon Int) running in the odontoblast (od) layer. Bar: 4 µm.

(c) Confocal laser microscopy and 3D reconstruction revealing a similar expression of $P2X_3$ *(red)* and p75 NTR *(green)*, a classical marker of trigeminal fibers innervating the dental pulp (Ad; C). Bar: 2 µm.

Furthermore, it should be pointed out that the complex effect of GAL is the result of differential activation of GAL receptors subtypes (R1 is inhibitory, and R2, excitatory). At present, the GAL receptor 1 is the only one identified on trigeminal C fibers, and it could be speculated that the effect of GAL is predominantly inhibitory by blocking the effect of substance P and calcitonin gene-related peptide (CGRP)[118] (Fig 4). Finally, it still cannot be excluded that a structural binding between odontoblasts and pulpal afferents could directly contribute (when stimulated following fluid movement within dentinal tubules) to the signal transduction via mechanotransducers (ASIC3) recently identified (at the gene expression level) in trigeminal ganglion neurons.[119]

The Odontoblast: A Target Cell for Nerve Endings

During development, the tooth pulp acquires a profuse sensory innervation originating from trigeminal ganglion. The major target area of trigeminal nerve endings is the odontoblast region of the crown, where the nerve fibers form a dense network of sensory axons. Nerve endings have been described as coiled around the cell bodies and the processes of odontoblasts within the dentinal canalicules.[12] The development of pulpal and dentinal innervation is closely linked to the developmental maturation of the teeth. Several studies on the localization of nerve fibers in human and murine teeth have shown that dental axonal growth and patterning take place in a spatiotemporally controlled manner and are closely linked with advancing tooth morphogenesis.[120] The development of sensory pulpal axons forming the subodontoblastic plexus of Raschkow and the appearance of nerve endings in the odontoblastic layer, predentin and dentin, are correlated with tooth eruption and root formation. In humans, this process is slow and full dental innervation occurs relatively late at the postnatal stages, many years after tooth eruption. Then it seems that growth and establishment of nerve terminals are controlled by local molecular signals.[121] The specificity of dental sensory innervation and the highly plastic aspect of the system imply that signals involved in the regulation of axonal growth must be selective in terms of sensory modalities. The molecular mechanisms allowing neurite outgrowth induction and establishment of target contact in the odontoblastic layer are poorly understood. However, there is some accumulating evidence that molecules involved in the development of the peripheral nervous system are

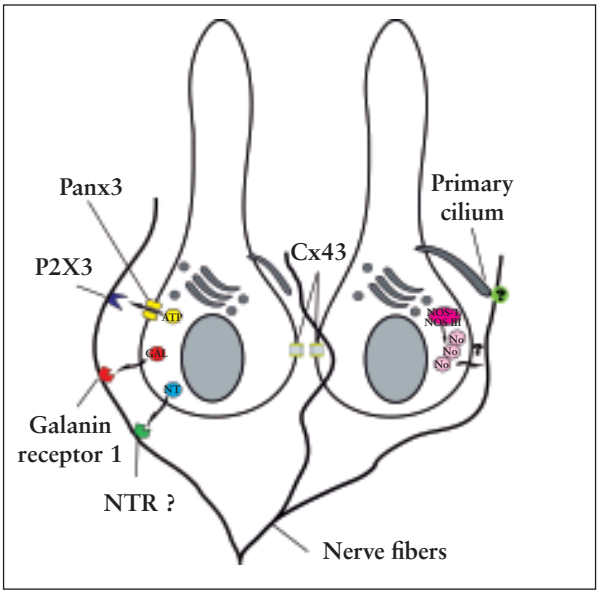

Fig 4 Schematic representation of the possible crosstalk between odontoblasts and nerve fibers. Gap junctions and Cx43 identified in odontoblast cell membranes could be involved in the transmission of information between these cells in the proximity of nerve terminals. Added to this, the expression of the ATP-permeable hemichannels Panx3 was identified in odontoblasts. Thus, the release of ATP in the extracellular space via Panx3 could stimulate the $P2X_3$ receptors identified on afferent nerve terminals situated in the odontoblastic layer. Other mediators released in the gap space, as GAL acting on the GAL receptor 1, or neurotensin (NT) receptors (NTR) which remain to be identified, could also modulate activity of the nociceptive fibers. The identification of NOS-I and NOS-III in odontoblasts indicates a putative role for NO as a cell mediator. The precise relationships between the odontoblast primary cilium and the nerve fibers are yet unknown.

also involved in dental axonal guidance. Therefore, it is plausible that molecular cues originating from odontoblasts might coordinate the establishment of the innervation in the odontoblast layer, predentin, and dentin.

During the development of the nervous system, growing nerve fibers are guided to their target by a combination of attractive and repulsive cues based on diffusible factors and contacts with cell surface and ECM protein. Among them, neurotrophic factors promote survival differentiation and maintenance of neurons in mammalian nervous system. The expression of nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) in the developing tooth pulp suggests that these molecules are key regulators of dental axonal guidance.[122] In rodents, the gene expression of NGF and GDNF is upregulated during postnatal stages in the dental papilla, particularly in the odontoblastic layer, predentin and dentin. To confirm the part played by neurotrophic factors during pulpal innervation, trigeminal ganglia were cocultured with dental pulp explants.[123] However, when the cocultures were treated with antineurotrophic blocking antibodies, no disruption of neurite outgrowth was observed. These results suggest that additional neuroregulatory molecules could be implicated in dental axonal guidance.

Signalling Molecules and Odontoblasts

The delayed establishment of the sensory innervation around the odontoblastic layer first requires the presence of neurorepelling factors. Different families of molecules have been identified in odontoblasts and could exert an inhibitory/repelling effect on neurite growth. These molecules include semaphorins, ephrins, and Bone Morphogenetic Proteins (BMP).[124,125] Semaphorins are a large and diverse family of repulsive/attractive proteins whose expression has been investigated during odontogenesis. Transcripts of several classes of semaphorins were already analysed by Real-Time Polymerase Chain Reaction (PCR) in tooth pulp tissue (semaphorins 3A, 3C, 3F, 4F, 5B, 6A, 6B, 6C). The higher levels have been observed earlier during tooth development (E13–E15), and their expression decreases in postnatal stages. Actually, a few numbers of semaphorins have been identified in odontoblasts: Sema3A, Sema4D, and Sema7A. Sema3A and Sema4D are two secreted molecules acting as chemorepellents of the peripheral nerve fibers.[126,127] Their transcripts have been identified in odontoblasts during the bell stage (E18).[128,129] Then, the expression of Sema3A decreases during later stages, whereas no obvious change in Sema4D expression has been observed.[129,130] Additionally, mRNAs of cell membrane-bound ephrin ligands A1 (ephA1) have been identified in preodontoblasts and odontoblasts. This protein, acting as a chemorepellent, may contribute to dental axonal guidance to the odontoblastic layer by defasciculation of dental axons.[131]

BMPs are a large family of signalling molecules influencing apoptosis, cell proliferation, and cell differentiation. BMP-7 can act as a chemorepellent and can collapse the growth cone of commissural neurons.[132] Transcripts of BMP-7 have been identified during mice tooth development in preodontoblasts (E18). This expression becomes weaker with advancing development (P1–P4).

Considering that the expression of these proteins is regulated in the odontoblastic layer mostly during the stages when this area is devoid of innervation, it is thus tempting to suggest that Sema3A, Sema4D, ephA1, and BMP-7 might prevent the arrival of pulpal nerve fibers close to odontoblasts and could explain the late innervation in this target zone. However, the precise part played by each of these molecules during dental pulp innervation remains to be determined, and their involvement in other developmental processes cannot be excluded.

The late arrival of nerve fibers in the odontoblastic layer requires also the presence of chemoattractive cues integrated by the pulpal nerve fibers. These signals can be brought by semaphorins, bifunctional proteins acting also as chemoattractant molecules. In particular, Sema7A is a membrane glycosylphosphatidylinositol (GPI)-anchored protein promoting axonal growth during the development of the olfactory system.[133] The expression of Sema7A has been clearly demonstrated in human odontoblasts during postnatal stages. This expression seems to be closely correlated with the process of pulpal and dentin innervation. Moreover, in coculture experiments, COS cells (immortalized cell line CV-1 derived from kidney cells of the African green monkey) transfected with a Sema7A plasmid construct are able to attract trigeminal nerve fibers.[134] These results suggest that Sema7A could be a good candidate to promote the final step of dentinal innervation by guiding developing axons to this final target field. Moreover, the neuregulin (NRG)-ErbB signalling pathway should be taken into account as a possible factor in dental pain. The growth factor NRG and the tyrosine kinase receptors ErbB are key regulators of axon-Schwann cell interactions. Moreover, interactions between nociceptive nerve fibers and NRG-ErbB have been correlated in transgenic mice that express dominant-negative Erb receptors in adult nonmyelinated Schwann cells.[135] A disruption of ErbB signalling leads to a progressive sensory neuropathy with a degeneration of unmyelinated axons and loss of heat and cold pain sensitivity. Transcripts of ErbB4 have been observed during rat molar development, and this expression is upregulated in the odontoblasts and subodontoblastic layer when the pulp nerves' outgrowth become intense in this area. These results strongly suggest that NRG-ErbB signalling may be involved in the regulation of odontoblastic layer innervation. This hypothesis is strengthened by the detection of NRG in neuronal cells of the trigeminal ganglion.[136]

ECM Protein and Plasticity of Pulpal Nerve Fibers

Dental nerve fibers form a highly plastic system since they constantly remodel throughout the life of the tooth. These changes occur with the shift from primary to permanent dentition, with aging, and with dental injury. A dental injury model used to study pulpal inflammatory processes has shown that sensory nerve fibers react to dentin injury by extensive sprouting of their terminal branches into the adjacent surviving pulp.[19] This response is localized in time and space. Its extent and duration are dependent on the severity and the nature of the injury, as well as the survival of odontoblasts. During minor dentin injuries, primary odontoblasts are not altered, and the sprouting of sensory nerve fibers runs under the lesion. This response can be related to the increase in dentinal sensitivity noted in human teeth after cavity drilling.[137] In contrast, a deep dentin cavity or a small pulp exposure leads to the destruction of primary odontoblasts. It is accompanied by a reduction of sensory innervation in the underlying pulp and by sprouting of sensory axons in the adjacent surviving pulp. Thus, the regeneration of pulpal nerve fibers could be under the control of factors originating from odontoblasts (Fig 5).

Many ECM proteins seem to be predominant molecules in axonal guidance by acting as promoters or inhibitors of neurite outgrowth and extension.[125] Among ECM proteins, several laminins (LNs) have been identified in odontoblasts (laminin α1, α2, β1 and γ1 subunits).[138,139] LNs are a large family of heterotrimeric glycoproteins composed of one α, one β, and one γ chain. In vitro studies have shown that LNs are potent inducers of neurite outgrowth during development and after axonal injury.[140,141] Recently, coculture experiments have demonstrated that LN-8 can promote the growth of trigeminal ganglion neurons.[138] Moreover, the expression of LNs seems to be increased during processes of dental nerve repair.[142] Thus, LNs elaborated by odontoblasts might constitute the key molecules for promoting and guidance of terminal neurite extension by mediating migration and attachment of trigeminal axons to the odontoblastic layer.

Reelin, another ECM protein, has been identified in mature human odontoblasts.[36] Reelin is a large ECM glycoprotein implicated both in embryonic and postnatal development. It plays a pivotal role in neuronal migration during development and could be involved in the modulation of adhesion processes of nerve endings in adults. In situ hybridization and immunohistochemistry performed on human tooth pulp tissues have shown a restricted

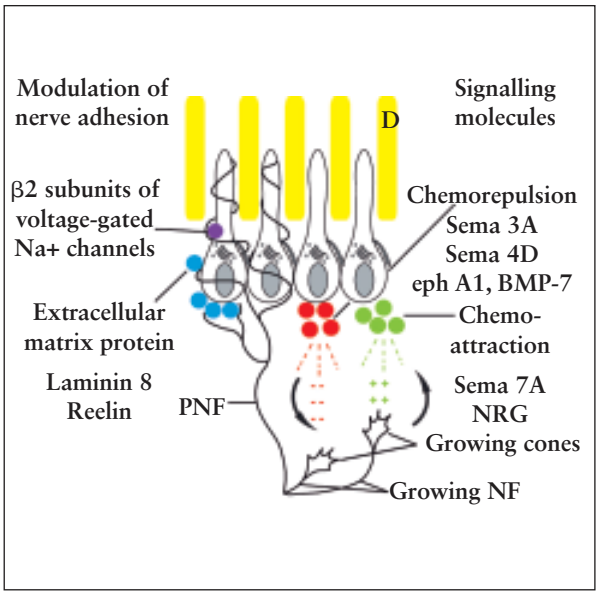

Fig 5 Schematic representation of the axonal guidance molecules secreted by odontoblasts during the establishment of the dentin/pulp complex innervation. Signalling molecules secreted by odontoblasts could diffuse in the extracellular environment and provide a chemoattractive or chemorepulsive signal to guide the growing pulpal nerve fibers in close contact to odontoblasts. The fibers could also be guided by contact-mediated mechanisms involving ECM proteins (as laminin 8 and reelin) acting on the modulation of adhesion process of nerve endings with their substrate. The modulation of the nerve adhesion could also be modulated by the β2– subunits of voltage-gated Na+ channels identified at the TW of the odontoblast layer. D: dentin; NF: nerve fibers; PNF: pulpal nerve fibers; Sema: semaphorin; eph: ephrin.

expression of reelin in the odontoblastic layer. In addition, cocultures of trigeminal axons with odontoblasts have demonstrated that a single neurite could be associated with odontoblasts through a varicosity (bead nerve terminal). Moreover, reelin is present in the microenvironment of the odontoblast cell membrane and colocalizes with nerve varicosities. This protein could also be involved in the modulation of adhesion processes between cells and nerve endings during the tooth life. This hypothesis is strengthened by the expression of VLDR, ApoER-2, CNR, and Dab-1 (assumed as reelin receptors and cytoplasmic adapter) in rat trigeminal ganglion. Reelin might have a pivotal role in the extension and branching of pulp axons into the target area as described in other tissues such as the hippocampus and the olfactory system.[143,144] Thus, by allowing a close contact between nerve fibers and odontoblasts, reelin could be an important molecule involved in the earliest step of nociceptive signal transduction, and this possibility should be explored.

Dental Pain, Odontoblasts, and Inflammatory Processes

Dentin sensitivity is enhanced by inflammation of the pulp, and this event is correlated with A-δ fibers responses.[18,20,77] This process is assumed to be the consequence of sensitization of nociceptors by inflammatory mediators. In this regard, it should be noted that odontoblasts are the first cells encountered by bacteria entering the dentin, and recent data have strongly suggested that they are involved in pulp inflammatory responses following dentin injury.[145] Bacterial byproducts such as lipoteichoic acid (LTA) or lipopolysaccharide (LPS) can be sensed by odontoblasts, and the recognition molecules include the Toll-like receptor family (TLRs). Among the latter, TLR2 and TLR4 genes are overexpressed by dentin-forming cells underneath carious lesions. Thereafter, TLR-activated receptors induce in odontoblasts the overexpression of chemokines such as CCL2 and CXCL10[146]; proinflammatory cytokines such as TNF-α and interleukin -1β[147]; or T-cell costimulatory molecules.[148] The local increase in inflammatory mediators triggers neuropeptides release (serotonin, CGRP) and contributes to increased pain sensitivity.[149] In addition, odontoblasts express phospholipase C (PLC)-coupled receptors (bradykinin receptors and ATP-binding metabotropic P2Y purinoreceptors) that are activated by extracellular signaling molecules released by inflammatory responses in the dental pulp.[69] Activation of these PLC-coupled receptors elicits inositol 1,4,5-trisphosphate (IP3)-induced Ca^{2+} release and subsequent store-operated Ca^{2+} channel (TRPC channel) opening after depletion of IP3-sensitive Ca^{2+} stores.[69] Finally, group I metabotropic glutamate receptors (mGluR5) expressed by human odontoblasts[150] and known to play a crucial role in nociceptive mechanisms associated with peripheral inflammation, may also be involved in this mechanism that remains to be elucidated in teeth. Thus, in the early steps of dentin damage (caries, attrition, abrasion, or restorative procedures), odontoblasts have features consistent with their being able to participate in pain perception.

Conclusions and Perspectives

It is well known that odontoblasts are involved in the dentin formation throughout the life of the tooth, but extensive lines of evidence outlined in this review

suggest that they conceivably could play a major role in dental pain. One of the most enigmatic features of these cells is that they are closely related to nerve endings; however, the nature of adhesion processes, the role of this intimate contact between them (including the primary cilium), and the molecules involved in this process are poorly understood. Nevertheless, biological signals from odontoblasts are probably transduced to axons and vice-versa. Indeed, sensory innervation occurs only in dentinal tubules with viable odontoblasts that maintain their columnar form only in these innervated regions (the root region is sparsely innervated and odontoblasts are cuboidal-shaped in contrast to elongated-shaped in the crown).[19] This kind of partnership, critical for odontoblast behavior, could be analogous to the role that neuregulin and its ErbBs receptors play in the control of cell morphology and in hyperalgesia.[135,151,152] Therefore, there is a need to identify the proteins that mediate signals between odontoblasts and nerves to determine if and which kind of trigeminal axons are specifically involved (particularly in the guidance to target odontoblasts), and to learn whether the cilium can act as a signal integrator. Further analysis is required of candidate transducing ion channels such as pannexins (also involved in intercellular calcium wave propagation) and of the mechanisms of interplay between mechano/thermo-sensitive ion channels themselves. For instance, TREK-1, when genetically inactivated, could provide evidence of its key role in tooth pain perception by modulating TRP channel sensitivity. Finally, the origin and ion composition of the dentinal fluid entrapped in calcified tubules have to be revisited, particularly the potential role of odontoblasts in the fluid transmembrane transport (involvement of chloride and/or aquaporin channels).[62,64,153,154] Nevertheless, the early step underlying dentin nociceptive signalling is probably a very intricate mechanism where odontoblasts likely play a pivotal role in the pulp/dentin complex as strain sensors by setting up a bridge between the neural and hydrodynamic theories previously proposed. This explains the difficulty in developing more effective therapeutic strategies for the treatment of dentin sensitivity.

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References

1. Bartold PM. Dentinal hypersensitivity: A review. Aust Dent J 2006;51:212–218.
2. Brannstrom M, Astrom A. The hydrodynamics of the dentine; its possible relationship to dentinal pain. Int Dent J 1972;22:219–227.
3. Brannstrom M, Linden LA, Astrom A. The hydrodynamics of the dental tubule and of pulp fluid. A discussion of its significance in relation to dentinal sensitivity. Caries Res 1967;1:310–317.
4. Charoenlarp P, Wanachantararak S, Vongsavan N, Matthews B. Pain and the rate of dentinal fluid flow produced by hydrostatic pressure stimulation of exposed dentine in man. Arch Oral Biol 2007;52:625–631.
5. Chidchuangchai W, Vongsavan N, Matthews B. Sensory transduction mechanisms responsible for pain caused by cold stimulation of dentine in man. Arch Oral Biol 2007;52:154–160.
6. Linsuwanont P, Versluis A, Palamara JE, Messer HH. Thermal stimulation causes tooth deformation: A possible alternative to the hydrodynamic theory? Arch Oral Biol 2008;53:261–272.
7. Pashley DH, Matthews WG, Zhang Y, Johnson M. Fluid shifts across human dentine in vitro in response to hydrodynamic stimuli. Arch Oral Biol 1996;41:1065–1072.
8. Vongsavan N, Matthews B. The permeability of cat dentine in vivo and in vitro. Arch Oral Biol 1991;36:641–646.
9. Matthews B, Andrew D, Amess T, Ikeda H, Vongsavan N. The functional properties of intradental nerves. In: Shimono M, Maeda T, Suda H, Takahashi K (eds). Dentin/Pulp Complex. Tokyo: Quintessence, 1996:146–153.
10. Gunji T. Morphological research on the sensitivity of dentin. Arch Histol Jpn 1982;45:45–67.
11. Holland GR. Morphological features of dentine and pulp related to dentine sensitivity. Arch Oral Biol 1994; 39(suppl):3S–11S.
12. Byers MR. Dental sensory receptors. Int Rev Neurobiol 1984;25:39–94.
13. Byers MR. Terminal arborization of individual sensory axons in dentin and pulp rat molars. Brain Res 1985;345:181–185.
14. Baume LJ. The biology of pulp and dentine. A historic, terminologic-taxonomic, histologic-biochemical, embryonic and clinical survey. Monogr Oral Sci 1980;8:1–220.
15. Ruch JV, Lesot H, Begue-Kirn C. Odontoblast differentiation. Int J Dev Biol 1995;39:51–68.
16. Carda C, Peydro A. Ultrastructural patterns of human dentinal tubules, odontoblasts processes and nerve fibres. Tissue Cell 2006;38:141–150.
17. Hildebrand C, Fried K, Tuisku F, Johansson CS. Teeth and tooth nerves. Prog Neurobiol 1995;45:165–222.
18. Närhi M, Jyväsjärvi E, Virtanen A, Huopaniemi T, Ngassapa D, Hirvonen T. Role of intradental A- and C-type nerve fibres in dental pain mechanisms. Proc Finn Dent Soc 1992;88(suppl 1):507–516.
19. Byers MR, Närhi M. Dental injury models: Experimental tools for understanding neuroinflammatory interactions and polymodal nociceptor functions. Crit Rev Oral Biol Med 1999;10:4–39.
20. Byers MR, Suzuki H, Maeda T. Dental neuroplasticity, neuro-pulpal interactions, and nerve regeneration. Microsc Res Tech 2003;60:503–515.
21. Couve E. Ultrastructural changes during the life cycle of human odontoblasts. Arch Oral Biol 1986;31:643–651.

22. Sasaki T, Garant PR. Structure and organization of odontoblasts. Anat Rec 1996;245:235–249.
23. Yoshiba K, Yoshiba N, Ejiri S, Iwaku M, Ozawa H. Odontoblast processes in human dentin revealed by fluorescence labeling and transmission electron microscopy. Histochem Cell Biol 2002;118:205–212.
24. Sigal MJ, Aubin JE, Ten Cate AR. An immunocytochemical study of the human odontoblast process using antibodies against tubulin, actin, and vimentin. J Dent Res 1985;64:1348–1355.
25. Nishikawa S, Kitamura H. Localization of actin during differentiation of the ameloblast, its related epithelial cells and odontoblasts in the rat incisor using NBD-phallacidin. Differentiation 1986;30:237–243.
26. Larsson PA, Howell DS, Pita JC, Blanco LN. Aspiration and characterization of predentin fluid in developing rat teeth by means of a micropuncture and micro-analytical technique. J Dent Res 1988;67:870–875.
27. About I, Laurent-Maquin D, Lendahl U, Mitsiadis TA. Nestin expression in embryonic and adult human teeth under normal and pathological conditions. Am J Pathol 2000;157:287–295.
28. Butler WT, Ritchie H. The nature and functional significance of dentin extracellular matrix proteins. Int J Dev Biol 1995;39:169–179.
29. MacDougall M, Simmons D, Luan X, Nydegger J, Feng J, Gu TT. Dentin phosphoprotein and dentin sialoprotein are cleavage products expressed from a single transcript coded by a gene on human chromosome 4. Dentin phosphoprotein DNA sequence determination. J Biol Chem 1997;272:835–842.
30. Maurin JC, Couble ML, Staquet MJ, et al. Microtubule-associated protein 1b, a neuronal marker involved in odontoblast differentiation. J Endod 2009;35:992–996.
31. Simon S, Smith AJ, Lumley PJ, et al. Molecular characterization of young and mature odontoblasts. Bone 2009;45:693–703.
32. Unterbrink A, O'Sullivan M, Chen S, MacDougall M. TGF beta-1 down regulates DMP-1 and DSPP in odontoblasts. Connect Tissue Res 2002;43:354–358.
33. Lu Y, Xie Y, Zhang S, Dusevich T, Bonewald LF, Feng JQ. DMP-1 targeted CRE expression in odontoblasts and osteocytes. J Dent Res 2007;86:320–325.
34. Allard B, Magloire H, Couble ML, Maurin JC, Bleicher F. Voltage-gated sodium channels confer excitability to human odontoblasts: Possible role in tooth pain transmission. J Biol Chem 2006;281:29002–29010.
35. Ibuki T, Kido MA, Kiyoshima T, Terada Y, Tanaka T. An ultrastructural study of the relationship between sensory trigeminal nerves and odontoblasts in rat dentin/pulp as demonstrated by the anterograde transport of wheat germ agglutinin-horseradish peroxidase (WGA-HRP). J Dent Res 1996;75:1963–1970.
36. Maurin JC, Couble ML, Didier-Bazes M, Brisson C, Magloire H, Bleicher F. Expression and localization of reelin in human odontoblasts. Matrix Biol 2004;23:277–285.
37. Byers MR, Närhi M, Dong WK. Sensory innervation of pulp and dentin in adult dog as demonstrated by autoradiography. Anat Rec 1987;218:207–215.
38. Calle A. Intercellular junctions between human odontoblasts. A freeze-fracture study after demineralization. Acta Anat (Basel) 1985;122:138–144.
39. Ushiyama J. Gap junctions between odontoblasts revealed by transjunctional flux of fluorescent tracers. Cell Tissue Res 1989;258:611–616.
40. Turner DF, Marfurt CF, Sattelberg C. Demonstration of physiological barrier between pulpal odontoblasts and its perturbation following routine restorative procedures: A horseradish peroxidase tracing study in the rat. J Dent Res 1989;68:1262–1268.
41. About I, Proust JP, Raffo S, Mitsiadis TA, Franquin JC. In vivo and in vitro expression of connexin 43 in human teeth. Connect Tissue Res 2002;43:232–237.
42. Kagayama M, Akita H, Sasano Y. Immunohistochemical localization of connexin 4 in the developing tooth germ of rat. Anat Embryol 1995;191:561–568.
43. Ingber DE. Cellular mechanotransduction: Putting all the pieces together again. FASEB J 2006;20:811–827.
44. Wheatley DN, Wang AM, Strugnell GE. Expression of primary cilia in mammalian cells. Cell Biol Int 1996;20:73–81.
45. Christensen ST, Pedersen LB, Satir P, Veland IR, Schneider L. The primary cilium coordinates signaling pathways in cell cycle control and migration during development and tissue repair. Curr Top Dev Biol 2008;85:261–301.
46. Haycraft CJ, Serra R. Cilia involvement in patterning and maintenance of the skeleton. Curr Top Dev Biol 2008;85:303–327.
47. Gerdes JM, Davis EE, Katsanis N. The vertebrate primary cilium in development, homeostasis and disease. Cell 2009;137:32–45.
48. Badano JL, Mitsuma N, Beales PL, Katsanis N. The ciliopathies: An emerging class of human genetic disorders. Annu Rev Genomics Hum Genet 2006;7:125–148.
49. Pazour GJ, Witman GB. The vertebrate primary cilium is a sensory organelle. Curr Opin Cell Biol 2003;15:105–110.
50. Praetorius HA, Spring KR. A physiological view of the primary cilium. Annu Rev Physiol 2005;67:515–529.
51. Nauli S, Kawanabe Y, Kaminski JJ, Pearce WJ, Ingber DE, Zhou J. Endothelial cilia are fluid shear sensors that regulate calcium signaling and nitric oxide production through polycystin-1. Circulation 2008;117:1161–1171.
52. Magloire H, Couble ML, Romeas A, Bleicher F. Odontoblast primary cilia: Facts and hypotheses. Cell Biol Int 2004;28:93–99.
53. Magloire H, Couble ML, Thivichon-Prince B, Maurin JC, Bleicher F. Odontoblast: A mechano-sensory cell [review]. J Exp Zool B Mol Dev Evol 2009;312B:416–424.
54. Satir P, Pedersen LB, Christensen ST. The primary cilium at a glance. J Cell Science 2010;123:499–503.
55. Ascenzi MG, Lenox M, Farnum C. Analysis of the orientation of primary cilia in growth plate cartilage: A mathematical method based on multiphoton microscopical images. J Struct Biol 2007;158:293–306.
56. Thivichon-Prince B, Couble ML, Giamarchi A, et al. Primary cilia of odontoblasts: Possible role in molar morphogenesis. J Dent Res 2009;88:910–915.
57. Delmas P, Padilla F, Osorio N, Coste B, Raoux M, Crest M. Polycystins, calcium signaling, and human diseases. Biochem Biophys Res Commun 2004;322:1374–1383.
58. Giamarchi A, Padilla F, Coste B, et al. The versatile nature of the calcium-permeable cation channel TRPP2. EMBO Rep 2006;7:787–793.
59. Tsiokas L, Kim S, Ong EC. Cell biology of polycystin-2. Cell Signal 2007;19:444–453.
60. Marshall WF, Kintner C. Cilia orientation and the fluid mechanics of development. Curr Opin Cell Biol 2008;20:48–52.
61. Davidson RM. Potassium currents in cells derived from dental pulp. Arch Oral Biol 1993;38:803–811.

62. Guo L, Davidson RM. Potassium and chloride channels in freshly isolated rat odontoblasts. J Dent Res 1998;77:341–350.
63. Allard B, Couble ML, Magloire H, Bleicher F. Characterization and gene expression of high conductance calcium-activated potassium channels displaying mechanosensitivity in human odontoblasts. J Biol Chem 2000;275:25556–25561.
64. Shibukawa Y, Suzuki T. Ionic currents in odontoblasts and dental pulp cells. In: Nakamura Y and Sessle BJ (eds). Neurobiology of Mastication: From Molecular to System Approach. Amsterdam: Elsevier Science BV, 1999:165–168.
65. Seux D, Joffre A, Fosset M, Magloire H. Immunohistochemical localization of L-type calcium channels in the developing first molar of the rat during odontoblast differentiation. Arch Oral Biol 1994;39:167–170.
66. Lundgren T, Linde A. Voltage-gated calcium channels and non voltage-gated calcium uptake pathways in the rat incisor odontoblast plasma membrane. Calcif Tissue Int 1997;60:79–85.
67. Westenbroek RE, Anderson NL, Byers MR. Altered localization of Cav1.2 (L-type) calcium channels in nerve fibers, Schwann cells, odontoblasts and fibroblasts of tooth pulp after tooth injury. J Neurosci Res 2004;75:371–383.
68. Shibukawa Y, Suzuki T. Ca2+ signaling mediated by IP3-dependent Ca2+ releasing and stored-operated Ca2+ channels in rat odontoblasts. J Bone Miner Res 2003;18:30–38.
69. Magloire H, Lesage F, Couble ML, Lazdunski M, Bleicher F. Expression and localization of TREK-1 K+ channels in human odontoblasts. J Dent Res 2003;82:542–545.
70. Honore E. The neuronal background K2P channels: Focus on TREK1. Nat Rev Neurosci 2007;8:251–261.
71. Murbartian J, Lei Q, Sando JJ, Bayliss DA. Sequential phosphorylation mediates receptor- and kinase-induced inhibition of TREK-1 background potassium channels. J Biol Chem 2005;280:30175–30184.
72. Lesage F, Lazdunski M. Molecular and functional properties of two-pore-domain potassium channels [review]. Am J Physiol Renal Physiol 2000;279:F793–801.
73. Patel AJ, Honore E. Properties and modulation of mammalian 2P domain K+ channels. Trends Neurosci 2001;24:339–346.
74. Hassinger TD, Guthrie PB, Atkinson PB, Bennett MV, Kater SB. An extracellular signaling component in propagation of astrocytic calcium waves. Proc Natl Acad Sci USA 1996;93:13268–13273.
75. Shibukawa Y, Suzuki T. Measurements of cytosolic free Ca2+ concentrations in odontoblasts. Bull Tokyo Dent Coll 1997;38:177–185.
76. Markowitz K, Bilotto G, Kim S. Decreasing intradental nerve activity in the cat with potassium and divalent cations. Arch Oral Biol 1991;36:1–7.
77. Markowitz K, Pashley DH. Discovering new treatments for sensitive teeth: The long path from biology to therapy. J Oral Rehabil 2008;35:300–315.
78. Son AR, Yang YM, Hong JH, Lee SI, Shibukawa Y, Shin DM. Odontoblast TRP channels and thermo/mechanical transmission. J Dent Res 2009;88:1014–1019.
79. Dhaka A, Viswanath V, Patapoutian A. TRP ion channels and temperature sensation. Annu Rev Neurosci 2006;29:135–161.
80. Cortright DN, Krause JE, Broom DC. TRP channels and pain. Biochim Biophys Acta 2007;1772:978–988.
81. Pedersen ST, Owsianik G, Nilius B. TRP channels: An overview. Cell Calcium 2005;233–252.
82. Park CK, Kim MS, Fang Z, et al. Function expression of thermo-transient receptor potential channels in dental primary afferent neurons: Implication in tooth pain. J Biol Chem 2006;281:17304–17311.
83. Okumura R, Shima K, Muramatsu T, et al. The odontoblast as a sensory receptor cell? The expression of TRPV1 (VR-1) channels. Arch Histol Cytol 2005;68:251–257.
84. Levine JD, Alessandri-Haber N. TRP channels: Targets for the relief of pain. Biochim Biophys Acta 2007;1772:989–1003.
85. Myers BR, Sigal YM, Julius D. Evolution of thermal response properties in a cold-activated TRP channel. PLoS One 2009;4:e5741.
86. Staaf S, Oerther S, Lucas G, Mattsson JP, Ernfors P. Differential regulation of TRP channels in a rat model of neuropathic pain. Pain 2009;144:187–199.
87. Okumura R, Shibukawa Y, Muramatsu T, et al. Sodium-calcium exchangers in rat ameloblasts. J Pharmacol Sci 2010;112:223–230.
88. Tsumura M, Okumura R, Tatsuyama S, et al. Ca^{2+} extrusion via Na^{+}-Ca^{2+} exchangers in rat odontoblasts. J Endod 2010;36:668–674.
89. Noel J, Zimmermann K, Busserolles J, et al. The mechano-activated K+ channels TRAAK and TREK-1 control both warm and cold perception. EMBO J 2009;28:1308–1318.
90. Davidson RM. Neural form of voltage-dependent sodium current in human cultured dental pulp cells. Arch Oral Biol 1994;39:613–620.
91. Joao SM, Arana-Chavez VE. Expression of connexin 43 and ZO-1 in differentiating ameloblasts and odontoblasts from rat molar tooth germs. Histochem Cell Biol 2003;119:21–26.
92. Murakami S, Muramatsu T, Shimono M. Expression and localization of connexin 43 in rat incisor odontoblasts. Anat Embryol 2001;203:367–374.
93. Fried K, Mitsiadis TA, Guerrier A, Haegerstrand A, Meister B. Combinatorial expression patterns of the connexins 26, 32, and 43 during development, homeostasis, and regeneration of rat teeth. Int J Dev Biol 1996;40:985–995.
94. Peracchia C. Chemical gating of gap junction channels; roles of calcium, pH and calmodulin. Biochim Biophys Acta 2004;1662:61–80.
95. Trexler EB, Bukauskas FF, Bennett MV, Bargiello TA, Verselis VK. Rapid and direct effects of pH on connexins revealed by the connexin 46 hemichannel preparation. J Gen Physiol 1999;113:721–742.
96. Bhatnagar M, Cintra A, Tinner B, et al. Neurotensin-like immunoreactivity in odontoblasts and their processes in rat maxillary molar teeth and the effect of pulpotomy. Regul Pept 1995;58:141–147.
97. Korkmaz Y, Baumann MA, Steinritz D, et al. NO-cGMP signaling molecules in cells of the rat molar dentin-pulp complex. J Dent Res 2005;84:618–623.
98. McCormack K, Davies R. The enigma of potassium ion in the management of dentine hypersensitivity: Is nitric oxide the elusive second messenger? Pain 1996;68:5–11.
99. Cook SP, McCleskey EW. Desensitization, recovery and Ca(2+)-dependent modulation of ATP-gated P2X receptors in nociceptors. Neuropharmacology 1997;36:1303–1308.

100. Huang YJ, Maruyama Y, Dvoryanchikov G, Pereira E, Chaudhari N, Roper SD. The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds. Proc Natl Acad Sci USA 2007;104:6436–6441.
101. Roper SD. Signal transduction and information processing in mammalian taste buds. Pflugers Arch 2007;454: 759–776.
102. Suzuki T. Cellular mechanisms in taste buds. Bull Tokyo Dent Coll 2007;48:151–161.
103. Staikopoulos V, Sessle BJ, Furness JB, Jennings EA. Localization of P2X2 and P2X3 receptors in rat trigeminal ganglion neurons. Neuroscience 2007;144:208–216.
104. Kim YS, Paik SK, Cho YS, et al. Expression of P2X3 receptor in the trigeminal sensory nuclei of the rat. J Comp Neurol 2008;506:627–639.
105. Alavi AM, Dubyak GR, Burnstock G. Immunohistochemical evidence for ATP receptors in human dental pulp. J Dent Res 2001;80:476–483.
106. Renton T, Yiangou Y, Baecker PA, Ford AP, Anand P. Capsaicin receptor VR1 and ATP purinoceptor P2X3 in painful and non painful human tooth pulp. J Orofac Pain 2003; 17:245–250.
107. Bao L, Locovei S, Dahl G. Pannexin membrane channels are mechanosensitive conduits for ATP. FEBS Lett 2004;572:65–68.
108. Fitz JG. Regulation of cellular ATP release. Trans Am Clin Climatol Assoc 2007;118:199–208.
109. Ma W, Hui H, Pelegrin P, Surprenant A. Pharmacological characterization of pannexin-1 currents expressed in mammalian cells. J Pharmacol Exp Ther 2009;328:409–418.
110. Penuela S, Bhalla R, Gong XQ, et al. Pannexin 1 and pannexin 3 are glycoproteins that exhibit many distinct characteristics from the connexin family of gap junction proteins. J Cell Sci 2007;120:3772–3783.
111. Thompson RJ, Macvicar BA. Connexin and pannexin hemichannels of neurons and astrocytes. Channels (Austin) 2008 Mar 29;2[epub ahead of print].
112. Mechenthaler I. Galanin and the neuroendocrine axes. Cell Mol Life Sci 2008;65:1826–1835.
113. Paakkonen V, Bleicher F, Carrouel F, et al. General expression profiles of human native odontoblasts and pulp-derived cultured odontoblast-like cells are similar but reveal differential neuropeptide expression levels. Arch Oral Biol 2009;54:55–62.
114. Ratcliffe CF, Qu Y, McCormick KA, et al. A sodium channel signaling complex: Modulation by associated receptor protein tyrosine phosphatase beta. Nat Neurosci 2000;3: 437–444.
115. Suzuki H, Iwanaga T, Yoshie H, et al. Expression of galanin receptor-1 (GALR1) in the rat trigeminal ganglia and molar teeth. Neurosci Res 2002;42:197–207.
116. Lazarov NE. Comparative analysis of the chemical neuroanatomy of the mammalian trigeminal ganglion and mesencephalic trigeminal nucleus. Prog Neurobiol 2002;66:19–59.
117. Fornaro M, Lee JM, Raimondo S, Nicolino S, Geuna S, Giacobini-Robecchi M. Neuronal intermediate filament expression in rat dorsal root ganglia sensory neurons: An in vivo and in vitro study. Neuroscience 2008;153: 1153–1163.
118. Zubrzycka M, Janecka A. Effect of galanin on substance P- and vasoactive intestinal polypeptide-induced nociceptive trigemino-hypoglossal reflex in rats. J Physiol Pharmacol 2007;58:479–486.
119. Hermanstyne TO, Markowitz K, Fan L, Gold MS. Mechanotransducters in rat pulpal afferents. J Dent Res 2008;87:834–838.
120. Luukko K, Moe K, Sijaona A, et al. Secondary induction and the development of tooth nerve supply. Ann Anat 2008;190:178–187.
121. Fried K, Nosrat C, Lillesaar C, Hildebrand C. Molecular signaling and pulpal nerve development. Crit Rev Oral Biol Med 2000;11:318–332.
122. Kvinnsland IH, Luukko K, Fristad I, et al. Glial cell line-derived neurotrophic factor (GDNF) from adult rat tooth serves a distinct population of large-sized trigeminal neurons. Eur J Neurosci 2004;19:2089–2098.
123. Lillesaar C, Eriksson C, Johansson CS, Fried K, Hildebrand C. Tooth pulp tissue promotes neurite outgrowth from rat trigeminal ganglia in vitro. J Neurocytol 1999; 28:663–670.
124. Dickson BJ. Molecular mechanisms of axon guidance. Science 2002;298:1959–1964.
125. Tessier-Lavigne M, Goodman CS. The molecular biology of axon guidance. Science 1996; 274:1123–1133.
126. Fuchikawa T, Nakamura F, Fukuda N, Takei K, Goshima Y. Protein tyrosine phosphatase SHP2 is involved in Semaphorin 4D-induced axon repulsion. Biochem Biophys Res Commun 2009;385:6–10.
127. Ulupinar E, Datwani A, Behar O, Fujisawa H, Erzurumlu R. Role of semaphorin III in the developing rodent trigeminal system. Mol Cell Neurosci 1999;13:281–292.
128. Abe M, Inagaki S, Furuyama T, Iwamoto M, Wakisaka S. Semaphorin 4D inhibits collagen synthesis of rat pulp-derived cells. Arch Oral Biol 2008;53:27–34.
129. Loes S, Kettunen P, Kvinnsland IH, Taniguchi M, Fujisawa H, Luukko K. Expression of class 3 semaphorins and neuropilin receptors in the developing mouse tooth. Mech Dev 2001;101:191–194.
130. Lillesaar C, Fried K. Neurites from trigeminal ganglion explants grown in vitro are repelled or attracted by tooth-related tissues depending on developmental stage. Neuroscience 2004;125:149–161.
131. Luukko K, Loes S, Kvinnsland IH, Kettunen P. Expression of ephrin-A ligands and EphA receptors in the developing mouse tooth and its supporting tissues. Cell Tissue Res 2005;319:143–152.
132. Charron F, Stein E, Jeong J, McMahon AP, Tessier-Lavigne M. The morphogen sonic hedgehog is an axonal chemoattractant that collaborates with netrin-1 in midline axon guidance. Cell 2003;113:11–23.
133. Pasterkamp RJ, Peschon JJ, Spriggs MK, Kolodkin AL. Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. Nature 2003;424:398–405.
134. Maurin JC, Delorme G, Machuca-Gayet I, et al. Odontoblast expression of semaphorin 7A during innervation of human dentin. Matrix Biol 2005;24:232–238.
135. Chen S, Rio C, Ji RR, et al. Disruption of ErbB receptor signaling in adult non-myelinating Schwann cells causes progressive sensory loss. Nat Neurosci 2003;6:1186–1193.
136. Fried K, Risling M, Tidcombe H, Gassmann M, Lillesaar C. Expression of ErbB3, ErbB4, and neuregulin-1 mRNA during tooth development. Dev Dyn 2002;224:356–360.
137. Anderson DJ, Matthews B, Shelton LE. Variations in the sensitivity to osmotic stimulation of human dentine. Arch Oral Biol 1967;12:43–47.

138. Fried K, Sime W, Lillesaar C, Virtanen I, Tryggvasson K, Patarroyo M. Laminins 2 (alpha2 beta1 gamma1, Lm-211) and 8 (alpha4 beta1 gamma1, Lm-411) are synthesized and secreted by tooth pulp fibroblasts and differentially promote neurite outgrowth from trigeminal ganglion sensory neurons. Exp Cell Res 2005;307:329–341.
139. Salmivirta K, Sorokin LM, Ekblom P. Differential expression of laminin alpha chains during murine tooth development. Dev Dyn 1997;210:206–215.
140. Luckenbill-Edds L. Laminin and the mechanism of neuronal outgrowth. Brain Res Rev 1997;23:1–27.
141. Wallquist W, Patarroyo M, Thams S, et al. Laminin chains in rat and human peripheral nerve: Distribution and regulation during development and after axonal injury. J Comp Neurol 2002;454:284–293.
142. Byers MR, Kvinnsland I, Bothwell M. Analysis of low affinity nerve growth factor receptor during pulpal healing and regeneration of myelinated and unmyelinated axons in replanted teeth. J Comp Neurol 1992;326:470–484.
143. Borrell V, Del Rio JA, Alcantara S, et al. Reelin regulates the development and synaptogenesis of the layer-specific entorhino-hippocampal connections. J Neurosci 1999;19: 1345–1358.
144. Teillon SM, Yiu G, Walsh CA. Reelin is expressed in the accessory olfactory system, but is not a guidance cue for vomeronasal axons. Brain Res Dev Brain Res 2003;140:303–307.
145. Durand SH, Flacher V, Romeas A, et al. Lipoteichoic acid increases TLR and functional chemokine expression while reducing dentin formation in in vitro differentiated human odontoblasts. J Immunol 2006;176:2880–2887.
146. Farges JC, Keller JF, Carrouel F, et al. Odontoblasts in the dental pulp immune response. J Exp Zool B Mol Dev Evol 2009;312B:425–436.
147. Keller JF, Carrouel F, Colomb E, et al. Toll-like receptor 2 activation by lipoteichoic acid induces differential production of pro-inflammatory cytokines in human odontoblasts, dental pulp fibroblasts and immature dendritic cells. Immunobiology 2010;215:53–59.
148. Hahn CL, Liewehr FR. Innate immune responses of the dental pulp to caries. J Endod 2007;33:643–651.
149. Ngassapa D, Närhi M, Hirvonen T. Effect of serotonin (5-HT) and calcitonin gene-related peptide (CGRP) on the function of intradental nerves in the dog. Proc Finn Dent Soc 1992;88(suppl 1):143–148.
150. Kim YS, Kim YJ, Paik SK, et al. Expression of metabotropic glutamate receptor mGluR5 in human dental pulp. J Endod 2009;35:690–694.
151. Birchmeier C, Nave KA. Neuregulin-1, a key axonal signal that drives Schwann cell growth and differentiation. Glia 2008;56:1491–1497.
152. Chen S, Velardez MO, Warot X, et al. Neuregulin 1-erbB signaling is necessary for normal myelination and sensory function. J Neurosci 2006;26:3079–3086.
153. Felszeghy S, Modis L, Németh P, et al. Expression of aquaporin isoforms during human and mouse tooth development. Arch Oral Biol 2004;49:247–257.
154. Hou J, Situ Z, Duan X. CIC chloride channels in tooth germ and odontoblast-like MDPC-23 cells. Arch Oral Biol 2008;53:874–878.