Trigeminal Sensory Neurons and Pulp Regeneration

ABSTRACT

The pulp-dentin complex is innervated by a high density of trigeminal neurons free nerve endings. These neuronal fibers are highly specialized to sense noxious stimuli such as thermal, mechanical, chemical, and biological cues. This robust alert system provides immediate feedback of potential or actual injury triggering reflex responses that protect the teeth from further injury. In the case of patients, pain is the most important experience that leads them to seek oral health care. The adequate removal of the etiology, such as caries, provides ample opportunity for the robust reparative and regenerative potential of the pulp-dentin complex to restore homeostasis. In addition to this elaborated surveillance system, evidence has accumulated that sensory neuronal fibers can potentially modulate various steps of the reparative and regenerative process through cellular communication processes. These include modulation of immunologic, angiogenic, and mineralization responses. Despite these orchestrated cellular events, the defense of the pulp-dentin complex may be overwhelmed, resulting in pulp necrosis and apical periodontitis. Regenerative endodontic procedures have evolved to restore the once lost function of the pulp-dentin complex. After these procedures, a large subset of successful cases demonstrates a positive response to sensitivity testing, suggesting reinervation of the canal space. This process is likely mediated through cellular and noncellular release of neurotrophic factors such as brain-derived nerve growth factor. In addition, these newly recruited nerve fibers appear equipped to sense thermal stimuli through nonhydrodynamic mechanisms. Collectively, the significance of innervation in the normal physiology of the pulp-dentin complex and its role in regeneration need to be better appreciated to promote further research in this area that could potentially bring new therapeutic opportunities. (J Endod 2020;46:S71–S80.)

KEY WORDS

Axonal targeting; neurite outgrowth; neurons; neurotrophins; regeneration; regenerative endodontics

NEURONS AS ACTIVE PARTICIPANTS IN DENTAL PULP REGENERATION

It has become evident that sensory neurons have numerous other functions than their sensorial putative role. Most evidence comes from studies that evaluated the interaction of neurons with non-neuronal cells elsewhere in the body [7]. It is well accepted that nociceptors represent the primary surveillance system in the body alerting us of potential or actual injury [1-7]. Thus, the high representation of nociceptors within the dental pulp suggests that, through evolutionary pressure, the protection and maintenance of dental pulp homeostasis play an important role in overall health and survival. This observation is supported by anthropological studies that have highlighted the significance of functional dentition for eating and defense of mammals in the wild throughout evolution [8]. Therefore, trigeminal sensory neurons are important structures in the pulp-dentin complex that have increased in both quantity and quality with mammalian evolution. Given the very important functions of innervation in normal pulp physiology and response to injury, a better understanding of neurogenesis in pulp regeneration is necessary. In this review, the role played by sensory neurons in repair and regeneration in vital pulp is discussed. In addition, evidence of neuronal regeneration and its mechanisms in regenerative endodontic procedures (REPs) is presented.

SIGNIFICANCE

The pulp-dentin complex is densely innervated with sensory neurons. In addition to detecting noxious stimuli, they have been shown to modulate many important reparative and regenerative processes. The understanding of these mechanisms could unravel new therapeutic strategies. Furthermore, pulp regeneration, in its strict sense, must include re-establishment of form and function of the innervation of the pulp-dentin complex.
associated with enhanced pain sensitivity in acute and chronic pulp inflammation. The known mechanisms underlying the recruitment of these nerve fibers include the release of neurotrophic factors such as nerve growth factor (NGF) by pulpal fibroblasts, odontoblasts, and/or demineralized dentin matrix. In addition to nociception, this enriched innervation at the foci of injury likely participates in the overall reparative and regenerative response to injury through the release of soluble factors that through a paracrine fashion regulate cellular function.

Innervation of the dental pulp is largely originated by medium to large neurons within the second and third divisions of the trigeminal ganglia, and it is composed of almost exclusively nociceptive fibers. There is also sympathetic autonomic innervation derived from the superior cervical ganglia that, within the pulp, primarily serve the role of vasocostriction. A large percentage of pulpal nociceptors are classified as peptidergic because these fibers contain a large repertoire of neuropeptides that are released upon neuronal activation in a calcium-dependent mechanism (Fig. 1A–D). The best recognized neuropeptide effect in the dental pulp is mediated by calcitonin gene-related peptide (CGRP) and substance P (SP), which upon being released promote vasodilatation and plasma extravasation, respectively. Thus, these 2 vasoactive neuropeptides directly participate in the inflammatory process. The mechanism for neuronal-generated inflammation, called neurogenic inflammation, is often intertwined with classic immunologic inflammatory processes because of a positive feedback loop. Therefore, it is hard to determine which event physiologically started first. Although inflammation and neurogenic inflammation have different mechanisms, they are often present together as part of an overall amplifying inflammatory response whose balance is crucial for repair and regeneration.

In addition to vascular effects, neuronal-derived soluble factors have been shown to modulate the overall inflammatory response through a direct cellular effect. For example, SP exerts stimulatory effects on macrophages and lymphocytes leading to the release of cytokines and inflammatory mediators. On the other hand, CGRP has been shown to have anti-inflammatory activities and decreased proliferation of lymphocytes. Also, in vitro studies showed that SP and CGRP lead to increased proliferation of dental pulp–derived fibroblasts, and CGRP potentiates the reparative dentinogenesis by stimulating the release of various bone morphogenic proteins. Deafferentation in vivo resulted in an accelerated loss of vital pulp, progression of infection, and development of apical periodontitis after experimental pulp exposure and a decrease in cellular proliferation after experimental cavity preparation.

Also, the anatomic position of pulpal free nerve endings in close relationship with odontoblasts and dentinal tubules provides ample opportunity for neuronal-odontoblastic communication with evidence of free nerve endings often extending into tubules of reactionary dentin in response to caries. Thus, in addition to nociception, sensory neurons actively regulate tissue homeostasis through neuronal communication with the other cells of the pulp-dentin complex via the release of soluble factors such as neuropeptides.

**SENSORY NEURONS AND REPS**

REPs are comprised of therapies tailored to the maintenance or re-establishment of a vital dental pulp after either reversible or irreversible injury to the native dental pulp. To date, most of these procedures rely on indirect or direct pulp capping procedures (ie, vital pulp therapies) or revitalization procedures (ie, therapy for pulp necrosis). Given the elaborate structure and function of the pulp-dentin complex, its regeneration after pulp necrosis is daunting, with many research groups focused on this task that has tremendous scientific and clinical implications. The currently used revitalization procedures rely on adequate disinfection of the root canal system and the recruitment of undifferentiated oral stem cells from the apical region either by provoking bleeding or by the placement of platelet-rich plasma or platelet-rich fibrin. Evidence has accumulated from many studies that these procedures promote a similar success rate regarding healing compared with conventional root canal therapies but have the advantage of promoting continued root development and re-establishment of vitality responses in approximately half of the cases. These vitality responses require targeting of the periapical neuronal terminals into coronal areas of the repaired tissue nearly 10–15 mm away from the closest nerve trunks. Indeed, evidence of such reinnervation or neurogenesis is observed in histologic studies of teeth treated with REPs.

**HISTOLOGIC EVIDENCE OF NEURONAL REGENERATION IN REPS**

The continued formation of mineralized tissues (ie, dentin) leading to the formation of a calcific bridge in cases of pulp capping procedures or continued root development in cases of pulpotomies or REPs has been the primary focus of researchers and clinicians for decades. It is not surprising that most initial histologic studies of teeth treated with these procedures focused on the identification of newly formed mineralized tissues. However, as stated previously, approximately half of the cases treated with REPs recover responses to sensitivity tests such as cold or electric pulp tester responses, suggesting reinnervation of the formed tissues. Although published evidence of nerve regeneration existed in animal models after REPs, it was first demonstrated in humans after a histologic assessment of a tooth demonstrating the presence of nerve fibers in a tooth previously diagnosed with complete pulp necrosis seen by staining with the pan-neuronal marker PGP9.5. In another histologic study, tooth #29 had been diagnosed with pulp necrosis and acute apical abscess but became asymptomatic after REP with evidence of continued root development and the presence of a calcific barrier under the bioceramic material. However, the tooth was extracted after 11 months because of orthodontic reasons. A careful histologic assessment was performed revealing a mixture of reparative and regenerative processes, including the formation of osteodentin, ectopic presence of cementum and periodontal ligament, rich vasculature, and, importantly, the presence of organized nerve fibers detected by immunoreactivity to neurofilament heavy antibody. These studies proved that nerve fibers were present in these 2 teeth treated with REPs but did not provide evidence that these were sensory fibers.

A more recent study evaluated the histologic outcome of a successful and an unsuccessful case (because of coronal leakage) treated with REPs. The successful case (tooth #29 previously diagnosed with nécrotic pulp and symptomatic apical periodontitis) was asymptomatic with normal responses to cold and electric pulp tests and has clear evidence of continued radiographic development up to 4 and a half years after REP. However, the tooth had to be extracted because of orthodontic reasons, and immunohistochemistry was performed revealing the presence of nerve fibers positive for CGRP, a marker for a subpopulation of sensory fibers that are highly expressed in the native pulp-dentin complex. In addition, similar to the naïve pulp-dentin complex, these fibers were also positive for the pan-neuronal marker class III beta tubulin (TUBB3), a Schwann cell marker (S100), and myelin basic protein, suggesting that these fibers were myelinated. For tooth #9, CGRP staining was not detected, but myelinated fibers were also present. In conclusion, this study
FIGURE 1 – A representative confocal image evaluating the expression patterns of CGRP and TRPV1 in nerve fibers located in the human coronal dental pulp. The inset indicates the area of the pulp represented by the image in A. (B–D) Magnified images from the area enclosed by the white square in A. (C) TRPV1 (red) and CGRP (green) immunoreactivities were often coexpressed in the same nerve fibers. Nerve fibers containing N52 are represented in blue. Modified with permission13.

FIGURE 2 – Representative hematoxylin-eosin staining of sections of (A) a healthy mature tooth #28, (B) REP treated tooth #29, and (C) REP treated tooth #9. Immunohistochemical staining showing von Willebrand factor (vWF) and podoplanin (PDPN) immunoreactivity in (D) a healthy mature tooth #28, (E) tooth #29, and (F) tooth #9 (F). Immunohistochemical staining showing colocalization of CGRP and TUBB3 in (G) a healthy mature tooth #28 and (H) tooth #29. (I) Tooth #9 shows TUBB3 immunoreactivity but no colocalization with CGRP. Immunohistochemical staining demonstrating colocalization of Schwan cell marker (S100) with TUBB3 in (J) a healthy mature tooth #28, (K) tooth #29, and (L) tooth #9. Immunohistochemical staining showing close proximity of blood vessels stained with vWF and a marker for antigen-presenting cells (human leukocyte antigen–antigen D related) in (M) a healthy mature tooth #28, (N) tooth #29, and (O) tooth #9. Immunohistochemical staining demonstrating close proximity of blood vessels stained with vWF and a pan-leukocytic marker (CD45) in (P) a healthy mature tooth, (Q) tooth #29, and (R) tooth #9. Arrows in each panel indicate positive expression of the various proteins. Modified with permission45.
demonstrated the presence of sensory fibers in a successful REP providing further insight into the capacity of axonal targeting from the apical region into the newly formed tissues after these procedures. The mechanism of this axonal targeting is not fully understood, but it appears that dental innervation is particularly equipped to display robust plasticity.

**NEUROPLASTICITY AND AXONAL TARGETING**

Human dental development occurs late after embryogenesis extending into adult life, representing a good example of postnatal organogenesis. Somatosensory innervation typically occurs during in utero embryonic development. However, the dental pulp becomes innervated much later in life, generally after initial tooth eruption. Another unique feature of the dental pulp innervation is the high density of exclusively nociceptive primary afferents. Furthermore, the innervation of the dental pulp is gained and lost as deciduous teeth exfoliate and permanent teeth erupt. Thus, there is a very dynamic pattern of axonal targeting through the shift between primary (deciduous) to permanent dentition and during the maturation of each of these sets of teeth.

The process of dental innervation during developmental maturation of teeth has been shown to be elegantly modulated by a cascade of molecular signals. Primary afferent branches accumulate around the apical papilla but do not enter the dental pulp proper until late in the cap stage of tooth development to finalize the branching of the primary afferents on the periphery of the pulp-dentin complex. These molecular signals are primarily mediated by a concert of neurotrophins released with specific temporal and spatial resolution, resulting in the unique highly specialized innervation of the dental pulp. Among these neurotrophins, NGF and brain-derived neurotrophic factor (BDNF) have been implicated in the development of pulp innervation. Therefore, trigeminal sensory neurons targeting the dental pulp are particularly equipped to follow gradients of neurotrophic factors, exhibiting robust axonal growth and targeting. The source of these neurotrophic factors could be local cells or extracellular matrix (i.e., dentin matrix).

**CELLULAR SOURCE OF NEUROTROPHIC FACTORS**

Stem cells from the apical papilla (SCAP) are, among other apically positioned mesenchymal stem cells (MSCs), likely to be the primary undifferentiated cells that populate the canal space after REPs in immature teeth. Importantly, mesenchymal stem cells (MSCs) have the ability of secreting numerous factors depending on the biological demand of the environment. These factors include cytokines, chemokines, growth factors, and neurotrophins. A study evaluated whether SCAPs could mediate axonal targeting. Using in vitro assays, including a microfluidic approach, and an in vivo innervation assay, it was demonstrated that SCAPs produce and secrete NGF, BDNF, and glial-derived nerve growth factor (GDNF). The expression of BDNF and GDNF was increased when these cells were in the presence, albeit not in direct contact, of trigeminal neurons. The secreted factors from SCAPs promoted robust axonal growth and targeting with axonal growth exceeding 100 μm in vitro. This effect was completely abolished when neutralizing antibodies against BDNF were used. Levels of BDNF in SCAPs cocultured with trigeminal ganglia (TG) neurons were 10-fold greater than the levels of GDNF and NGF. Also, these results are consistent with the up-regulation of BDNF seen in dental pulp stem cells used in an animal model resulting in complete regeneration of the dental pulp innervation in an animal model of regenerative endodontics. In addition, bone marrow stem cells have been shown to secrete BDNF, leading to increased neuronal survival of cortical neurons exposed to nitric oxide and to promote functional recovery by promoting axonal sprouting and targeting in a spinal injury model.

An elegant study showed that dental pulp stem cells are capable of differentiating into Schwann cells in vitro. Similar to the study mentioned previously, these cells released substantially greater levels of BDNF compared with NGF and GDNF, and they promoted survival and neurite outgrowth of cultured dorsal root ganglia neurons. In addition, using a 3-dimensional neural construct, the newly differentiated Schwann cells formed a column that appeared to guide neurites in a linear fashion, also resulting in their myelination. Thus, BDNF appears to be preferentially released by MSCs when in the presence of injured neurons leading to increased survival and axonal targeting. However, it is likely to act in concert with other factors released that are part of the rich secretome of MSCs.

**NEUROTROPHIC FACTORS IN DENTIN MATRIX**

Although the presence of neurotrophic factors has been well demonstrated in tooth development, it was unclear if they are sequestered and “fossilized” in the extracellular matrix of human dentin as described for various other molecules. It has been shown that these dentin-derived molecules can be released by either demineralizing carious lesions or chemical agents and have profound effects mediating repair and regeneration of the pulp-dentin complex. Austad and colleagues demonstrated that dentin matrix proteins (DMPs) isolated from whole extracted human teeth contained many neurotrophic factors, including NGF, BDNF, GDNF, neurotrophin-3, and neurotrophin-4, that promoted the axonal growth of rat primary cultured neurons in a concentration-dependent manner. Another study showed that there was a difference in neurotrophic factors between radicular and coronal dentin. It was found that NGF is the most abundant neurotrophic factor with 3-fold increased expression in radicular dentin. Similarly, BDNF and neurotrophin-3 are more abundant in radicular than coronal dentin. Conversely, GDNF is more abundant in coronal dentin, whereas NT4 is equally distributed. DMPs promoted neurite outgrowth in vitro and axonal targeting in vivo, with a greater effect observed by radicular dentin extracts. It is noteworthy that EDTA was used in these studies to solubilize DMPs, and it is also used in regenerative procedures with the same intent. Thus, the robust effect of DMPs on neurite outgrowth and axonal targeting could, at least in part, be 1 of the mechanisms by which the pulplike tissue becomes reinervated in patients after REPs.

**NEUROPHYSIOLOGY AND RESPONSES TO SENSITIVITY TESTS IN REPS**

In normal healthy teeth, pulpal responses to cold stimuli depends on the inward movement of fluid within dentinal tubules and the subsequent activation of low-threshold Aβ fibers that extend up to 300μm into dentinal tubules. This hydrodynamic theory of dentinal pain postulates that the application of either cold or hyperosmotic solutions onto the surface of a tooth triggers fluid movement and subsequent neuronal depolarization. However, this theory cannot explain cold sensitivity after REPs because a restorative material blocks neuronal access to coronal dentinal tubules. Instead, the cold detection observed in a subset of REPs could involve the direct detection of temperature change by dental afferent neurons, which are known to express thermosensitive ion channels such as transient receptor potential ankyrin type 1 (TRPA1) and transient receptor potential melastatin 8 (TRPM8). These channels are activated by temperatures below 17°C and 25°C, respectively, and could be involved in the
FIGURE 3 – SCAPs mediate axonal growth and targeting through a BDNF-mediated mechanism. (A) TG neurons extend axonal projections toward SCAPs in culture. A microfluidic neurite isolation device was used to evaluate the targeting of trigeminal neurons toward an SCAP culture. Axonal projections into the opposing chamber of the isolation device were seldom seen in TG neurons cultured in the absence of SCAPs. However, there were evident axonal projections toward the opposing chamber containing SCAPs (10^5 cell/mL). Furthermore, there was an increase in both the number and distance of projections when the density of SCAPs was increased (3 × 10^5 cells/mL). TG neurons are visualized by immunocytochemistry with staining against the neuronal marker N52 (green), whereas cellular nuclei were visualized by the nuclear stain TOPRO (blue). (A) Images were acquired by confocal microscopy with a 10× objective as representative images of replicate experiments. (B) A neutralizing antibody against BDNF completely blocks the SCAP-mediated increase in neurite outgrowth. Rat primary trigeminal neurons were cultured in the absence (control) or presence of 10^5 SCAPs/mL. Furthermore, the cocultures were maintained in the presence of neutralizing antibodies against NGF (α-NGF), GDNF (α-GDNF), and BDNF (α-BDNF) or in the presence of a cocktail of these antibodies (Ab mix) for 3 days. A neurite outgrowth assay was used to quantify changes in axonal growth. Treatment with α-BDNF completely blocked the increase in neurite outgrowth evoked by SCAPs. Importantly, a mixture of the neutralizing antibodies (Ab mix) did not result in further reduction in neurite outgrowth. Data were analyzed with 1-way analysis of variance with the Bonferroni post hoc test (groups with different lowercase letters have statistically significant differences, P < .05).

FIGURE 4 – Pulp temperatures after cold stimulation of normal and endodontic-treated teeth. A thermocouple probe was placed through the apical foramen into the pulp chamber of untreated and endodontic-treated extracted immature teeth. (A) A cotton pellet saturated with EndoIce was applied to occlusal surfaces for 5 or 10 seconds, and changes in temperature were recorded (n = 6/group). Data are presented as the mean ± standard deviation of the recorded temperatures. **P < .01 and ***P < .001; 1-way analysis of variance with the Tukey post hoc test. (B) Placement of the thermocouple probe in the pulp chamber was confirmed radiographically before cold stimulation. Modified with permission.
direct transduction of nociceptive signals upon cold stimulus.75,78,80,81. Another possible mechanism could involve the release of adenosine triphosphate (ATP) by neighboring cells, similar to that normally observed in odontoblasts85, and its detection by the purinergic receptors such as ionotropic channel P2X3 known to be expressed in trigeminal neurons86–90. Thus, trigeminal neurons are equipped to respond to thermal changes through nonhydrodynamic mechanisms.

Using an ex vivo model, it was found that the application of a cotton pellet saturated with EndoIce (Hygenic, Akron, OH) onto the occlusal surface of an extracted intact immature human premolar resulted in the temperature change from 37°C14 to 9°C91. This drop in temperature was further increased to as low as 16°C if the tooth received a coronal restoration as recommended in REPs that includes a 3-mm layer of mineral trioxide aggregate (Fig. 4A and B). This finding points out that the thermal insulating capacity of dentin is lost when a coronal restoration of mineral trioxide aggregate and composite resin is placed. More importantly, this decrease in temperature is sufficient to reach the activation threshold for TRPM8 and TRPA1 channels was significantly increased when TG neurons were cultured with SCAPs, suggesting that these stem cell release factors result in neuronal gain of function (Fig. 5A and B). Lastly, it was found that SCAP release ATP, independent from cellular lysis or death, when exposed to temperatures below 37°C. The released ATP is capable of activating purinergic channels in TG neurons, and this activation is increased in the presence of SCAPs. Altogether, the expression of TRPA1 and TRPM8 in TG neurons and their gain of function in the presence of SCAPs and the possible temperature-dependent release of ATP from dental pulp cells are 2 alternative hypotheses for cold detection mechanisms after clinical REPs.

CONCLUSIONS

Innervation is a key component of the pulpdentin complex that, in addition to its sophisticated sensorial function, modulates vascular, immunologic, and dentinogenic responses to injury. There is strong evidence that demonstrates that current REPs result in the formation of a pulp-like tissue that contain sensory innervation that is consistent with responses to vitality testing seen in a subset of treated cases. The reinnervation of the pulp canal space in these procedures requires the recruitment of apically positioned free nerve endings through axonal guidance. This is likely mediated by released neurotrophic factors from cells, such as SCAP and DPSCs, and dentin. Also, these newly recruited neuronal fibers may be modulated by stem cells resulting in the establishment of alternative thermosensitive mechanisms. This neurosupportive function of oral stem cells transcends dentistry because they have been shown to have a strong therapeutic value in models of neurodegenerative diseases. Lastly, the true regeneration of a fully functional pulp-dentin complex should comprise full restoration in its form and function (restitutio ad integrum), and that includes its innervation. Given the importance of innervation for pulp homeostasis, repair, and regeneration, further research is warranted to add to an already existing body of knowledge in angiogenesis and mineralization.

ACKNOWLEDGMENTS

The author denies any conflicts of interest related to this study.

REFERENCES


