From the Department of Neuroscience
Karolinska Institutet, Stockholm, Sweden

On Functions of Neurotrophic Factors in Taste Buds and Teeth

Irina Vukmanovic Nosrat, DDS

Karolinska Institutet

Stockholm 2017

Cover picture: A dental pulp cell, which acquired neuronal phenotype in culture. This neuron expresses the neuron-specific marker PGP 9.5 and is able to maintain this characteristics for an extended period of time.

ISBN 978-91-7676-907-2
Printed by E-Print AB 2017

Karolinska Institutet

Department of Neuroscience

On Functions of Neurotrophic Factors in Taste Buds and Teeth

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Atrium, Nobels väg 12B.

Fredagen den 15 december, 2017, kl 13.00

av

Irina Vukmanovic Nosrat
Leg. Tandläkare

Huvudhandledare:
Professor Lars Olson
Karolinska Institutet
Department of Neuroscience

Fakultetsopponent:
Professor Päivi Kettunen
University of Bergen
Department of Biomedicine

Betygsnämnd:
Professor/Handkirurg Mikael Wiberg
Umeå Universitet
Institutionen för integrativ medicinsk biologi

Professor Mårten Risling
Karolinska Institutet
Department of Neuroscience

Universitetslektor Elisabet Åkesson
Karolinska Institutet
Department of Neurobiology

Stockholm 2017

For my husband Christopher,

my grown children Philip and Stephan,

and my parents Dusanka and Zelimir

ABSTRACT

Background: Chemosensory disorders affect approximately 15% of the U.S. population and an estimated 200,000 individuals visit a doctor each year for problems with their ability to taste or smell (NIDCD). Among the common causes of taste problems are radiation therapy, chemotherapy, exposure to certain chemicals and medications, head trauma and surgical injuries. Tastants are detected by taste buds, which are specialized collections of cells. Taste bud development and innervation has been an active research front and several key molecules involved in these processes have been elucidated. Neurotrophins, in particular brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) were among the first to be identified as playing a role in taste buds. BDNF and NT-3 are expressed in developing and adult rodent tongues in a temporospatially specific manner. BDNF mRNA is found in the gustatory epithelium during development and in adult taste buds, NT-3 mRNA in the surrounding epithelium in rodents. Neurotrophins are also expressed in a temporospatially specific manner during tooth morphogenesis. Nerve growth factor (NGF), BDNF and glial cell line-derived neurotrophic factor (GDNF) are expressed in developing rodent teeth.

Aims: To examine the expression of mRNA encoding neurotrophic factors in the developing human taste system and teeth, to assess the role of neurotrophic factors in the formation and innervation of taste buds and teeth, and to explore possible consequences of neurotrophic factor expression in cultured dental pulp cells (DPCs).

Results: Neurotrophic factor expression patterns are described in the developing human tongue and compared to those of rodents. BDNF was found in the first-trimester in the same areas as in rodents; developing gustatory epithelium and taste buds, and in additional areas such as the subepithelial mesenchyme. Human NT-3 mRNA expression patterns were largely similar to those of rodents, except that taste buds also expressed NT-3 mRNA during development and in adults. In both rodents and humans, BDNF was expressed prior to innervation of gustatory papillae, and thus serves as a very early marker of the gustatory epithelium. Our study showed wider expression patterns of both BDNF and NT-3 in the human gustatory system (paper I) compared to rodents. Next, we showed that taste papillae in BDNF/NT-3 double KO mice were smaller and less innervated compared to $BDNF^{-/-}$ mice, indicating specific gustatory roles for both neurotrophins (paper V). Studies of developing human teeth showed that NGF, BDNF, NT-3, neurotrophin-4 (NT-4), GDNF and neurturin (NTN) were expressed in the tooth organ and surrounding mesenchyme (paper III).

Interactions of neurotrophic factors from the dental pulp and trigeminal, motor and dopamine (DA) neurons were analyzed. DPCs promoted survival and neurite outgrowth from trigeminal neurons in cocultures, and prolonged neural survival *in vitro*. DPCs also promoted motoneuron survival in a rodent model of spinal cord injury (paper II), as well as the survival of embryonic DA neurons *in vitro* (paper IV).

BDNF is the main neurotrophic factor in the gustatory system, but NT-3 plays a role as well in both humans and rodents, which knockout studies were able to detect. The tooth provides an excellent model to study molecular events in cells during organ formation, and to examine how neurotrophic factors promote innervation during development.

Key words: gustation, gustatory, taste buds, taste disorder, neurotrophin, NGF, BDNF, NT-3, NT-4, GDNF, NTN, innervation, development, tooth, dental, odontogenesis, spinal cord injury, knock-out, transgenic.

TABLE OF CONTENTS

ABBREVIATIONS

ATP	Adenosine Triphosphate
BDNF	Brain-derived neurotrophic factor
BMP	Bone morphogenic protein
DA	Dopamine
DPCs	Dental pulp cells
E	Embryonic day
FGF	Fibroblast growth factor
GAP-43	Growth-associated protein 43
GDNF	Glial cell line-derived neurotrophic factor
Gli	Glioma-Associated Oncogene Family Zinc Finger
GPCR	G protein-coupled receptor
IP3R3	Inositol 1,4,5-trisphosphate receptor
K8, K14	Cytokeratin 8, Cytokeratin 14
NF	Neurotrophic factors
NGF	Nerve growth factor
NTN	Neurturin
NT-3	Neurotrophin-3
NT-4	Neurotrophin-4
P	Post natal day
P13K	Phosphatidylinositol 3-kinase
P2X3	Purinergic Receptor P2X 3
PGP 9.5	Protein gene product 9.5, Ubiquitin carboxyl terminal hydrolase-1
PLCß2	Phospholipase Cß2
PLC-γ1	Phospholipase C-γ1
PROP	6-n-propylthiouracil
Ptc	Patched
PTC	Phenylthiocarbamide
Shh	Sonic hedgehog
Smo	Smoothened
Sox	SRY (Sex Determining Region Y)
T1R2	Taste receptor type 1 member 2 (TAS1R2)
T1R3	Taste receptor type 1 member 3 (TAS1R3)
T2R	Taste receptor type 2 (TAS)
TGF	Transforming growth factor
TRPA1	Transient receptor potential cation channel, subfamily A, member 1
TRPM3, 5, 8	Transient receptor potential cation channel, subfamily M, member 3, 5, 8
TRPV1-4	Transient receptor potential cation channel, subfamily V, member 1-4
Trk	Tyrosine kinase receptor
VM	Ventral mesencephalon
Wnt	Wingless-Type MMTV Integration Site Family
Zeb	Zinc Finger E-Box Binding Homeobox

LIST OF PUBLICATIONS

I. Nosrat IV, Lindskog S, Seiger A, Nosrat CA, Lingual BDNF and NT-3 mRNA expression patterns and their relation to innervation in the human tongue: Similarities and differences compared with rodents. Journal of Comparative Neurology 2000, 417:133-52.

II. Nosrat IV, Widenfalk J, Olson L, Nosrat CA, Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons *in vitro*, and rescue motoneurons after spinal cord injury. Developmental Biology 2001, 238:120-32.

III. Nosrat I, Seiger A, Olson L, Nosrat CA, Expression patterns of neurotrophic factor mRNAs in developing human teeth. Cell & Tissue Research 2002, 310:177-87.

IV. Nosrat IV, Smith CA, Mullally P, Olson L, Nosrat CA, Dental pulp cells provide neurotrophic support for dopaminergic neurons and differentiate into neurons *in vitro*; implications for tissue engineering and repair in the nervous system. European Journal of Neuroscience 2004, 19:2388-98.

V. Nosrat IV, Agerman K, Marinescu A, Ernfors P, Nosrat CA, Lingual deficits in neurotrophin double knockout mice. Journal of Neurocytology 2004, 33:607-15.

INTRODUCTION

Neurotrophic factors are instrumental for development of neurons, both in the central and the peripheral nervous system. They are secreted by target tissues (either neuronal or nonneuronal) and provide trophic support of neurons, which in turn innervate the corresponding tissues (Levi-Montalcini et al 1969, Olson 1967). The studies in this thesis will focus on neurotrophic factors in the taste system and the dental organs.

Neurotrophic factors

Neurotrophic factors (NF) are endogenous soluble proteins that regulate survival, growth, morphological plasticity, or synthesis of proteins for differentiated functions of neurons (Hefti et al 1993) and include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin 4 (NT-4), ciliary neurotrophic factor (CNTF), fibroblast growth factors (FGFs), insulin growth factor (IGF), epidermal growth factors (EGFs), transforming growth factors (TGFs), and more. They are synthesized in different locations, such as in neurons, glia or in the target tissues. In neurons, NFs are transported retrogradely, as well as anterogradely (von Bartheld et al 1996a, von Bartheld et al 1996b). Disturbances of neurotrophic signaling pathways and oxidative stress may impair development of the brain or other organ systems, and contribute to neurodegenerative diseases (Forman et al 2004, Uttara et al 2009).

Neurotrophins and their receptors

Neurotrophins control survival, development, and a wide range of neuronal functions in both the central and the peripheral nervous system. They act as survival factors for sympathetic and neural crest derived sensory neurons. Deleting neurotrophins during development control the numbers of surviving neurons. Sensory neurons receive a limited number of NF from both peripheral and central sources, thereby ensuring the survival of neurons adequately connected with their targets (Skaper 2012, Thoenen et al 1987).

NGF, the first member of neurotrophins, was discovered in the early 1950s (Levi-Montalcini 1987, Thoenen & Barde 1980). The other neurotrophins in mammals consist of BDNF (Barde et al 1982, Leibrock et al 1989), NT-3 (Ernfors et al 1990b, Hohn et al 1990, Jones & Reichardt 1990, Maisonpierre et al 1990, Rosenthal et al 1990), and NT-4 (Hallböök et al 1991, Ip et al 1992). NT-5 is the mammalian equivalent of NT-4. NT-4 and NT-5 exhibit many similar properties in several *in vitro* assay systems, hence the nomenclature NT-4/5 (Berkemeier et al 1991).

Identified as the first receptor for NGF (Chao et al 1986, Johnson et al 1986, Radeke et al 1987), p75 was later found to bind to all NGF family ligands with similar affinity and thus better described as a low-affinity neurotrophin receptor (Ibanez et

al 1992, Rodriguez-Tebar et al 1992, Ryden et al 1995). Neurotrophins are synthesized as proneurotrophins, which are cleaved to biologically active proteins. It has been shown that proneurotrophins bind with high affinity to p75, which mediates apoptosis (Lee et al 2001), while the mature forms promote survival. Three tyrosine kinase receptors (trk) have been identified which bind the neurotrophins with high affinity. TrkA binds NGF, trkB BDNF and NT-4, and trkC primarily NT-3, although both trkA and trkB can also bind NT-3 in some organs (Barbacid 1994).

Upon binding of a neurotrophin, the trk receptor becomes dimerized, which causes its phosphorylation at different sites in the intracellular domain, and thereby activation (Treanor et al 1995). This is critical (Schlessinger & Ullrich 1992) in order to activate three major signal transduction pathways: Ras, phosphatidylinositol 3-kinase (PI3K) and phospholipase C-γ1 (PLC-γ1) (Bucci et al 2014, Kaplan & Miller 2000, Pawson & Nash 2000).

Due to their exceptionally complex cytoarchitecture, neurons are particularly dependent on intracellular transport. Disturbances of transport mechanisms can lead to neuronal stress and cell death (Perlson et al 2010, Wang et al 2013). Alzheimer's disease is characterized by accumulation of β-amyloid, which causes impaired retrograde transport of BDNF/trkB activated complexes. This in turn alters synaptic plasticity, which is important for memory (Poon et al 2011, Poon et al 2013). Huntington's disease is characterized by impairment of movement and cognitive decline and caused by expansions in the Huntingtin (Htt) protein, which alters retrograde transport of trkB in striatal dendrites (Liot et al 2013).

Sensory neurons – peripheral nervous system

NT-3 and trkC are expressed during development of the neural tube, as well as in migrating neural crest cells (Kahane & Kalcheim 1994, Tessarollo et al 1994, Williams 1993). NGF is expressed in trigeminal and dorsal root ganglia, and plays an important role for one functional subtype of the nociceptive system (Lewin & Mendell 1993, Lewin & Mendell 1994). Around 75% of the sensory neurons in the DRG and in the trigeminal ganglion require NGF during development (Pearson et al 1983), while in mature DRG neurons this number decreases to 40% (Verge et al 1989). TrkB is found throughout the peripheral nervous system. Both neural crest derived sensory neurons and neuronal placode-derived ganglia, encompassing the geniculate, petrosal and nodose ganglia, are responsive to BDNF (Lindsay et al 1985), in line with the expression of BDNF in taste buds. Among neural crest-derived neurons, BDNF- and NGF-responsive populations overlap, while proprioceptive neuronal populations respond to NT-3 (Wright et al 1997). Muscle spindle and Golgi tendon organ afferents are missing in NT-$3^{-/-}$ mice, indicating that muscle afferents require NT-3 (Ernfors et al 1994). Further, BDNF and NT-4 stimulate regeneration of retinal ganglion cells in the adult retina (Sawai et al 1996).

Sympathetic neurons – peripheral nervous system

NGF is crucial for the development of the sympathetic paravertebral chain (Arias et al 2014, Levi-Montalcini & Cohen 1960). Similar to NGF-responsive sensory neurons, many sympathetic neurons are first dependent on NT-3 (Birren et al 1993, Dechant et al 1993). NGF is still required in adults for maintenance of sympathetic neurons, while this is not the case for NGF- responsive sensory neurons (Lewin & Barde 1996).

Central nervous system

NGF mRNA is expressed in different brain regions, such as in the dentate gyrus and the pyramidal cell layer of the hippocampus, indicating that NGF is produced by neurons in this region (Ayer-LeLievre et al 1988). Similarly, BDNF is expressed in hippocampus, e.g. in neurons of CA2, CA3, the hilar region of the dentate gyrus, and in the pyramidal layers of the cerebral cortex (Ernfors et al 1990b). BDNF mediates activity-dependent processes in the brain, such as neuronal differentiation and growth, synapse formation and plasticity, and higher cognitive functions (Park & Poo 2013). TrkB is also found throughout the central nervous system. Cholinergic neurons of the basal forebrain express p75 and respond to NGF, while cholinergic neurons in striatum lack the p75 receptor and NGF sensitivity (Koh & Loy 1989, Springer et al 1987). Motoneurons can be rescued by the administration of BDNF and to some extent by NT-4 and NT-3 (Henderson et al 1993, Sendtner et al 1992, Yan et al 1992). However, GDNF, not belonging to the neurotrophin family, has a much higher potential (Henderson et al 1994).

The glial cell-derived neurotrophic factor (GDNF) family

This family of ligands consists of GDNF (Lin et al 1994), Neurturin (NTN) (Kotzbauer et al 1996), artemin (ARTN), and Persephin (PSP) (Milbrandt et al 1998) and are a subfamily of ligands within the TGF-ß superfamily that have neurotrophic properties (Lin et al 1993). The GDNF receptor (GFR) α family encompasses GFRα1, GFRα2, GFRα3 and GFRα4 which bind to GDNF, neurturin, artemin, and persephin, respectively (Saarma 2000). However, alternate receptor interactions also occur (Baloh et al 1998). After ligand engagement, the GNDF-GFRα complex activates c-ret tyrosine kinase (Airaksinen et al 1999, Enokido et al 1998, Jing et al 1996, Klein et al 1997, Lindahl et al 2001). GDNF is crucial for embryonic midbrain DA (ventral mesencephalon), spinal motor, cranial sensory, sympathetic, and hindbrain noradrenergic neurons (Liu et al 2013b, Moore et al 1996), and plays key roles also in non-neuronal tissues such as the kidney and gastrointestinal tract (Nosrat et al 1996).

The ret receptor tyrosine kinase plays a role in differentiation and survival of central nervous system neurons, development of kidneys, and the enteric nervous system (Durbec et al 1996, Treanor et al 1996). Further, ret mutations are associated with Hirschsprung's disease, resulting in intestinal obstruction and

constipation (Edery et al 1994), and also with sporadic thyroid carcinomas (Goodfellow & Wells 1995).

TASTE SYSTEM

Taste buds, their innervation and neurotrophic factors

Three different types of gustatory papillae reside on the dorsal surface of the mammalian tongue. The fungiform papillae are located on the dorsal surface of the anterior two-thirds, the foliate papillae on the lateral-posterior part, and the circumvallate papillae on the posterior part of the tongue. The fourth type of papillae, which do not contain taste buds, are the filiform papillae, covering most of the dorsal two-thirds of the tongue's surface (Doty 2015). Taste buds not associated with papillae, are found in the soft palate, epiglottis, pharynx and larynx and amounts to 25% of the total number of taste buds (Nosrat 1998). Sweet taste receptors are also expressed in the gut, pancreas, bladder, brain, bone and adipose tissue. In the gut, this receptor has been suggested to be involved in glucose sensing and the maintenance of glucose homeostasis (Laffitte et al 2014).

The fungiform papillae and the anterior part of the foliate papillae are innervated by chorda tympani, a branch of the facial nerve (CN VII) (Kandel et al 2013). The taste buds in the nasoincisor duct and on the soft palate are supported by the greater petrosal nerve (CN VII). The circumvallate papillae and the posterior part of the foliate papillae are supplied by the glossopharyngeal nerve (CN IX). Finally, epiglottis, pharynx and larynx are innervated by the vagus nerve (CN X). The ganglia for cranial nerves VII, IX and X are the geniculate, petrosal and nodose ganglion, respectively (Doty 2015, Sollars & Hill 2000). The tongue also receives motor innervation from efferent nerve fibers of the hypoglossal nerve (CN XII) (Kandel et al 2013). Autonomic parasympathetic ganglia are located along the lingual and glossopharyngeal nerves (Chibuzo et al 1980), and sympathetic innervation of the tongue is supplied by axons from the superior cervical ganglion (Wang & Chiou 2004). The somatosensory innervation of the tongue is provided by the lingual nerve (CN V) innervating the anterior two thirds of the tongue and the glossopharyngeal nerve (CN IX) providing nerve supply to the posterior tongue (Doty 2015, Kandel et al 2013).

BDNF is expressed during early development of the taste buds and is one of the first biomarkers of taste buds. It is observed in the taste placodes in a temporospatially orderly manner from E15 to E17 in rats, with a peak at E16 and being almost absent at E21 (Nosrat & Olson 1995). However BDNF is expressed again at higher levels in adult rats (Nosrat et al 1996a). BDNF null mice show severe defects in morphology and taste perception, indicating the crucial role it is playing in the taste system (Nosrat et al 1997a). Overexpression of BDNF in adult mice leads to increased taste bud size, elevated taste cell number and denser gustatory innervation, indicating

that BDNF is the most potent neurotrophic factor in the taste system (Nosrat et al 2012).

Taste bud formation

Taste buds arise directly from the oral epithelium (Barlow & Northcutt 1995). Papillae formation is nerve independent, but maintenance requires innervation (Farbman 1972, Farbman & Mbiene 1991, Ito et al 2010). Taste bud cells originate from Cytokeratin 14-positive perigemmeal cells that can undergo proliferation. These cells are located immediately outside the taste buds, give rise to mature taste buds or give rise to new surrounding keratinocytes. Inside the taste bud, daughters of these cells switch on Cytokeratin 8, while they lose Cytokeratin 14 expression (Okubo et al 2009). Cytokeratin 8 positive taste bud primordia are observed on the dorsal surface of the anterior tongue at 13 days of gestation in mice and possess elongated cells in epithelial placods (Mbiene & Roberts 2003). Several neurotrophins are expressed in taste buds. However, only BDNF and NT-3 show a distinct expression pattern in the developing and adult taste buds and the surrounding lingual epithelium (Nosrat et al 1996a, Nosrat & Olson 1995, Rodriguez-Serrano et al 2014). Absence of neurotrophins BDNF, NT-3 or NT-4 results in reduced numbers of taste papillae and taste buds, reduced taste bud innervation and loss of somatosensory innervation (Liebl et al 1999, Nosrat et al 1997a).

Other growth factors and morphogenes are also expressed in developing taste buds and play a role in taste bud formation and development. The Sonic hedgehog (Shh) gene is exclusively expressed in undifferentiated type IV cells of the taste buds and has been suggested to play an important role in their proliferation (Liu et al 2013a, Miura et al 2006). After Shh moves out of the taste buds, it binds to Patched (Ptc) which is expressed in epithelial cells on the basal side outside the taste buds (Miura et al 2001), Smoothened (Smo) is liberated. This leads to intracellular signaling, activating Gli transcription factors and activation of Shh target genes (Saha & Schaffer 2006). While Shh is expressed inside taste buds, Shh target genes Ptc 1 and Gli 1 are expressed by K14-positive progenitor cells adjacent to the buds (Liu et al 2013a, Miura et al 2001). Gli protein mediates transcriptional activity of Shh protein (Ingham & McMahon 2001), and overexpression of Gli leads to basal cell carcinoma (Hutchin et al 2005). These findings suggest that Shh may play a role in taste bud renewal and regeneration.

Shh is also important for differentiation. Taste bud stem cells residing outside of the taste buds self-renew and generate transit amplifying cells. These cells eventually exit the cell cycle, and enter the taste buds as post-mitotic precursor cells. These post-mitotic Shh-expressing basal cells differentiate into type I, type II and type III cells (Miura & Barlow 2010, Miura et al 2014). This is the current model of differentiation or taste bud cell turn-over. Finally, a study has shown that Shh also plays a critical role in patterning of taste papillae during development, having a repressive function (Mistretta et al 2003), while in adults Shh promotes taste cell differentiation (Castillo et al 2014).

Multiple members of the Wnt/β-catenin signaling pathway (Gaillard & Barlow 2011, Iwatsuki et al 2007), Sox2 (Ito & Nosrat 2009, Okubo et al 2006) and Wilms' tumor 1 protein (Gao et al 2014) are also expressed in taste buds. However, their exact functions are still poorly understood.

Several regulators are expressed differentially in taste papillae located on the anterior two thirds of the tongue, compared to the posterior part. Sox17 is essential for formation of endoderm (Kanai-Azuma et al 2002), and lineage tracing of Sox 17-Cre mice have shown that taste buds on the posterior tongue (circumvallate and foliate papillae) are of endodermal origin, while taste papillae on the anterior two thirds of the tongue (fungiform papillae) are derived from ectodermal cells (Rothova et al 2012). BMP-4 is expressed in the posterior papillae while it is absent in papillae on the anterior two thirds of the tongue (Nguyen & Barlow 2010). A similar pattern is observed for keratin 20 (Zhang & Oakley 1996), and FGF signaling (Petersen et al 2011). Also, it has been shown that Pax9 is crucial for circumvallate papillae formation, indicating its endodermal origin (Kist et al 2014).

Taste bud morphology

Taste buds are sensory end organs, consisting of a collection of 50-100 elongate epithelial cells (Type I, Type II and Type III) and a small number of proliferative basal cells (Type IV). They perceive the five basic taste qualities sweet, salt, sour, bitter and umami (Japanese for "pleasant savory taste"), as well as the taste for carbonation (Chandrashekar et al 2009, Sternini 2013).

Type I cells, also called "dark cells", absorb and degrade neurotransmitters. They are the most abundant cells in taste buds and have also been proposed to function as support cells. Type I cells extend lamellate processes around other types of taste cells, suggesting that they serve to functionally isolate other taste cell types. The glial glutamate transporter GLAST is expressed by type I cells, indicating an involvement in glutamate uptake (Lawton et al 2000). The presence of nucleoside triphosphate diphosphohydrolase-2 (NTPDase2), a plasma membrane-bound nucleotidase, suggests hydrolysis of extracellular ATP (Bartel et al 2006). Salty taste may also be transduced by some Type I cells (Chaudhari & Roper 2010), but the extent of this is still uncertain.

Type II (receptor) cells are the second most prevalent cell type. They have a large round nucleus, and express T1R and T2R taste receptors. They also express signaling molecules for taste transduction such as gustducin (Boughter Jr et al 1997), the downstream components phospholipase Cß2 (PLCß2) and type 3 inositol 1,4,5-trisphosphate receptor (IP3R3)(Clapp et al 2001, Miura et al 2007). Sweet, bitter and umami taste compounds activate receptor cells, induce them to release ATP through pannexin1 (Panx1) hemichannels, which in turn excites the ATP receptors P2X2 and P2X3 (Huang et al 2011b).

Type III (presynaptic) cells are the least abundant cell type (Chaudhari & Roper 2010), representing only 6-10% of the cells in taste buds and containing many

substances that are commonly found in neurons. They have synaptic contacts with the gustatory nerve fibers and express synaptic membrane protein 25 (SNAP25) (Yang et al 2000), neural cell adhesion molecule (NCAM) (Nelson & Finger 1993), neuron specific enolase (NSE), and ubiquitin carboxyl terminal hydrolase 9.5 (PGP 9.5). This implies that type III cells participate in transmission of information to the nervous system. Presynaptic cells also release serotonin (5-HT), which in turn inhibits receptor cells. Sour compounds and carbonation directly activate Type III cells (Huang et al 2011a). Sour-sensing cells are therfore also able to perceive the taste of carbonation (Chandrashekar et al 2009). Synaptobrevin, a vesicle-associated membrane protein (VAMP), colocalizes with SNAP25, 5-HT, PGP 9.5, and IP3R3 (Yang et al 2004). Syntaxin is also expressed in type III cells, supporting the theory that taste cells use classical SNARE machinery for neurotransmitter release (Yang et al 2007).
Type IV basal cells are undifferentiated Shh-expressing basal cells (precursors) of taste cell types I, II and III (Miura et al 2014). They are located at a distance from the taste pore and do not extend processes into them (Farbman 1965). Vismodegib, an FDA approved treatment for basal-cell carcinoma, is an inhibitor of the hedgehog pathway. Vismodegib-treated mice have reductions in taste bud size and numbers of taste cells, including PLCß2- and α-gustducin-expressing cells, which detect sweet, bitter and umami tastants (Yang et al 2014).

Taste transduction of salt, sweet, bitter, umami, and sour taste

Salty tastants break up into Na^+ and Cl^- ions, and Na^+ permeates through amiloride-sensitive epithelial Na^+ channels, (ENaC), where amiloride functions as a potent inhibitor (Heck et al 1984). Knocking out ENaC leads to impaired salt detection (Chandrashekar et al 2010). It has been suggested that Na^+ detecting cells are among Type I cells (Vandenbeuch et al 2008).

The perception of sweet, bitter and umami tastes is initiated by the interaction of sapid molecules with G protein-coupled receptors (GPCRs) in the microvilli of the taste receptor cells (TRCs). α-gustducin, an α-subunit of G protein, is selectively expressed in approximately 25-30% of the Type II cells. GPCRs interact with α-gustducin. This causes the Gβγ subunits to be freed from the taste GPCR and interact with phospholipase, PLCβ2. This in turn stimulates the synthesis of the second messenger inositoltriphosphate (IP3), which opens IP_3R3 ion channels on the endoplasmic reticulum, releasing Ca^{2+} into the cytosol. The increased intracellular Ca^{2+} causes taste-selective cation channel TRPM5 to produce a depolarizing generator potential. Also, ATP is secreted through the gap junction hemichannels into the extracellular space (Chaudhari & Roper 2010). Another second messenger, cAMP, causes an influx of cations upon exposure to sweet, bitter and umami. cAMP signaling seems to modulate the secretion of amine neurotransmitters in presynaptic cells (Type III) (Roberts et al 2009).

Two unrelated families of receptors (T1Rs and T2Rs) transduce sweet, aminoacid (umami), and bitter tasting compounds. The TRPM5 is expressed in the same cells

as T1R and T2R, and TRPM5 mutants do not respond to sweet, amino acid, or bitter stimuli (Zhang et al 2003b).

The sweet taste receptor is a heterodimer of two GPCRs where T1R2 is paired with T1R3. The sweet receptors are expressed in all three gustatory papillae, but to different degrees. All fungiform papillae contain sweet receptors, while only 10% of circumvallate papillae contain receptors for sweet compounds (Hoon et al 1999). Natural and artificial sweeteners activate the same receptor (Li et al 2002, Nelson et al 2001), albeit through different mechanisms (Margolskee 2002).

T2R receptors, which encompass about 25 T2R subtypes, transduce bitter tastes. T2Rs are not expressed in the same taste cells as T1R1, T1R2 and T1R3. In rodents, T2Rs are found in 15-20% of TRCs in taste buds of circumvallate and foliate papillae and the palate, but in very few TRCs of fungiform papillae (Hoon et al 1999, Margolskee 2002). Sensitivity to bitter flavor has evolved as a protective mechanism against poisonous compounds.

Umami is the taste of glutamate, certain other amino acids and nucleotides (Chaudhari et al 2009). Three GPCRs have been proposed as taste receptors for umami. A heterodimer of T1R1 and T1R3 responds to glutamate and amino acids. Taste-mGluR4 is a taste-specific variant of a metabotropic glutamate receptor, which is expressed in rat taste buds from the posterior tongue (Chaudhari et al 1996). The third receptor for umami is a truncated mGluR1 (San Gabriel et al 2005). Umami-sweet interactions have been investigated *in vitro* and it was shown that sensitivity to sucrose was increased by monosodium glutamate and umami active peptides (Shim et al 2015).

The cells that detect sour express the polycystic kidney disease 2-like 1 protein (PKD2L1)(Bushman et al 2015, Chaudhari & Roper 2010, Frings 2010). However, the mechanism by which this tastant is detected is still poorly understood. Type III cells also detect carbonation, which is a novel taste modality. In water, CO_2 leads to HCO_3^- and H^+ ions as enhanced by carbonic anhydrase (Chandrashekar et al 2009), where the protons acidify the environment (Chandrashekar et al 2009, Chaudhari & Roper 2010).

Number of fungiform papillae and bitter receptors are increased in supertasters

Based on the capability to perceive the chemical compounds phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP), people are divided into non-tasters and tasters (Bartoshuk et al 1994). PTC is toxic, and thus PROP has replaced it. Non-tasters do not perceive bitterness of PROP at all. Medium tasters sense the bitterness but don't mind it, while supertasters perceive a very intense bitter taste after ingestion of PROP. Since the "non-tasters" still perceive taste, but not PROP, we could also call them "non-PROP tasters".

Around 25% of the population are "supertasters" (Bartoshuk et al 1998), and considering this high ratio, we could denote them as "high-tasters". Accordingly, the tasters could be referred as "medium-tasters" and the "non-tasters" as "low-tasters".

Kale, Brussels sprouts and other vegetables are perceived much stronger by the "super-tasters" or "high-tasters". PTC and PROP are synthetic compounds, but are chemically similar to isothiocyanates found in broccoli, cabbage and other Brassica vegetables (Drewnowski & Gomez-Carneros 2000). Supertasters have a higher number of fungiform papillae and taste buds. Women have more fungiform papillae and, accordingly, supertasting abilities are more prevalent among them. Supertasters also perceive more burn from alcohol and capsaicin (Bartoshuk et al 1994). Non-tasters experience less bitterness from alcohol compared to supertasters. The intensity from an alcohol probe correlates with either PROP bitterness or the number of fungiform papillae (Duffy et al 2004). Supertasters also feel more creaminess from fats in food and thereby tend to avoid fatty foods (Bartoshuk 2000). The prevalence of dental caries in preschool children is increased in children of mothers who are non-tasters (Alanzi et al 2013).

Our laboratory has generated novel transgenic mouse lines in which the gustducin promoter drives overexpression of BDNF in taste buds. Such mice have a larger number of taste cells, and larger taste buds with a more dense innervation (Nosrat et al 2012). Our data show that these BDNF overexpressing mice are also able to detect sucrose at lower concentrations compared to wild type (Nosrat et al 2016), and thus can be used to study supertasting in mice.

Bitter perception is mediated by a family of 25 TAS2R (T2R) taste receptors. The most studied receptor, TAS2R38, is associated with the ability to taste PTC and PROP (Bufe et al 2005). Food liking strongly correlates with PROP taster ability but not as much with TAS2R38 genotypes. Ability to taste PROP is defined by polymorphisms in TAS2R38, which is a pleiotropic gene and influences multiple phenotypic traits such as bitter and non-bitter tastes (Robino et al 2014).

Neurotransmitters in the gustatory system

There are several neurotransmitters expressed in the taste buds. ATP is released from taste buds and activates P2X2/P2X3 receptors on taste nerves (Bo et al 1999, Finger et al 2005, Kataoka et al 2006), as well as P2Y receptors on taste cells (Baryshnikov et al 2003, Huang et al 2009, Kataoka et al 2004). Adenosine is a breakdown product of ATP signaling, and is required for normal sweet taste function in the posterior tongue. Its receptor, adenosine receptor A2BR is particularly expressed in type II taste cells in circumvallate papillae and modulates sweet taste (Kataoka et al 2012). Serotonin (Jaber et al 2014, Kim & Roper 1995, Yee et al 2001), gamma-amino butyric acid,,acetylcholine, and norepinephrine are also expressed. Gamma-amino butyric acid and serotonin are inhibitory transmitters in taste buds and presumably have a modulating role in taste responses (Huang et al 2011a). Glutamate is a candidate neurotransmitter (Roper 2013). Neuropeptides are also expressed in the taste buds, which include cholecystokinin (Herness et al 2002), galanin (Seta et al 2006), glucagon-like peptide (GLP) (Elson et al 2010, Shin et al 2008), neuropeptide Y (Zhao et al 2005) and vasoactive intestinal peptide (Shen et al 2005). The glucagon-like peptide-1 (GLP-1) is released from the taste buds upon stimulation with sweet compounds. Also, GLP-1R$^{-/-}$ mice have reduced

neuronal and taste behavioral responses to sweeteners. This indicates that GLP-1 is functioning as a neurotransmitter for sweet taste in addition to the main neurotransmitter ATP (Takai et al 2015).

Taste disorders

Taste disorders are defined as hypogeusia which is a decreased ability to taste, ageusia meaning a complete loss of taste perception, hypergeusia referring to increased gustatory sensitivity and the most common taste dysfunction, dysgeusia, which is impaired taste sensation. In this taste disorder taste stimuli are often perceived as metallic or bitter. The aetiology of taste disorders include head trauma, infections of the upper respiratory tract, exposure of harmful chemicals, iatrogenic causes such as dental treatment or radiation exposure, medicines, and glossodynia, also known as the "Burning Mouth Syndrome" (Hummel et al 2011).

One example of a medication-induced taste disorder is the use of chemotherapy given to patients suffering from advanced basal cell carcinoma, which is also the most common cancer. Basal cell carcinoma is caused by alterations in the Shh signaling pathway caused most commonly by mutations in Ptc. With the loss of function of Ptc, the inhibition of signaling activity of Smo is lost, resulting in uncontrolled proliferation of basal cells. Vismodegib is a chemotherapy drug which is an inhibitor of Smo and tumor shrinkage is observed in patients taking this medicine. Unfortunately, adverse events such as dysgeusia, alopecia, fatigue and weight loss are also observed and many patients discontinue taking the drug (Sekulic et al 2012). Our lab has shown that overexpression of BDNF in taste buds prevents dysgeusia following the treatment, and preserves taste bud morphology and innervation. These BDNF overexpressing mice show a higher expression of Shh, gustducin, Ki67, Troma-1 (cytokeratin 8), T1R3, GAP-43, NCAM and P2X3 in the taste buds, compared to wild type mice (Palacios et al 2017). The findings indicate that BDNF or its mediators may be used for treatment of taste dysfunction, but further studies need to be undertaken.

NEUROBIOLOGY OF TEETH

Vertebrate dentition

Most vertebrates have the ability to renew their dentition multiple times throughout life (polyphyodonts), while the majority of mammals can replace their teeth only once (diphyodonts) (Whitlock & Richman 2013). Rodents develop only one set of teeth (monophyodonty), but their incisors grow and wear away continuously throughout their lives and constantly produce enamel. Birds have lost the ability to form teeth about a million years ago (anodonty) (Sire et al 2008). The shape of the teeth depends on its use. Animals use their teeth for mastication, grooming and defense (Szalay & Seligsohn 1977). Mammalian teeth have more cusps and are more complex, while fish and reptiles have a larger number of simple teeth. Among

mammals, molars are generally simpler in carnivores (e.g. lions), while herbivores (e.g. pandas) have complex crown topography (Jernvall & Thesleff 2012).

The origin of teeth, tooth development and signaling molecules

Teeth form through intricate series of reciprocal interactions between the ectodermal-derived oral epithelium and the underlying neural crest-derived ectomesenchyme. The initial inductive signal resides in the epithelial cells of the dental lamina placodes, followed by mesenchymal condensation (Hildebrand et al 1995, Sharpe 2001). Until recently, it has been thought that DPCs and odontoblasts derive from the neural crest (Miletich & Sharpe 2004). However, it has been shown that a significant number of mesenchymal stem cells are derived from peripheral nerve-associated glia which produce pulp cells and odontoblasts (Kaukua et al 2014).

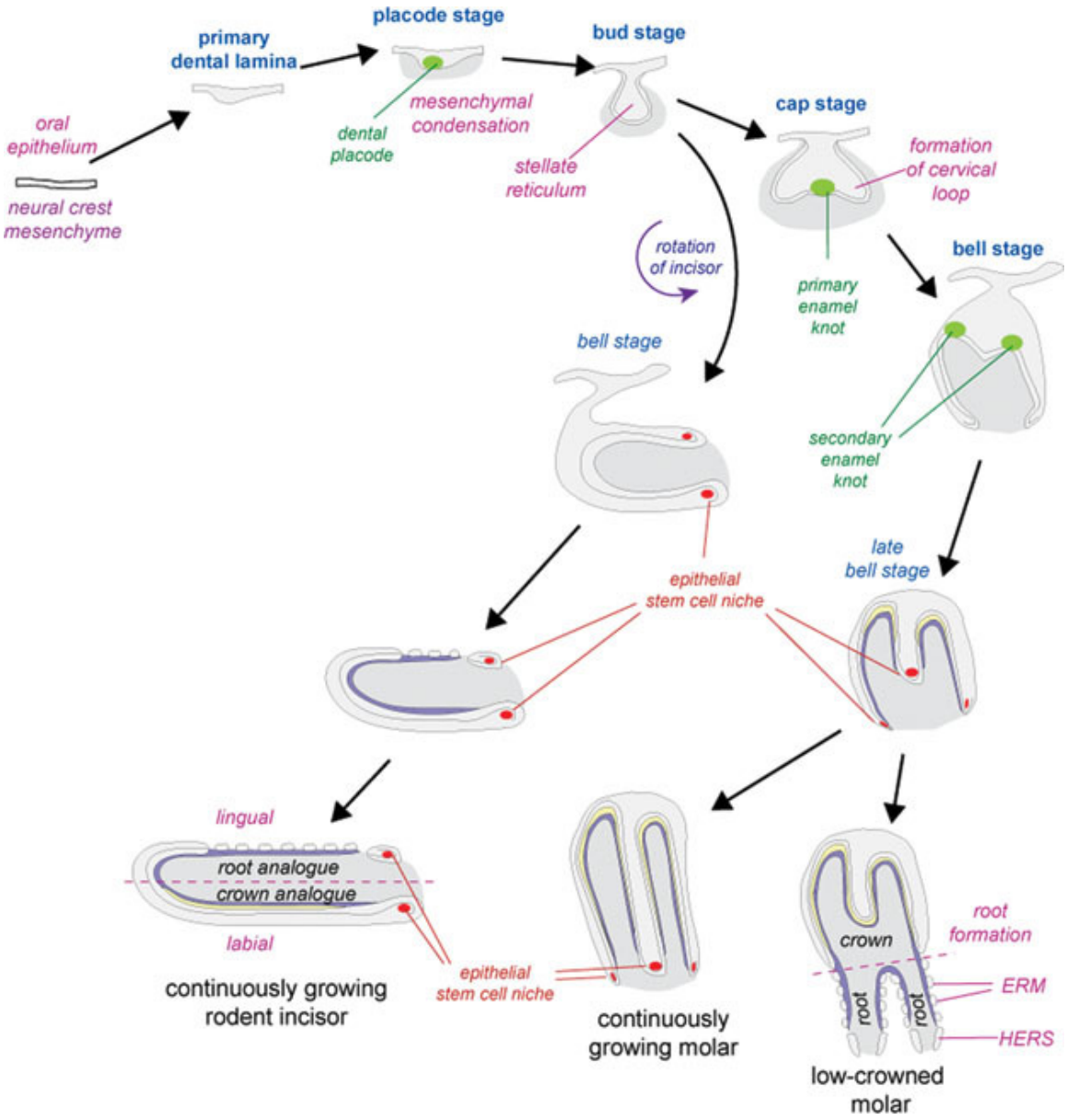

Fig 1. Development of molar and incisor teeth. With permission, as summarized by Thesleff and Tummers 2009.

The first morphological sign of tooth development is the formation of the dental lamina (Fig. 1)(Thesleff & Tummers 2009), a thickening of the oral epithelium at the site of the future tooth, which occurs at around E11 for murine first molars. This thickening expresses Shh that acts to increase cell proliferation (Hardcastle et al 1998). Subsequently, the proliferating dental lamina invaginates further into the underlying ectomesenchyme, thereby forming epithelial buds (E 13, bud stage). During this stage, the dental epithelium segregates into two distinct cell lineages, the peripheral basal cells and the loosely arranged stellate reticulum located in the middle (Thesleff & Tummers 2008). Until E12 the instructive information for tooth development resides in the epithelium, but at E12 the mesenchyme starts to

condense around the forming bud and takes over the instructive role (Lumsden 1988, Mina & Kollar 1987, Tucker & Sharpe 1999). The mesenchymal condensation is controlled by both chemical signals and mechanical compression. The initial effects of cell compaction are mediated by suppression of the mechanical signaling molecule RhoA. Fgf8 and Sema3f, produced by the dental epithelium, attract and repulse mesenchymal cells, respecively (Mammoto et al 2011). The condensed mesenchyme expresses signaling molecules and transcription factors such as Bmp4, Msx1, and Pax9 (Chen et al 1996, Peters et al 1998, Satokata & Maas 1994). The dental mesenchyme then signals back to the developing tooth and induces the formation of the enamel knot seen at the tip of the bud. This cluster of cells express signaling molecules such as Shh, Fgf4, Fgf8, Fgf9, Bmp2, Bmp4, Bmp7, and Wnt10b, and as such, has been classified as an important signaling center (Kettunen & Thesleff 1998, Kratochwil et al 2002, Sarkar & Sharpe 1999, Vaahtokari et al 1996). Fgf15 and Fgf20 have recently been discovered in the enamel knot, while Fgf16, Fgf17, and Fgf18 are expressed in other regions of the tooth germ (Porntaveetus et al 2011). Shh is also expressed in developing human primary dentition (Hu et al 2013). Mutations in Wnt10a and Eda cause taurodontism (enlarged pulp chamber) and tooth agenesis (He et al 2013, Yang et al 2015). Endosulfatases Sulf 1 and Sulf2 are important modulators for desulfation of heparin sulfate proteoglycans (HSPGs), for the activation of the Wnt signaling in odontoblasts, and for production of the dentin matrix (Hayano et al 2012). Molars go on to form secondary and tertiary enamel knots, while incisors only have one knot, indicating that the number of enamel knots determine the cuspal pattern of the resulting tooth (Vaahtokari et al 1996). Bmp7 antagonist USAG-1 (uterine sensitization-associated gene-1) inhibits Bmp7 binding to their cognate receptors and thereby also participates in tooth patterning and morphogenesis (Kiso et al 2014).

At E14-15 the dental epithelium acquires the cap shape (cap stage). The mesenchyme diverges into two different pathways, the dental papillae which later gives rise to odontoblasts and dental pulp, and the peripheral dental follicle giving rise to the cementoblasts and periodontal tissue (Thesleff & Tummers 2008). TGF-ß2 is expressed in the epithelial cells and the enamel knot at E14-16. It is also detected in odontoblasts and ameloblasts (Li et al 2008). Edar (Ectodysplasin A receptor), a member of the tumor necrosis factor receptor family, is also involved in tooth development, as is also Eda, belonging to the tumor necrosis factor ligand family. It has been shown that Edar/Eda interactions are important for the enamel knot formation (Tucker et al 2000). Transcription factors Zeb1 and Zeb2 are expressed in the tooth germ at the cap stage, and participate in neural crest-derived morphogenesis (Shin et al 2012).

After the cap stage, the tooth germ progresses to the bell stage (starting around E17), and epithelial cells differentiate into enamel-secreting ameloblasts. The enamel organ consists of the outer dental epithelium, stellate reticulum, stratum intermedium and inner dental epithelium (Lesot & Brook 2009, Tummers & Thesleff 2009). The dental mesenchyme (to become dental papilla) is adjacent to the inner dental epithelium of the enamel organ and both of them are surrounded by mesenchymal dental follicle cells. During the cap and bell stages the lateral sides of

the epithelial bud envelops the dental mesenchyme. The leading edge of the epithelium is called the cervical loop. Enamel and dentin are secreted by ameloblasts and odontoblasts, respectively. After the crown formation is completed, root formation is initiated and cementoblasts, that have differentiated from the dental follicle, form cementum (Thesleff & Tummers 2008). In non-continuously growing teeth the cervical loop is modified when root formation is commenced, which is not the case in continuously growing incisors, as in rodents. In molars, stellate reticulum and stratum intermedium disappear leaving only a double layer of basal epithelium known as Hertwig's epithelial root sheet (see below). Tetraspanin transmembrane proteins, such as p53 apoptosis effector, are involved in the morphogenesis of the epithelium and the enamel formation. p53 apoptosis effector is expressed in the dental lamina and stellate reticulum at the bud and cap stages and interacts likely with the enamel knot (Neupane et al 2014). Mutations in SPARC related modular calcium binding protein have been identified in a family with oligodontia and microdontia, showing that this protein plays an important role in the development of permanent and deciduous teeth (Alfawaz et al 2013).

Dental stem cells involved in tooth development

Dental stem cells have a major role in the developing tooth organ. Stem cells have the ability to self renew and continuously produce one or several differentiated cell types throughout the lifetime of an organism (Donovan & Gearhart 2001). They are controlled by particular microenvironments known as 'niches', which maintain and control stem cell activity (Spradling et al 2001). The neurovascular bundle represents a mesenchymal stem cell niche found in the mouse incisor. The mesenchymal stem cells originate from periarterial cells and are regulated by Shh secreted from the sensory nerves in the neurovascular bundle (Zhao et al 2014).

Cells in the apical end of the tooth proliferate and differentiate into mesenchyme-derived odontoblasts and epithelium-derived ameloblasts. It has been suggested that epithelially derived stem cells, that give rise to the ameloblasts in the incisor teeth in rodents, reside in the area of the cervical loop of the crown analogue side. The cervical loop consists of a central core of stellate reticulum cells surrounded by a layer of basal epithelial cells contacting the dental mesenchyme. Mesenchymal FGF-10 (and FGF-3) provides proliferative signals from mesenchyme to epithelium. FGF-10 also stimulates Lunatic Fringe expression, which affects Notch signaling, and thereby the cell fate of stem cells in the cervical loop (Harada et al 1999).

Rodent incisors demonstrate continuous growth, exhibiting a constant stem cell compartment and expression of FGF-10. By contrast, molars begin to form roots after completion of crown formation, and FGF-10 disappears during this transitional stage. Overexpression of FGF-10 in the transitional stage of molar germs inhibits the formation of Hertwig's epithelial root sheet. The expression pattern of FGF-10 suggests its role in facilitating the diversification of tooth size, number of cusps and whether the teeth are in a state of continuous (rodent incisors) or limited growth (e.g. molars) (Yokohama-Tamaki et al 2006).

Many studies have shown the potency of dental stem cells. The transcription factor Sox2, a specific marker for epithelial stem cells, is expressed in the cervical loop, and Sox2+ cells contribute to the renewal of ameloblasts as well as all other epithelial cell lineages of the tooth (stratum intermedium derived from stratum reticulum cells) (Juuri et al 2012). Another study has shown that bovine dental follicle cells are capable to form cementum like matrix indicating that they contain cementoblast progenitors (Handa et al 2002). The dental follicle gives rise to several tissues of the periodontium, including cementum, the periodontal ligament and/or alveolar bone. Wnt5a regulates dental follicle stem cells of the periodontium (Xiang et al 2014). A study performed in alligators (embryos and hatchling to 3-y-old) showed that the dental lamina organization is more complex in alligators than in any other family that also forms successional teeth. B-catenin, the intracellular signal transducer in the Wnt signaling pathway, is also expressed (Wu et al 2013). Further, epithelial cell rests of Malassez (ERM) are quiescent epithelial remnants of Hertwig's epithelial root sheath (HERS), that didn't completely disappear after root formation. ERM can differentiate into ameloblast-like cells and generate enamel-like tissue (Shinmura et al 2008). HERS/ERM cells express stem cell markers, such as Nanog, stage-specific embryonic antigen-4 and octamer-binding transcription factor 3/4 (Nam et al 2011). Octamer-binding transcription factor 3/4 and B lymphoma Mo-MLV insertion region 1 are involved in the root development of mouse molars (Nakagawa et al 2012).

The post-natal dental pulp contains a population of multipotent stem cells with the capacity to differentiate into several different cell lineages *in vitro* and *in vivo*, including glia and nerve cells. They are also able to regenerate a dentin-pulp-like complex that is composed of mineralized matrix with tubules lined with odontoblasts, and blood vessels embedded in fibrous tissue (Gronthos et al 2002, Gronthos et al 2000, Miura et al 2003, Nosrat et al 2001b). Also, stem cells from the apical papilla of the root, combined with periodontal ligament stem cells from human exfoliated deciduous teeth, have the capacity to generate dentin and periodontal ligament, including cementum and Sharpey's fibers (Miura et al 2003, Shi et al 2005, Sonoyama et al 2006, Yen & Sharpe 2008).

Finally, we have shown that rat and human post-natal DPCs acquire neuronal morphology in culture, expressing neuronal markers PGP 9.5 and ß-III tubulin, and show distinct electrophysiological properties (Nosrat et al 2004b). These findings suggest that adult neuronal stem cells derived from dental pulp may provide an opportunity for the development of autologous cell engraftment strategies for the treatment of disease and tissue repair in the nervous system.

Tooth innervation, neurotrophic factors and semaphorin

Trigeminal axons are present before any histological signs of tooth formation are visible. When epithelial changes occur in areas where dental ridges will form (Lefkowitz et al 1953), axons are present in the maxillary and mandibular processes. Simultaneous with dental epithelium thickening and the ectomesenchyme condensation underneath the epithelium, axons grow towards the mesenchyme,

divide into lingual and buccal branches and extend towards the epithelium (Luukko et al 1997b),

Innervation of the mandibular and maxillary processes is observed even before the tooth buds appear. This seems to be important for the initiation and patterning of the tooth germs (Kollar & Lumsden 1979). The mammalian tooth starts becoming innervated in the bell stage, with thin nerve fibers entering the mesenchyme, from which the dental pulp and follicle develop (Mohamed & Atkinson 1983, Pearson 1977). In the late bell stage, after dentin and enamel formation have started, PGP 9.5-positive fibers, and sensory calcitonin gene-related peptide- and substance P-positive fibers are seen. When root formation commences, sympathetic neuropeptide Y-positive fibers are also observed. Axons are present in the cuspal dentinal tubuli several days before a molar erupts and by the time the teeth have become functional most of the innervation in the cusps is established (Byers 1980).

Sensory nerve fiber ingrowth into the dental pulp occurs around P4-5 in rodents (Moe et al 2008). It has been suggested that NF in the dental pulp initiate and orchestrate the dental pulp innervation. NF are important modulators of neuronal survival, plasticity, and target innervation and play important roles in axon growth and synaptogenesis (Lewin & Barde 1996, Poo 2001, Tucker et al 2001). NGF is required for differentiation of cranial neural crest cells into tooth organs as shown by *in vitro* organ culture studies (Amano et al 1999). All neurotrophins (NGF, BDNF, NT-3 and NT-4) and other NF, such as GDNF and neurturin, are also expressed in developing human and rodent tooth germs (Luukko 1998, Nosrat et al 1997b, Nosrat et al 2002). GDNF has been proposed to be involved in the regulation of innervation of teeth postnatally (Luukko et al 1997a, Nosrat et al 1998). NGF, BDNF, and GDNF mRNA transcripts are highly upregulated in the DPCs at the time of the dental pulp innervation, indicating that NGF, BDNF and GDNF may play a role in innervation (Luukko et al 1997a, Luukko et al 1997b, Nosrat et al 1998, Nosrat et al 1997b). In support of a trophic role for DPCs, we have shown that DPCs promote the survival of sensory neurons of the trigeminal ganglion, motoneurons of the spinal cord (Nosrat et al 2001b), and DA neurons (Nosrat et al 2004b).

Although nerve fibers are observed in the tooth germ already during embryonic stages, they innervate the dental pulp only after the onset of enamel formation, which in mice occurs around P3-4 (Moe et al 2008, Mohamed & Atkinson 1983). Studies of semaphorin-3A-deficient mice show that nerve fibers reach the presumptive pulp-dentin border area earlier than normally. This indicates that semaphorin-3A acts as a chemorepellant to control timing of the innervation of the dental pulp (Moe et al 2012).

TGF-beta 1 mediates regulation of NGF and both are upregulated in pulp tissues after tooth trauma and injury, suggesting that they are involved in the differentiation of odotoblast-like cells (Byers et al 1992, Tziafas & Papadimitriou 1998, Yongchaitrakul & Pavasant 2007). Another study has shown that pulp inflammation leads to upregulation of BDNF in trigeminal ganglion suggesting that BDNF may play a role in plasticity at the first-order trigeminal synapses (Tarsa et al 2010). The complement system, which is an important part of innate immunity and

one of the first to elicit immune responses, is active during carious lesions and plays an important role in regeneration of dentin and pulp via interaction of the active Complement C5a fragment and pulp progenitor cells. It has been shown that the C5a receptor (C5aR) modulates NGF secretion by pulp fibroblasts which were stimulated by lipoteichoic acid in order to mimic dental caries. Interestingly, there is a negative correlation between the secretion of NGF from pulp fibroblasts and C5aR activation (Chmilewsky et al 2016b). On the other hand, another study has shown that C5aR acts as a positive modulator of BDNF and the neurite outgrowth which is observed after lipoteichoic acid stimulation, and thus can be attributed to this neurotrophic factor (Chmilewsky et al 2016a).

Nerve supply in mature teeth

The tooth is innervated by sensory and sympathetic nerve fibers originating from the trigeminal and the superior cervical ganglion, respectively, and also to some extent by parasympathetic nerve fibers (Hildebrand et al 1995, Olgart 1996) through the otic ganglion (Segade & Suarez-Quintanilla 1988). The dental pulp is mostly supplied by sensory, nociceptive myelinated Aß, A∂ and unmyelinated C-fibers (Chung et al 2013).

Innervation is necessary for development and maintenance of organs and tissues, and an excellent model to demonstrate this is the gustatory system. BDNF- and NT-3 null mice have severe loss of innervation of taste papillae and somatosensory innervation of the epithelium, respectively. Absence of BDNF causes distorted taste buds and malfunctioning taste perception (Nosrat et al 1997a).

Dental nociception

Nociception is the perception of pain and nociceptors are seemingly non-specialized nerve endings that initiate the sensation of pain (Purves & Augustine 2011). Dental pain is derived from the dental tubuli and dental pulp. Three different hypotheses, the nerve conduction theory, the hydrodynamic theory, and the theory that odontoblasts function as nociceptors, have been proposed (Chung et al 2013). In order to arrive at a diagnosis of pain, history, clinical examination, and tests should be undertaken. These include application of dry ice, a percussion test and a mobility test (Ehrmann 2002). The three, not necessarily mutually excluding, theories of the origin of pain perception in teeth are:

1. ***The neural theory***

   Nerve endings that penetrate dentinal tubules respond directly to external stimuli. Temperature-sensitive transient receptor potential channels (TRP channels) emphasize the direct transduction of noxious temperatures by dental primary afferent neurons. Several temperature-sensitive TRP channels are expressed by trigeminal ganglion neurons and, more specifically, in dental primary afferents (Chung et al 2013).

Electrophysiological recordings during application of capsaicin suggest functional expression of TRPV1 and TRPV2 in rodent primary afferent neurons. It has been shown that dental afferent neurons express TRPV1 and TRPV2 more abundantly relative to the trigeminal ganglion neurons, which is consistent with the traditional view that most dental afferent neurons are nociceptors (Tarsa et al 2010).
Cold stimuli are a frequent cause of tooth pain and it is known that subpopulations of Aδ- and C-fiber neurons are responsive to cold stimuli. TRPM8 and TRPA1 receptors are activated by temperatures of 25˚C and 17˚C, respectively (Tominaga & Caterina 2004). Also, the cold receptors TRPM8 and TRPA1 are co-expressed within subpopulations of TRPV1-positive dental afferent neurons, which might explain why it is difficult to discriminate between hot and cold stimuli applied to the teeth (Chung et al 2013).

2. The hydrodynamic theory

Mechanical forces generated by fluid movements within the dentinal tubules are detected by nerve endings near the dentin and cause dental pain. Several cellular mechanical force transducer molecules are expressed in dental afferents, such as TRPV1, TRPV2 and TRPA1. Other mechanotransducers are TRPV4 and TRPM3, reported only in trigeminal ganglion neurons, but not in dental primary afferents. 75% of mandibular pulpal nerves respond to mechanical stimulation of exposed pulp (Chung et al 2013). Pulsating pain experienced with chronic pulpitis suggests that tooth pain might be induced by hydrostatic pressure applied to inflamed pulp tissue surrounded by hard dentin structures (Heyeraas & Berggreen 1999). TRPA1 is implicated in both cold hyperalgesia and mechanosensation, which might explain why dental pain elicited by a light puff of air is often confused with cold nociception (Chung et al 2013).

3. The odontoblast transducer theory

Odontoblasts are proposed to have a sensory function in this concept and be capable of detecting thermal or mechanical stimuli, thereby serving as pain transducers. They express several members of the TRP channels such as TRPV1-4, TRPM3 (Son et al 2009), TRPM8, as well as TRPA1 (El Karim et al 2011). The odontoblasts also possess direct excitability, demonstrated by expression of voltage-gated Na^+ channels, voltage-gated K^+ channels, calcium-activated K^+ channels, Na^+/Ca^{2+} exchanger, and TREK-1 channels. Signaling between odontoblasts and underlying primary afferents has been demonstrated. Expression of purinergic P2X3 (Alavi et al 2001) and P2X7 receptors (Itoh et al 2011) in nociceptive dental primary afferents has been detected. The close relationship of odontoblasts with trigeminal sensory axons, suggests that ATP could mediate painful signaling between odontoblasts and neurons. It has been suggested that purinergic receptors could be targeted to develop novel analgesics (Burnstock 2006).

SPECIFIC AIMS

- Identify neurotrophins and their receptors in the developing and adult taste system
- Evaluate the roles of BDNF and NT-3 in taste papillae formation
- Identify neurotrophic factors in developing human teeth
- Evaluate production, release and biological activity of neurotrophic factors in dental pulp
- Assess dental pulp cell neurotrophic activity, function and support for trigeminal, dopamine and motor neurons
- Evaluate dental pulp maintenance and innervation *in oculo*

MATERIALS

Embryonic human tongue, embryonic human teeth, and adult human tongue biopsies

Embryonic human tissue pieces were obtained following elective first-trimester abortions, which was approved by the Human Ethics Committee of the Karolinska Institutet (Sweden) and was also in accordance with NIH guidelines. The age was determined by examination of anatomical landmarks (such as the appearance and maturity of the central nervous system, extremities, start of ossification of certain bones) and size of the abortus according to the atlas of England (England 1990), see also (Åkesson et al 1998, Falci et al 1997).

Human tongue biopsies were obtained from healthy, non-smoking young adult volunteers (20–30 years old). Punch biopsies (Biopsy Punch, Sylak, Norrköping, Sweden) were obtained under local anesthesia with Xylocaine-adrenalin (Astra, Sweden; lidocaine 20 mg/ml + adrenalin 12.5 μg/ml) from the anterior part of the tongue, rapidly frozen on dry ice and the biopsy area was sutured. All procedures were in accordance with the Human Ethics Committee of the Karolinska Institutet, Stockholm, Sweden and also with NIH guidelines.

Human dental pulp cells

Human DPCs were obtained from patients undergoing routine extractions of third molars of young adults. The pulp tissue was dissected and processed for cell culture experiments.

Homozygous BDNF$^{-/-}$ x NT-3$^{-/-}$ mice

BDNF and NT-3 heterozygous mice were crossbred to generate BDNF/NT-3 heterozygous mice, which in turn were crossbred to generate BDNF $^{-/-}$ x NT-3$^{-/-}$ mice. PCR (polymerase chain reaction)-based genotyping was used to identify the transgenic mice. The mice were analyzed at P0. Samples were immersion fixed in 4% paraformaldehyde (PFA) overnight.

Sprague-Dawley rats

Sprague–Dawley rats were obtained from Charles River breeders. Procedures were in accordance with the local Animal Research Committee of Stockholm, the Institutional Animal User Committee (IAUC) at the University of Michigan and University of Tennessee Health Science Center.

P1, P3 and adult female 250-300 g Sprague-Dawley rats were used for retrograde tracing of GDNF and for intraocular transplant experiments.

Dental molar pulp tissue from 5–8 days old rat pups, were used in cell culture and graft experiments.

Timed pregnant Sprague-Dawley rats

Timed pregnant Sprague-Dawley rats were used in order to obtain ventral mesencephalon (VM) for the DA neuron survival experiments. The day on which the rats were sperm-positive (a detectable sperm plug) was designated the first day of gestation (E1). To accurately estimate the age of the embryos, crown-rump length was also measured (Dunnett & Björklund 1992, Olson et al 1983), and embryos removed at E14.

METHODS

Radioactive in situ hybridization

In the human tongue study, oligonucleotide probes complementary to human BDNF [two non-overlapping 50-mer probes, GenBank accession number M37762, starting from bases 333 and 419, (Jones & Reichardt 1990), pig BDNF (GenBank accession number X16713, bases 250–298, (Leibrock et al 1989)], human NT-3 [two probes, GenBank accession number M37762, starting from bases 225 and 740; (Jones & Reichardt 1990)], and rat NT-3 (Ernfors et al 1990a) were synthesized (DNA technology, Aarhus, Denmark) and 3'-end labeled with ^{35}S-dATP. In the human teeth study, non-overlapping 50-mer oligonucleotide probes complementary to human NGF [GenBank accession number V01511, starting at bases 2149 and 2245, (Ullrich et al 1983)], human BDNF [Gen- Bank accession number M37762, starting at bases 333 and 419, (Jones & Reichardt 1990)], pig BDNF [GenBank accession number X16713, bases 250–298, (Leibrock et al 1989)], human NT-3 [GenBank accession number M37762, starting of bases 225 and 740, (Jones & Reichardt 1990)], and human GDNF [GenBank accession number L19063, starting at bases 256 and 340, (Lin et al 1993)] were synthesized (DNA technology, Aarhus, Denmark) and 3'-end labeled with ^{35}S-dATP. In the spinal cord transplantation study, an oligonucleotide probe complementary to GDNF [nucleotides 540–589, (Lin et al 1993)] was used. A 50-mer random probe was used as control [Scandinavian Gene Synthesis; (Nosrat & Olson 1995)]. Procedures for in situ hybridization were in general as described by Dagerlind et al. (Dagerlind et al 1992).

Fetal human tissue and adult tongue biopsies were immersion fixated in a formalin-picric acid mixture (4% PFA, 0.4% picric acid) for 1.5 hours at room temperature in darkness. Serial sections (14 μm) of the human fetal tissues and tongue biopsies were cut on a cryostat and mounted onto coated slides (ProbeOn, Fisher). Sections were hybridized with specific and control probes for 16-20 hours at 42^{O} C.

Slides were dipped in photographic emulsion (Kodak NTB2) and exposed for 4-10 weeks. After developing, the slides were counterstained with cresyl violet or toluidine blue and mounted with Entellan.

Evaluation and processing of in situ hybridized tissues

Positive control procedures were as follows 1) use of two different non-overlapping probes for a given mRNA species, 2) observation of correct labeling in known areas, and 3) microscopy performed by two independent observers who agreed on all findings. Negative controls included 1) labeling with a random control probe, 2) failure of specific probes to label irrelevant structures, and 3) the probes functioning as controls to each other since they had similar GC contents. Cells were regarded as positive if they had accumulations clearly above the surrounding background level, detectable using dark-field microscopy and a primary magnification of x10 or less.

A digital Nikon EXM1200 digital camera was also used, photomicrographs of the sections were scanned (SprintScan, Power Macintosh 9500), and digitally processed as for photoprints regarding brightness and contrast.

Immunohistochemistry

Human/mouse fetal tissue, adult human tongue biopsies, and P0 BDNF$^{-/-}$ x NT-3$^{-/-}$ double knockout tissues were immersion fixed for 3-4 hours or overnight.

Sections (14 µm thick) were collected on chrome alum gelatin-coated slides or Superfrost Plus slides (Fisher) and processed according to the indirect immunofluorescence technique (Coons 1958, Hökfelt et al 1973). Sections were incubated with primary antibodies for 24–48 hours in a humified chamber at 4^{0} C. Antisera against PGP 9.5 (PGP; Biogenesis and Chemicon) and Growth-associated protein 43 (GAP-43, Chemicon) was used. After rinsing in phosphate-buffered saline (PBS), sections were incubated with appropriate secondary antibody conjugated to fluorescein isothiocyanate (FITC, Molecular Probes) in a humidified chamber for 1.5 hours at room temperature in the dark. Following a final rinse in PBS, sections were mounted in glycerol/PBS.

Human and rat dental pulp cells and ventral mesencephalon cells

Human and rat DPCs and rat VM cells were fixed in 4% PFA in PBS for 30 – 60 min, washed with PBS, preincubated in 5% goat serum, and/or horse serum, 5% bovine serum albumin, and 0.3% Triton X-100 for 1 h, followed by overnight incubation with the different antisera in 0.3% Triton X-100 and blocking solution. Antibodies against PGP 9.5 (Biogenesis), growth-associated protein 43 (GAP, Chemicon), Synaptophysin (Chemicon), ß-III tubulin (Covance), glial fibrillary acidic protein (GFAP; Sigma), TH (Pel-Freez), S-100 (Sigma), and nestin (Chemicon) were utilized. Chamber slides were subsequently washed in PBS, incubated for 1 h with rhodamine or FITC-conjugated secondary antibodies. Alternatively, sections were incubated with biotinylated secondary antibodies, which were detected using an avidin-biotin complex (Vectastain ABC Elite); peroxidase activity was visualized with a diaminobenzidine (DAB) peroxidase substrate (Vector VIP kit, Vector Laboratories). Photographs were captured by a fluorescence microscope (Nikon 80i, Tokyo, Japan). Taste buds were analyzed using ImageJ software.

Cell culture procedure

Trigeminal ganglia and dental pulps (DPCs) were dissected and placed in a sterile petri dishes containing ice-cold DMEM/F12, 10% fetal bovine serum (FBS; GIBCO BRL), L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 mg/ml), amphotericin (2 mg/ml, initial 2 weeks), and gentamicin (50 mg/ml). Tissue pieces

were mechanically dissociated and rinsed twice with Earl's balanced salt solution (EBSS) without calcium or magnesium. Tissue fragments were then digested for 20–30 min with 0.25 mg/ml trypsin, and were further mechanically dissociated with fire-polished Pasteur pipettes and seeded on poly D-lysine (100 mg/ml) treated flasks.

Trigeminal ganglia and dental pulp co-culture

Dissociated trigeminal tissue fragments were plated onto chamber slides with DPCs that had been subcultured at least once. After letting trigeminal cells settle for 30 min, excessive cells were removed by aspirating the medium in order to minimize the number of nonneuronal cells in the cocultures, specifically that of Schwann cells.

Skin fibroblast and dental pulp

Skin fibroblast cultures were obtained from abdominal skin from adult rats (Kawaja et al 1992), which was shaved, washed with 70% ethanol, excised and cut into fine pieces, washed several times, and added into culture flasks.

Cell cultures were kept in a humidified incubator at 37^0 C with 5% CO_2, and the medium was changed every 2 days. The medium contained either 1% or 10% FBS When the skin fibroblasts reached confluency, they were subcultured by trypsinization (0.25% trypsin) and were used in the experiments after 1 or 2 passages.

Ventral mesencephalon and dental pulp cultures and cocultures

Rat fetuses were decapitated at E14, and using a lateral approach the VM was dissected (Dunnett & Björklund 1992). Tissue pieces were mechanically dissociated, and tissue fragments were trypsinized for 10 min with 0.1mg/mL trypsin. Dissociated VM cells were seeded into 12-well plates that contained inserts with cultured DPCs.

At different time intervals, VM cells were fixed with 4% PFA in PBS, blocked with a solution containing 5% heat-inactivated normal goat serum, 5% heat-inactivated normal horse serum, 5% bovine serum albumin and 0.3% Triton X-100 in PBS. Cells were then incubated with antibodies against tyrosine hydroxylase (TH; Pel-Freez) in blocking solution overnight at 4^0 C. Cells were subsequently processed (VectaStain ABC Elite system), and visualized (diaminobenzidine substrate kit; Vector Laboratories), and the ones with clear labeling in each cell culture well were counted. The software package for the digital camera (Spot, Diagnostic Instruments) was used to measure cell size and the length of the longest primary neurite on representative TH-positive cells.

Cell nuclei labeling

Dissociated cells from the trigeminal ganglion and DPCs were incubated for 30 min at 37^0 C in the dark in culture medium containing 10 g/ml bisbenzimide to label nuclear DNA (Hoechst, Sigma) to enable distinction between the contributions from different tissue components in the coculture experiments, and for transplantation experiments into the spinal cord.

DPCs and trigeminal cells were also cultured separately for a period of 1 month to study possible cytotoxic effects of bisbenzimide on these cells. Cells appeared normal without nuclear condensations, indicating that the labeling technique did not induce apoptosis or other overt cytotoxic effects at the concentration used.

Growth factor inhibiting antibodies and 6-OHDA treatment

Blocking antibody studies with dopamine neurons and dental pulp cells

Cells of the VM were plated directly on confluent cultures of DPCs, and one hour later antibodies blocking NGF, BDNF, and GDNF were added to the cultures. Cells were fixed after 4 days and TH-positive neurons were counted.

Culture (DPCs and cells from VM) treatments with 6-OHDA and cell counts

DPCs and cells from E14 VM were seeded onto 12-well plates for 24 h. Cocultures of dental pulp and VM cells, and VM cells alone were then exposed to a culture medium containing 6-OHDA (5 or 10 mM), and TH-positive cells were counted as described above.

Intraocular transplantations

Dental pulps were dissected into 6 – 8 pieces each, and were kept in DMEM/F12 until transplanted. Rats (150 g females) received 1 drop of 0.1% atropine in each eye for dilating the irises. The transplantation was carried out according to Olson and colleagues (1983). Intraocular grafting was performed under either ether-anesthesia or Isofluorane inhalation. Recipient rats received a small cut of the cornea with an iris scalpel and the pulpal tissue was injected with a glass pipette into the lateral angle of the anterior chamber of the eye. This procedure causes no bleeding, the cornea heals well and the eye and vision is routinely not harmed. The grafts attach to the anterior surface of the iris, from where they become vascularized. After 5 and 10 weeks, the animals were anesthetized and both transplants and irises were dissected and processed for immunohistochemistry and Falck-Hillarp histochemistry.

Falck–Hillarp histochemistry

Irises from the host rats were evaluated using Falck–Hillarp fluorescence

histochemistry for visualization of monoamines (Corrodi & Jonsson 1967, Falck et al 1962) to detect catecholamine-containing nerve fibers. Dental pulp grafts were carefully removed, the irises were stretch-prepared as whole mounts on glass slides, and reacted with gaseous PFA. The density of the sympathetic, noradrenergic nerve fiber plexus was estimated semiquantitatively on a blind basis using a fluorescence microscope and appropriate filters. The value 100 represents the density of noradrenergic sympathetic nerve fibers in a normal iris.

Spinal cord transplantations and motoneuron survival assay

A subpopulation of motoneurons in the lumbar enlargement was prelabeled with FluoroGold (Fluorochrome, CO) under general halothane (Fluothane; AstraZeneca) anesthesia, by cutting the peroneal nerve above the level of the knee joint and putting the proximal end of the nerve in 2% FluoroGold-Ringer's solution for 30 s.

FluoroGold labeling was performed ipsilateral to a spinal cord hemisection (Novikova et al 1996). The peroneal motoneurons are located in a column of cells extending through spinal cord segments L4–L6 (Swett et al 1986). To estimate the number of motoneurons sending axons into the peroneal nerve, the motoneuron columns of two noninjured animals were also labeled bilaterally using FluoroGold.

Three days after labeling, laminectomy was performed under general halothane (Fluothane) anesthesia to expose the L5 spinal cord segment. The exposed spinal cord was hemisected, cutting the right side completely, and dental pulp tissue, collected from first molars, was cut in pieces and gently inserted into the spinal cord lesions. Hemostasis was achieved and muscle and skin were sutured in layers. Antibiotics (Borgal; Hoechst) were given before surgery and the following week postoperatively. To avoid urinary tract infections, manual emptying of the urinary bladder was carried out three times a day the first week and twice a day thereafter.

4 weeks after injury spinal cords and grafts were dissected, postfixed in fixative for 1 h, and transferred to 10% sucrose solution for cryoprotection. Spinal cord segments L2–S1 were sectioned and the relative number of motoneurons was calculated by counting labeled motoneurons on every other section.

Retrograde transport of 125I-GDNF

Human GDNF protein (Amgen, Thousand Oaks, USA) was labeled with Iodine-125 using the Bolton & Hunter reagent (Amersham Biosciences, USA), to a specific activity of 161 mCi/nmol. 50 ng of Iodine-labeled GDNF protein in 2µL of PBS solution was used for each injection. As a control, ^{125}I- GDNF in a 100-fold stronger concentration of cold GDNF was used.

Pups received injections of ^{125}I-GDNF subcutaneously in the vibrissae area, in the area of the inferior alveolar nerve and into the maxillary teeth. Adult rats received injections subcutaneously in the vibrissae area, each consisting of 50 ng of labeled

GDNF. 24 h after tracer injections, animals were anaesthetized with nembutal (200 mg/kg body weight) and perfused with 4% PFA. Cryostat sections of trigeminal ganglia (and the site of injection) were mounted on gelatin-coated slides, which were dehydrated, dried, dipped in photographic emulsion (Kodak NTB-2) and exposed for 5-6 weeks at -20^0 C. Slides were subsequently developed (Kodak D19 developer) and counterstained with cresyl violet. Accumulations of silver grains over trigeminal neurons were counted. Counts were performed on every third slide and images recorded (Nikon EXM1200 digital camera).

RNA extraction and RNAse protection assay

Total RNA was extracted from DPCs (RNeasy mini kit; Qiagen) from both primary cultures and subcultured cells. NGF, BDNF, and GDNF ribo-probes, and a 100 base-pair marker (RNA Century marker templates; Ambion) were transcribed *in vitro* and labeled with ^{32}P-UTP (Amersham) using linearized DNA templates. RNase protection assay was performed (Ambion: RNase protection assay). The samples were hybridized, and RNase digestion was performed. After gel electrophoresis on a 6% polyacrylamide gel, the bands were visualized (Storm 840). The expression of NF were compared to each other and to that of cyclophilin, and the bands were quantified using NIH-Image software.

Reverse transcription PCR of DPCs

Rat dental pulps were dissected, tissue pieces were mechanically dissociated and washed twice with Earl's balanced salt solution without calcium or magnesium. Tissue fragments were then digested for 20-30 min with 0.25 mg/mL trypsin, and were further mechanically dissociated with fire-polished Pasteur pipettes, and seeded on poly-D-lysine-treated flasks and glass chamber slides (Falkon). DPCs were cultured in medium containing 5% FBS. DPCs that had been subcultured at least two times were used in these experiments.

Human dental pulps were dissected from extracted teeth and processed as above, but 20% serum was used. The cells had been passaged at least three times.

Human and rat dental pulp cell total RNA was prepared using Trizol reagent (Invitrogen), and total RNA was treated with DNase (RQ1 DNase, Promega, 1 h) prior to reverse transcription. cDNA synthesis was performed using the SuperScript double-stranded cDNA synthesis system (Invitrogen), followed by PCR. The rat primer pairs were rat NGF (GenBank Accession number XM_227525), rat BDNF (GenBank Accession number NM_012513), and rat GDNF (GenBank Accession number NM_019139). Human NGF (GenBank Accession number NM_002506), human BDNF (GenBank Accession number NM_000514), and human GDNF (GenBank Accession number NM_170732) were used for the human teeth. PCR was performed and all PCR products were sequenced at the University of Michigan DNA sequencing core to verify that correct DNA fragments had been amplified.

Cell nuclei labeling with bisbenzimide and striatal grafts of DPCs

In order to detect DPCs after being grafted into the CNS in rats, DPCs were incubated with a culture medium containing 10 mg/mL bisbenzimide (Sigma) for 30 minutes. The cells were rinsed with HBSS and culture medium, and kept overnight in an incubator. The following day, cells were trypsinized and resuspended. Anaesthetized (Nembutal, 30mg/kg weight) adult female Sprague-Dawley rats received stereotactic injections of bisbenzimide-labeled DPCs into the right striatum. The stereotactic coordinates used were AP 0.0 mm; L ±3.4 mm, DV -0.5 mm. Control animals received DPCs that had been fixed in 4% PFA and washed prior to grafting. After 6 weeks, rats were perfused with 4% PFA in PBS; brains were dissected and postfixed in the same medium for 2h, cryosectioned and mounted onto gelatin-coated slides. Slides were mounted with coverslips with glycerol-PBS. Bisbenzimide labeled DPCs were visualized with a UV filter.

RESULTS AND DISCUSSION

Paper I: BDNF and NT-3 are expressed in developing and adult human tongues

In this study, we examined if BDNF and NT-3 mRNA transcripts are expressed in developing and adult human taste papillae, and compared expression patterns between human and rodent taste papillae.

Several studies have demonstrated that BDNF and NT-3 are expressed in developing and adult rodent tongues in a temporospatially specific manner. BDNF labeling is associated with developing and adult taste buds that receive gustatory innervation, NT-3 with surrounding epithelial structures that receive general somatosensory innervation (Nosrat et al 1997a, Nosrat et al 2001a). Specific losses of innervation found in BDNF and NT-3 knockout mice confirm that BDNF is related to gustatory innervation and NT-3 to somatosensory innervation of the rodent tongue (Nosrat et al 1997a).

BDNF and NT-3 mRNA expression in the developing human tongue

During the first trimester, BDNF and NT-3 mRNA expression show specific temporospatial patterns in the human tongue. In the early stages, BDNF mRNA is expressed in the developing fungiform and circumvallate papillae before the arrival of nerve fibers. Previous studies have shown that cytokeratin 20 is selectively expressed in developing human taste buds from postovulatory week 8 (Witt & Kasper 1999). We show that BDNF is expressed prior to this stage, indicating that such BDNF-positive cells might be very early taste bud progenitor cells. BDNF mRNA labeling is also observed in the lingual epithelium, subepithelial mesenchyme and in the muscle fibers, suggesting that BDNF might also be related to initiation and establishment of the lingual epithelial innervation and subsequent taste bud development. In the absence of gustatory nerves, mammalian taste buds do not fully differentiate. In rodents, a similar pattern was seen indicating that BDNF and NT-3 are important components for the establishment of the innervation (Nosrat 1998, Oakley 1991, Ringstedt et al 1999). Studies with BDNF-overexpressing mice (Nosrat et al 2012) have shown that BDNF enables invasion of target by gustatory nerve fibers. Previous studies have shown that development of taste papillae in humans occur during weeks 6 and 7 and that development of fungiform taste buds takes place during weeks 8 and 9 (Witt & Reutter 1996, Witt & Reutter 1997), which is in accordance with our findings. BDNF mRNA labeling was detected in the taste buds once they were developed and intraepithelial nerve fibers were associated with the developing taste papillae and taste buds. Clusters of positive cells were observed in the soft palate as well, presumably being components of the palatal taste buds. In rodents, other morphogenetic genes have also been studied. Shh, bone morphogenetic proteins (BMP) 2 and 4, FGF and SOX2 are also expressed in the developing rodent tongue (Hall et al 1999, Jung et al 1999).

NT-3 mRNA labeling was also present from the earliest stages and also underwent a shift of expression pattern. NT-3 mRNA labeling was mainly observed in the lingual epithelium, subepithelial mesenchyme and developing muscles of the tongue. At a later stage, NT-3 mRNA labeling was observed mainly in the epithelium and the labeling became stronger, particularly in the apical surface of the developing taste papillae. At subsequent stages, labeling became weaker but still observable in the same structures as in the previous stage. BDNF and NT-3 mRNA were also found in other areas of the human embryo, such as in the dorsal root ganglia and in the sensory epithelium of the developing inner ear, similar to what is seen in rodents (Pirvola et al 1992).

Synaptic interactions between developing taste bud cells and nerve fibers are elementary, and coupled to the developmental presence of NF and their receptors (Kinnamon et al 2005). BDNF is down-regulated between P5 and P10, due to a reduction in progenitor cells, which coincides with a concurrent loss of innervation density. This indicates that there is a refinement of innervation, during postnatal development, leading to increased specificity of connections (Huang et al 2015).

BDNF and NT-3 mRNA expression in the adult human tongue

Strong BDNF mRNA labeling was observed in the fungiform taste buds and weak labeling in the epithelium surrounding the taste bud proper, suggesting preserved roles for BDNF for the initiation as well as maintenance of lingual gustatory innervation. Positive cells were also observed in non-gustatory filiform papillae.

NT-3 mRNA labeling was seen in the epithelial ridges of the non-gustatory filiform papillae and in the epithelium of the top and lateral surfaces of the fungiform papillae. NT-3 mRNA labeling in fungiform papillae was mainly detected in the same areas in which BDNF mRNA labeling was seen, but it was weaker than the BDNF mRNA labeling and the area was generally smaller.

Adult human tongue epithelium generally contained less PGP 9.5 immunoreactive fibers in comparison with rodents. Many PGP 9.5 positive nerve fibers were observed in fungiform taste buds, reaching very close to the outer surface.

Similarities and differences between rodent and human tongue

In the adult human biopsies, NT-3 mRNA is co-expressed in many areas with BDNF mRNA, which is not seen in rodents, suggesting overlapping roles for NT-3 and BDNF in the human tongue. It could also suggest that there is more overlap of the kinds of nerves stimulated by BDNF and NT-3, respectively. BDNF and NT-3 transcripts were also detected in additional areas both during development and in the adult lingual epithelium. BDNF mRNA labeling was expressed in the developing subepithelial mesenchyme, the adult filiform papillae and the epithelium surrounding the taste buds proper in fungiform papillae, which is not seen in rodents. NT-3 mRNA was not observed in rodent taste buds, while in humans it was detected both in developing and adult taste buds.

Previous studies have shown that rodent taste buds also receive perigemmal innervation (Finger 1986, Kinnamon 1987, Nosrat et al 1997a, Yamasaki et al 1984), which is not the case in humans. This specific innervation could be substituted by somatosensory nerve fibers entering the taste bud proper. We have seen nerve fibers that end in the proximity of the taste pore area in human taste buds, and some of these nerve fibers might be NT-3 dependent somatosensory nerve fibers. In the human tongue, BDNF and NT-3 might work in concert to support gustatory and/or somatosensory innervation.

Paper II: Dental pulp cells rescue trigeminal neurons and motoneurons

Here, we studied expression of NF in the dental pulp and how this tissue might interact with trigeminal and motor neurons. We also studied how dental pulp survives when grafted into different environments, how it affects host tissue, and if it can become re-innervated when grafted.

We analyzed neurotrophic factor mRNA expression by RNAse protection assay of cultured DPCs obtained from 5-6 days old rat pups and found that pulp cells produce several potent NF *in vitro*. NGF, BDNF, and GDNF mRNA are expressed by DPCs in culture and become upregulated when subcultured. Previous studies had shown a specific temporal pattern of neurotrophic factor expression in developing teeth (Nosrat 1998, Nosrat et al 1997b). The general expression of NF in teeth diminishes during development and at the time of innervation of the dental pulp, only NGF, BDNF and GDNF were expressed, indicating that these NF might be involved in its innervation (Nosrat et al 1998). Previous studies have suggested that NGF is involved in guidance of trigeminal axons to developing teeth and participates in the establishment of a pulpal innervation (Luukko et al 1997a). GDNF on the other side, is proposed to be involved postnatally in the regulation of tooth innervation (Luukko et al 1997c).

We developed an *in vitro* model in which dental pulp cell-trigeminal neuron interactions were studied without the addition of exogenous NF into the culture system. When DPCs were cocultured with trigeminal neurons, they promoted survival and neurite outgrowth from these neurons, visualized by PGP 9.5 and synaptophysin immunoreactivity. The trigeminal neurons appeared bipolar and the neurite outgrowth was specifically directed towards some, but not all of the DPCs, extending neurite branches over them. Trigeminal neurons did not give rise to neurite outgrowth when cultured with skin fibroblasts and almost all died after 3 days in culture. Previous studies have shown that trigeminal neurons die after 48 h in culture despite supplementing the culture medium with 10% FBS (Ulupinar et al 2000). Schwann cells are a source of trophic support for developing motor and sensory neurons (Riethmacher et al 1997), but constitute only a small fraction of cells in the trigeminal ganglia and were not able to provide sufficient neurotrophic support for the trigeminal neurons with the conditions used by us. Interestingly, the

trigeminal neurons survived for several weeks when cocultured with DPCs, indicating that NF secreted by DPCs provide neurotrophic support and are pivotal in regulating neuronal survival, differentiation, axonal growth and path-finding of trigeminal neurons.

Transplantation to the anterior chamber of the eye enables characterization of grafted tissues and the interactions between grafts and the host iris, including revascularization, growth and re-innervation (Olson et al 1983). Developing molar and incisor tissues from embryonic stages have been grafted to study interactions involved in tooth development (Fleming 1952, Kollar & Baird 1970a, Kollar & Baird 1970b, Kollar & Lumsden 1979, Lumsden 1979, Yoshikawa & Kollar 1981). We showed that when postnatal dental pulp tissue was grafted intraocularly, it induced collateral sprouting from existing iris nerve fibers into the grafted tissue as has also been shown with other peripheral tissue grafts (Olson & Malmfors 1970). Another study has shown that when developing molar tissues from E10 to E16 are grafted to the anterior chamber of the eye, they develop into complete crowns with normal crown shapes (Lumsden 1979). When rat molar and incisor tooth germs from E18-21, transplanted to the anterior chamber of the eye, was studied up to 6 months, normal mineralization was observed and distinct dentin and enamel structures developed (Granholm 1984). Additionally, long-term tooth grafts show osteogenic growth *in oculo* (Fleming 1953). We suggest that the dental pulp does so by producing NF. The iris has an exceptionally rich innervation, including fibers from the trigeminal, ciliary and sympathetic ganglia (Olson et al 1988). Recent evidence shows that the sympathetic nervous system regulates hematopoietic stem cell emergence (Fitch et al 2012). Sympathetic signaling also controls bone remodeling and osteoclast activation as occurs in tooth movement (Kondo et al 2013). Innervation of the ectopically transplanted dental pulp tissue was visualized by PGP 9.5 positive nerve fibers. The transplanted dental pulp tissue also contained blood vessels, indicating angiogenesis. Hard tissue that formed had a dentin- and osteodentin-like character, and in several areas each graft contained patterned tubuli and a predentin zone. Dental pulp grafts also increased the density of host iris catecholaminergic nerve fibers visualized by Falck-Hillarp fluorescence histochemistry. This could be attributed to the presence of the NF produced and released by the dental pulp.

When dental pulp was instead grafted to the hemisected spinal cord, we found that it promoted motoneuron survival, presumably by providing neurotrophic support. *In situ* hybridization showed GDNF mRNA labeling of the grafted dental pulp tissue. Previous studies have suggested that GDNF is a potent survival factor for motoneurons (Henderson et al 1994, Oppenheim et al 1995), and together with BDNF and NT-4, promotes regeneration of rubrospinal axons, reduces the necrotic zone, and supports motoneuron survival after spinal cord injury (Kobayashi et al 1997).

We propose that dental pulp-derived NF play a crucial role in orchestrating innervation of the dental pulp. Our findings also suggest that DPCs might constitute

a viable source of cells for transplantation in situations when NF have been shown to offer benefits in CNS disorders or injuries.

Paper III: Neurotrophic factors are expressed in developing human teeth

Teeth develop through sequential and reciprocal interactions between the oral epithelium and the neural crest-derived mesenchyme (Slavkin 1991). In this study we examined the expression pattern of NGF, BDNF, NT-3, NT-4, GDNF and NTN mRNA in developing human teeth during gestational weeks 6.5-11, representing early stages of tooth development, from bud to cap stage. We distinguished distinct and developmentally shifting patterns of expression for several NF.

Expression of NGF mRNA was found at 6.5 weeks of gestation and was localized in the mesenchyme below the oral epithelium. This suggests that NGF in the mesenchyme is functioning as a trophic factor for the arriving sensory neurons innervating the oral epithelium. It should be noted that NGF might be expressed even before this stage, since 6.5 weeks was the earliest stage examined. At 9 weeks, NGF was localized in the dental papillae, indicating that NGF is an important chemoattractant for the arriving sensory nerves. It has been shown that NGF is crucial for survival of sensory and sympathetic neurons (Levi-Montalcini 1987). At 10 weeks, NGF mRNA was found in the human dental papillae and in the dental follicle. Previous studies have shown that NGF knockout mice lack innervation of the dental pulp, periodontal ligament and gingiva (Byers et al 1997), and that trkA knockout mice are also devoid of nerve fibers (Matsuo et al 2001). Our findings of NGF mRNA in the dental follicle show that NGF may play an important role also in humans for innervation of the presumptive periodontal ligament, based upon earlier studies which indicate that the dental follicle gives rise to the periodontal ligament (Thesleff & Tummers 2008). However, NGF mRNA expression was rather weak in the developing human deciduous teeth compared to rodents (Luukko et al 1997a, Mitsiadis & Luukko 1995), suggesting that BDNF and NT-3, both highly expressed in developing human teeth, may be substituting for the early roles of NGF.

A recent study has explored the temporospatial distribution of NGF and $p75^{NTR}$ and has shown that NGF is expressed in the epithelium even at later stages (Mitsiadis & Pagella 2016) than our study found. The novel study found that NGF TrkA is expressed in differentiated cells such as preameloblasts/ameloblasts secreting enamel matrix and in odontoblasts secreting dentine matrix, while in contrast, $p75^{NTR}$ is localized in the dental epithelium (Mitsiadis & Pagella 2016). Addition of NGF to dental pulp cultures causes incorporation of BrdU in human DPCs, in contrast to BDNF, NT-3 and NT-4/5, indicating that NGF plays a role in proliferation of DPCs (Mizuno et al 2007). We propose that NGF, in addition to stimulating innervation, influences differentiation and proliferation of dental pulp and dental follicle cells during development of human tooth germs.

BDNF mRNA expression was observed at all stages included in our study in the area of the enamel knot, in the stellate reticulum in the area of the cervical loop, in the epithelial cells of the cervical loop, and in the inner dental epithelium. At 6.5 weeks of gestation, broad BDNF mRNA labeling was detected in the mesenchyme of maxillary and mandibular processes. At 8.5 weeks, BDNF mRNA was localized in the mesenchyme lateral to the tooth anlage, lateral to the outer dental epithelium. BDNF mRNA was also observed in the inner dental epithelium. At 9 weeks, BDNF labeling persisted in the mesenchyme lateral to the tooth and in the inner dental epithelium. In addition, BDNF was also localized lateral to the dental lamina, in the outer dental epithelium, dental follicle, in the area of the enamel knot, stellate reticulum in the area of the cervical loop and in the epithelial cells of the cervical loop. At 10 and 11 weeks, BDNF expression was similar to that at 9 weeks with some weaker and some stronger areas of BDNF. At 11 weeks, BDNF mRNA was below detection level in the mesenchyme of the maxillary and mandibular processes. A study has shown that NF added to human dental pulp cultures cause an increase of dentin sialophosphoprotein, alkaline phosphatase, osteopontin, type I collagen and BMP-2, as well as mineral depositions, with BDNF and NT-3 leading to the highest elevations. NGF also stimulated DNA synthesis by human pulp cells. These findings indicate that NF control hard-tissue related protein expression, calcification and proliferation in DPCs. The neurotrophins may also function as a differentiation factor of those cells (D'Souza et al 1997, Mizuno et al 2007). Our findings show the importance of BDNF during morphogenesis and presumably also in differentiation and proliferation of the inner dental epithelium in particular.

NT-3 mRNA expression was found in the dental papilla, dental follicle, dental lamina, cervical loop and in the oral epithelium. At 6.5 weeks of gestation, NT-3 mRNA labeling was seen in the mesenchyme of the anterior parts of the developing maxillary and mandibular processes, followed by expression in the dental papilla and oral epithelium at 7 weeks. At 9 weeks, NT-3 mRNA was still expressed in the oral epithelium and in the subepithelial mesenchyme. NT-3 mRNA was also observed in the mesenchyme lateral to the tooth anlage, extending to the dental papilla. NT-3 expression was stronger in the dental papilla than that of NGF and GDNF. NT-3 mRNA was also expressed in the dental lamina. At 10 weeks, NT-3 expression resembled that at 9 weeks, but expression in the dental papilla became stronger, specifically in the posterior teeth. NT-3 labeling was also observed in the cervical loop area. At 11 weeks, NT-3 expression became weaker in general and was below detection level in the oral epithelium and in the subepithelial mesenchyme.

All neurotrophins, including NT-3, are expressed in rodent tooth germs and induce neurite outgrowth from trigeminal ganglion explants, indicating their role in innervation and tooth development (Luukko et al 1997a). A recent study has shown that NT-3 and anti-NogoA promote axonal regeneration after spinal cord injury when delivered using a nanoparticle delivery system (Elliott Donaghue et al 2016). We have previously shown that DPCs express all neurotrophins and that they rescue motor neurons after spinal cord injury (Paper II). We propose that NT-3 may play a crucial role in innervation of the dental pulp in humans during development.

NT-4 mRNA labeling was detected in the oral epithelium, dental lamina, inner and outer dental epithelium, stellate reticulum, and the enamel knot during all stages of our study. At 6.5 weeks of gestation, NT-4 expression was observed in the oral epithelium and this labeling continued at 7 weeks. Additionally, at 7 weeks NT-4 mRNA was localized also in the dental lamina and at 8.5 weeks it was observed in the inner and outer dental epithelia. At 9 and 10 weeks, NT-4 expression persisted in the inner and outer dental epithelia. Strong labeling was observed lateral of the cervical loop. NT-4 mRNA was also observed in the oral epithelium, dental lamina and stellate reticulum. At 11 weeks, NT-4 was observed in the enamel knot in addition to the prior stages. A study has shown that NT-4 knockout mice have a decreased enamel matrix width and ameloblastin expression, which is important for ameloblast differentiation and enamel formation (Yoshizaki et al 2008). Hence, we propose that NT-4 may be important for maturation of the inner dental epithelium, which harbor pre-ameloblasts.

GDNF mRNA was found in the dental papillae, subepithelially in the anterior parts of the oral cavity, the inner dental epithelium, dental follicle, and the enamel knot at different stages. At 6.5 weeks of gestation, GDNF mRNA was observed in different regions of the oral cavity, mostly located in the mesenchymal structures. At 8.5 weeks, GDNF was observed in the dental papilla and on the medial side to the dental follicle. At 9 weeks, GDNF was located subepithelial of the oral epithelium, in the entire dental follicle, and in the inner and outer dental epithelium.

NTN mRNA labeling was seen in the oral and lingual epithelia, the subepithelial mesenchyme, inner dental epithelia and the cervical loop area during different stages. At 6.5 weeks of gestation, NTN expression was not observed in the dental structures, only in the oral and lingual epithelia. At 8.5 weeks, NTN was similar to the previous stage in addition to the expression in the dental lamina. At 9 and 10 weeks, NTN was observed subepithelial of the oral epithelium, in the inner dental epithelium, and in the area of the cervical loop. At 11 weeks, NTN mRNA was below detection level.

Cardiomyocytes transfected with adenovirus encoding GDNF have increased amounts of sympathetic, tyrosine hydroxylase positive nerves, indicating that GDNF stimulates development of sympathetic nerves (Fu et al 2013). Kidney development is regulated by a series of reciprocal inductive events in which uteric bud cells require GDNF for differentiation (Pichel et al 1996, Saxen & Sariola 1987, Velagapudi et al 2012). It has also been shown that GDNF is essential for proper enteric innervation (Pichel et al 1996). GDNF and NTN are important for the development of cranial parasympathetic ganglia, including the submandibular ganglia (Enomoto et al 2000). Parasympathetic nerves are necessary for maintenance of epithelial progenitor cells during the development of salivary glands, as well as for their regeneration (Knox et al 2013, Knox et al 2010). A study has shown that NTN is necessary for both normal cholinergic innervation of the heart and normal vagal responses (Downs et al 2016). Other studies on $NTN^{-/-}$ mice have shown reduced parasympathetic innervation, indicating that NTN is a neurotrophic factor for parasympathetic neurons (Heuckeroth et al 1999). We propose that GDNF

and NTN may have similar roles in the innervation of the tooth germ, as the teeth contain both sympathetic and parasympathetic nerve fibers in addition to the sensory fibers, as shown by earlier studies (Hildebrand et al 1995, Olgart 1996).

Expression patterns of neurotrophic factors in developing human teeth

	NGF	BDNF	NT-3	NT-4	GDNF	NTN
Mesenchyme	+	+	+		+	+
Oral epithelium			+	+		+
Dental lamina			+	+		
Dental papillae	+		+		+	
Dental follicle	+	+	+		+	
Enamel knot		+		+	+	
Stellate reticulum		+		+		
Cervical loop		+	+			+
Inner dental epithelium		+		+	+	+
Outer dental epithelium				+		

Previous studies have shown that neurotrophin mRNA labeling is generally weak in prenatal stages in rodents, while postnatally, NGF, BDNF and GDNF expression is strong prior to the initiation of dental pulp innervation and persists for several days after that initiation (Luukko et al 1997a, Nosrat et al 1997b). The findings here show that BDNF and GDNF mRNA are expressed at higher levels and that NGF expression is weaker in the developing human teeth compared to rodents. This suggests that BDNF and GDNF might have additional roles in the development of human teeth. Previous studies have shown differences also between the human and rodent

gustatory systems, where BDNF and NT-3 have broader and to some extent overlapping expression patterns in the developing and adult gustatory papilla (Paper I)(Nosrat et al 2000).

In humans, the dental follicle becomes innervated in the early cap stage (Christensen et al 1993), which was also observed in our study. In addition, we showed that specifically BDNF and GDNF mRNAs are expressed in the dental follicle during the time of its innervation (NGF mRNA expression was weak), indicating that BDNF and GDNF might be involved in tooth innervation also in humans. We have previously shown that DPCs provide neurotrophic support for trigeminal neurons *in vitro* (Paper II)*(Nosrat et al 2001b),* which also implies that the NF are pivotal for innervation of the dental pulp.

GDNF knockout studies have shown that odontoblasts, ameloblasts, enamel matrix and predentin fail to fully develop and differentiate. On the other hand, innervation of the tooth germs was normal in GDNF mutant mice, indicating that GDNF does not control innervation, but rather is crucial for morphogenesis (de Vicente et al 2002).

Development of organs and its innervation is dependent on neurotrophic factor and its receptors, including the mechanoreceptors. A study has shown that Ruffini afferents are dependent on BDNF and Merkel afferents on NGF and NT-3 (Fundin et al 1997). Another study has also shown that BDNF is important for the development of periodontal Ruffini endings. BDNF null mice show malformation of these mechanoreceptors at P3, indicating a role for BDNF in postnatal development of these structures (Alkhamrah et al 2003).

Here we showed that multiple NF are expressed in the developing human teeth, and we hypothesize, based on the expression patterns, that there might be a degree of functional redundancy among some of the NF and that they might be involved in both development and innervation of the teeth.

Paper IV. Dental pulp cells rescue dopaminergic neurons

Here we asked whether GDNF is retrogradely transported to trigeminal neuron cell bodies from areas where there is a high GDNF expression *in vivo*. We also studied dental pulp cell growth and protein expression *in vitro*, how DPCs interact with DA neurons, and the feasibility for cell replacement therapies in CNS using DPCs.

We report that when ^{125}I-GDNF is injected into whisker pads of neonatal and adult rats, into the developing maxillary teeth, or into the area of the inferior alveolar nerve, the labeled GDNF is retrogradely transported to neuron cell bodies in the trigeminal ganglion, indicating that GDNF might act as a target-derived neurotrophic factor in the trigeminal system and play a role in innervation of the whisker pads.

Neurons are produced in excess during development and a given target is able to support only a limited number of neurons, while the rest of the neurons die shortly after they have reached their target, in a process known as apoptosis. The neurons that do reach the target, are supported by target-derived neurotrophic factor,

retrogradely transported to the cell body (Barde 1989, Ibáñez et al 1993, Tomac et al 1995b).

All four neurotrophins, NGF, BDNF, NT-3, NT-4, and GDNF mRNA are expressed in embryonic whisker follicles and NT-4 protein promotes neurite outgrowth from trigeminal ganglia explants. This indicates that these NF orchestrate the innervation of the whisker pad by the trigeminal ganglion (Ibáñez et al 1993, Trupp et al 1995). In our study we provided evidence that GDNF is involved in this process also in adult rodents. Another study has shown that GDNF induces neurite elongation and regeneration of trigeminal ganglion cells *in vitro,* while GDNF antibodies reduce neurite elongation, indicating that GDNF may participate in elongation or regeneration in trigeminal neurons (Kishimoto et al 2012). Conditional GFRα1 knockout mice have shorter dendrites and show a decrease in postsynaptic specializations in hippocampal neurons, indicating that GDNF-GFRα1 signaling plays a crucial role for proper hippocampal neuronal development (Irala et al 2016).

We further show here that NGF, BDNF and GDNF mRNA are expressed by DPCs *in vitro*, both when cells were cultured from dental pulp tissue from 6-8-day-old rat pups and when they were cultured from adult human dental pulp. Previous studies showed that all neurotrophins, GDNF and NTN are expressed in developing rat and human teeth (Lillesaar et al 2001, Luukko et al 1997a, Luukko et al 1997c, Nosrat et al 1998, Nosrat et al 1996b, Nosrat et al 2001b). NF seem to be involved in both tooth development and tooth innervation. NGF has been shown to be required for differentiation of cranial neural crest cells into tooth organs (Amano et al 1999), innervation is severely reduced in NGF or trkA knockout mice (Byers et al 1997, Matsuo et al 2001), and anti-NGF treatment of neonatal rat pups reduces the amount of sensory axons in the dental pulp (Qian & Naftel 1996). We propose that NF play an important role in orchestrating the process of dental pulp innervation in rodents.

When DPCs were cocultured with embryonic DA neurons, there was a higher survival rate of DA neurons, larger soma sizes, larger numbers and increased lengths of primary neurites, indicating the ability of DPCs to affect both survival and phenotype of DA neurons *in vitro*. We also demonstrated that blocking NGF, BDNF and GDNF antibodies decreased the number of surviving DA neurons cocultured with DPCs. BDNF and GDNF protect DA neurons, while it is possible that NGF influences DA neurons indirectly through the glial cells that are present in the culture. Previous studies have shown that NGF regulates proliferation of glial cells (Zhang et al 2003a). It has also been shown that there is no major loss of DA neurons in either BDNF- or GDNF knockout mice, indicating roles of other factors for the early development of DA neurons, such as the transcription factor Nurr-1 (Zetterstrom et al 1997), whereas adult DA neurons can be protected by GDNF (Tomac et al 1995a). Our studies suggest that a combination of trophic factors, such as supplied by DPCs may exert a better trophic effect on DA neurons than single factor treatments.

Previous studies have shown that different types of cells transplanted into CNS can be used to produce and release neurotransmitters or NF, and that such cells might

be used in the treatment of CNS disorders (Olson 1997). Engraftment of genetically engineered cells for biodelivery of NGF to the basal forebrain in patients with Alzheimer's disease is safe (Eriksdotter-Jonhagen et al 2012, Ferreira et al 2015, Karami et al 2015), and patients show elevated cholinergic signaling markers, as well as improved cognitive test results. This suggests that encapsulated NGF-releasing cells may be a beneficial treatment for Alzheimer's disease patients (Ferreira et al 2015, Karami et al 2015). DPCs produce NF and could be similarly used in cell transplantation therapies in CNS disorders with the possible advantage of providing a cocktail of trophic support, rather than one specific transgenic trophic protein. Other natural sources of considerable amounts of NF are the developing kidney, the carotid body (Nosrat et al 1996b) and Sertoli cells (Widenfalk et al 1997). Here we show that bisbenzimide-labeled DPCs grafted into striatum survived for several weeks. We also show that DPCs can protect DA neurons against 6-OHDA *in vitro*.

Interestingly, culture of DPCs alone, led to cells with neuronal and glial phenotypes. DPCs from 6-8-day-old rat pups and adult human DPCs obtained from patients undergoing routine tooth extractions differentiate into and maintain a neuronal morphology, expressing the neuron-specific markers PGP 9.5 and β-III tubulin. S-100- and GFAP-positive cells were also observed in dental pulp cell cultures, potentially Schwann cells and/or other glial cells. β-III tubulin form the microtubules in neurons and is important for neurite outgrowth (Tucker et al 2008). The S100 protein family exert multiple functions, such as being involved in proliferation, differentiation, regulation of apoptosis, and inflammatory processes (Donato et al 2013). GFAP is the major intermediate filament in astrocytes and is important for proliferation, morphology and motility (Moeton et al 2016). Here we show that these proteins are also expressed in both rat and human DPCs. By grafting DPCs with or without a guiding scaffold to a site of CNS injury it would thus be possible to provide a cocktail of trophic factors as well as neural and astroglial cells that together may improve functional outcome.

Paper V: Deletion of BDNF and NT-3 cause lingual deficits

We studied the specific roles of BDNF and NT-3 in gustatory and somatosensory innervation of the tongue by taking advantage of knockout animals. We showed that lack of BDNF and NT-3 (P0) have additive effects leading to more severe gustatory and somatosensory deficits, indicating separate roles for the two neurotrophins for the innervation of the tongue.

The fungiform and circumvallate papillae were severely malformed and reduced in size, and the fungiform papillae were significantly reduced in number in $BDNF^{-/-}$ $xNT\text{-}3^{-/-}$ mice compared to $BDNF^{-/-}$ and wild type mice. The fungiform papillae received scarce innervation, and we did not observe any PGP 9.5-positive taste cells or taste buds in $BDNF^{-/-}xNT\text{-}3^{-/-}$ material. There was a total loss of intraepithelial nerve fibers in the circumvallate papillae in the double knockout mice, while there

were still some intraepithelial nerve fibers present in the circumvallate papillae of BDNF$^{-/-}$ mice. This indicates a need for the presence of both gustatory and somatosensory nerve components for normal development of taste buds and fungiform papillae.

There were some remaining nerve fibers in the core part of the circumvallate and fungiform papillae, which could indicate that not all nerves in the gustatory papillae are gustatory or somatosensory. The remaining nerve fibers could be contributions from the autonomic nervous system, supported by potent NF for the autonomic nervous system, such as GDNF and NTN, which are also expressed in the developing tongue, e.g. in the lingual epithelium, von Ebner's salivary glands, lingual ganglia and muscle tissue. Their receptors were also found in the lingual ganglia. NGF expression is very vague, which resembles the NT-3 labeling in the early prenatal tongue and is diffuse in the adult tongue (Nosrat 1998, Nosrat et al 1996a). Previous studies have shown that NT-3 is expressed in the developing geniculate ganglion (Ernfors et al 1992), and BDNF mutant mice lacking either one or both alleles have a decreased number of geniculate ganglion neurons (Patel & Krimm 2010). The lack of NT-3 in our study could contribute to the additional loss of gustatory neurons in the geniculate ganglion and lead to a more severe phenotype in BDNF$^{-/-}$xNT-3$^{-/-}$ mice, as the geniculate ganglion contains cell bodies of the chorda tympani.

TrkB is the receptor for both BDNF and NT-4 and mice have been generated with deletion of both ligands. BDNF$^{-/-}$xNT-4$^{-/-}$ mice have severely degenerated gustatory papillae and severely reduced innervation with only a few remaining nerve fibers innervating the papillae. However, taste papillae were still able to develop in the earliest stages of development during E13.5 to E15.5 in BDNF$^{-/-}$xNT-4$^{-/-}$ mice. This shows that BDNF and NT-4 are not needed for early taste development and that taste bud development is nerve-independent. Shh appears to control stem cell proliferation in taste buds and Sox2 appears to play a role in keeping taste bud progenitor cells undifferentiated. These markers are able to act before the arrival of nerve fibers and in the absence of BDNF. At later stages, taste buds become dependent on nerve fibers and in the absence of BDNF and innervation, taste buds degenerate (Ito & Nosrat 2009).

Overexpression of BDNF under the control of the nestin promoter, decreases size and numbers of taste papillae. Gustatory fibers reached the base of the tongue where ectopic BDNF was observed, but did not continue to the gustatory papillae. This shows that appropriate BDNF-dependent innervation is needed for taste bud development (Ringstedt et al 1999). A similar effect has been shown in studies with overexpression of BDNF and NT-4 in non-taste epithelia, which leads to decreased innervation of taste papillae despite an increased number of geniculate ganglion neurons. Instead, increased innervation of targets is observed in structures that usually do not receive gustatory innervation, such as filiform papillae (Krimm et al 2001).

It is well established that BDNF attracts innervation under normal conditions during development (Nosrat et al 1997a, Nosrat et al 1996a, Nosrat & Olson 1995). Ephrins control axon terminal positioning and EphB1 and EphB2 null mice show decreased

innervation of gustatory papillae, indicating that they are important signaling factors in the taste system (Treffy et al 2016). Sema3A has the opposite effect and prevents geniculate ganglion axons from penetrating fungiform papillae before E17, thus repels the axons from their target (Vilbig et al 2004). Taste bud development depends on BDNF, NT-3 and NT-4 (Ito & Nosrat 2009, Nosrat 1998, Nosrat et al 1996a, Nosrat & Olson 1995, Nosrat et al 2004a) and signals through trkB, trkC (Barbacid 1994) and p75 (Ibanez et al 1992, Ryden et al 1995). Adult $p75^{-/-}$ mice show reduced number of taste buds and cell bodies in the geniculate ganglion, indicating that p75 is necessary for normal taste bud development (Krimm 2006).

Our findings indicate functional roles for both BDNF-dependent gustatory and NT-3-dependent somatosensory innervation during the development of taste papillae. We speculate that BDNF overexpressing mice, which have larger taste buds, increased number of taste cells and denser innervation (Nosrat et al 2012) may be used as a model to study adverse events on taste perception following different drug treatments such as chemotherapy. These Gust-BDNF transgenic mice have increased taste perception and are able to taste sucrose at lower concentrations than wild type mice (Nosrat et al 2016). We propose that BDNF and/or its mediators may be used to mitigate dysgeusia following chemotherapy.

CONCLUSIONS

1. BDNF mRNA and NT-3 mRNA are expressed in human tongues *in vivo* and play significant roles in development, maintenance, and innervation of taste buds and somatosensory structures. BDNF mRNA is expressed in developing taste papillae before the arrival of any nerve fibers. BDNF mRNA is mainly expressed in the gustatory epithelium and later in taste cells, and NT-3 mRNA is mainly expressed in the somatosensory epithelium in both humans and rodents. BDNF and NT-3 transcripts exhibit a similar general expression pattern between the two species. One noteworthy difference is that while NT-3 mRNA is expressed mainly in extragemmal (outside taste buds) structures in rodents, in humans its expression is also found in some taste cells.

2. Neonatal rat dental pulp cells express NGF, BDNF and GDNF mRNA. The cells also secret the corresponding trophic proteins in experimental co-culture studies in which they rescue trigeminal neurons and embryonic dopamine neurons. Dental pulp cell implants also rescue motoneurons *in vivo*, and when placed in the anterior chamber of the eye, stimulate iris nerve fibers to innervate the implant. DPCs also stimulate neurite outgrowth from trigeminal neurons, and interestingly produce dentin-like mineralized tissue *in oculo*.

3. NGF, BDNF, NT-3, NT-4, GDNF and NTN mRNA are expressed *in vivo* during development in human tooth germs and possibly participate in both morphogenesis and innervation of the teeth.

4. While dental pulp cells promote the survival of embryonic dopamine neurons *in vitro*, inhibitory antibodies to NGF, BDNF and GDNF decrease the number of surviving dopamine neurons co-cultured with dental pulp cells. These cells also protect dopamine neurons against the neurotoxin 6-hydroxy-dopamine *in vitro*.

5. The fungiform and circumvallate papillae become severely distorted in the absence of both BDNF and NT3, and the lingual innervation becomes severely reduced.

ACKNOWLEDGEMENTS

This work has been performed at the Department of Neuroscience, Karolinska Institutet, and, in part, at the University of Michigan. I wish to thank:

Lars Olson, my supervisor, for giving me the opportunity to begin my graduate studies at the department of Neuroscience and continuing it at a distance. For teaching me neurobiology, being a good listener, giving me valuable input and excellent feedback on my drafts, and in particular for having patience with me to finish my thesis.

Christopher Nosrat, for teaching me different techniques and giving me the opportunity to work with him on his projects.

Co-authors **Johan Widenfalk**, **Sven Lindskog**, **Åke Seiger**, **Karin Agerman**, **Patrik Ernfors**, **Christopher Smith**, **Patrick Mullally** and **Andrea Marinescu**.

Eva Lindqvist for teaching me immunohistochemistry, in situ hybridization, perfusions and enthusiasm.

Karin Lundströmer for technical assistance.

Karin Lagerman for administrative work and correspondence.

Stefan Brene for always being friendly.

Anna Josephson for her never failing enthusiasm.

Jutta Kopp for the interesting conversations during lunch hours, violin lessons with the boys and bike tours.

The husbandry staff for taking care of the laboratory animals in the vivarium.

Ida Engqvist for administrative work.

Steven Friedrichsen, the dean of the College of Dental Medicine at Western University of Health Sciences, for his support and his emphasis on being a lifelong learner.

Bradley Henson, at Western University of Health Sciences, for his encouragement, support and all the good advice.

My colleagues at Western University of Health Sciences **Sandra Farah-Franco**, **Jim Borke**, **Jill Lewis** and **Bob Hasel** for their support and encouragement.

My family **Christopher**, **Philip** and **Stephan** for support and inspiration.

My parents **Dusanka** and **Zelimir** for guiding me in my life.

Work described in this thesis was supported by: The Swedish Medical Research Council (LO); Swedish National Board of Health and Welfare; Swedish Dental and Medical

Associations; Karolinska Institute Research Foundations (C.A.N, L.O.); David and Astrid Hagelén's Foundation; Kapten Artur Eriksson's Foundation; the Karolinska Institute Distinguished Professor Award (LO); USPHS; School of Dentistry – University of Michigan; Rackham Faculty Grants – University of Michigan; Michigan Parkinson's Foundation, and the National Institute on Deafness and Other Communication Disorders. LO is currently supported by an ERC grant.

REFERENCES

Airaksinen MS, Titievsky A, Saarma M. 1999. GDNF family neurotrophic factor signaling: four masters, one servant? *Molecular and cellular neurosciences* 13: 313-25

Åkesson E, Kjaeldgaard A, Seiger Å. 1998. Human embryonic spinal cord grafts in adult rat spinal cord cavities: survival, growth, and interactions with the host. *Expl. Neurol.* 149: 262-76

Alanzi A, Minah G, Romberg E, Catalanotto F, Bartoshuk L, Tinanoff N. 2013. Mothers' taste perceptions and their preschool children's dental caries experiences. *Pediatric dentistry* 35: 510-4

Alavi AM, Dubyak GR, Burnstock G. 2001. Immunohistochemical evidence for ATP receptors in human dental pulp. *Journal of dental research* 80: 476-83

Alfawaz S, Fong F, Plagnol V, Wong FS, Fearne J, Kelsell DP. 2013. Recessive oligodontia linked to a homozygous loss-of-function mutation in the SMOC2 gene. *Archives of oral biology* 58: 462-6

Alkhamrah BA, Hoshino N, Kawano Y, Harada F, Hanada K, Maeda T. 2003. The periodontal Ruffini endings in brain derived neurotrophic factor (BDNF) deficient mice. *Archives of histology and cytology* 66: 73-81

Amano O, Bringas P, Takahashi I, Takahashi K, Yamane A, Chai Y, Nuckolls GH, Shum L, Slavkin HC. 1999. Nerve growth factor (NGF) supports tooth morphogenesis in mouse first branchial arch explants. *Developmental dynamics : an official publication of the American Association of Anatomists* 216: 299-310

Arias ER, Valle-Leija P, Morales MA, Cifuentes F. 2014. Differential contribution of BDNF and NGF to long-term potentiation in the superior cervical ganglion of the rat. *Neuropharmacology* 81: 206-14

Ayer-LeLievre C, Olson L, Ebendal T, Seiger Å, Persson H. 1988. Expression of the b-nerve growth factor gene in hippocampal neurons. *Science (New York, N.Y.)* 240: 1339-41

Baloh RH, Tansey MG, Lampe PA, Fahrner TJ, Enomoto H, Simburger KS, Leitner ML, Araki T, Johnson EM Jr, Milbrandt J. 1998. Artemin, a novel member of the GDNF ligand family, supports peripheral and central neurons and signals through the GFRalpha3-RET receptor complex. *Neuron* 21: 1291-302

Barbacid M. 1994. The trk family of neurotrophin receptors. *J. Neurobiol.* 25: 1386-403

Barde YA. 1989. Trophic factors and neuronal survival. *Neuron* 2: 1525-35

Barde YA, Edgar D, Thoenen H. 1982. Purification of a new neurotrophic factor from mammalian brain. *EMBO Journal* 1: 549-53

Barlow LA, Northcutt RG. 1995. Embryonic origin of amphibian taste buds. *Dev. Biol.* 169: 273-85

Bartel DL, Sullivan SL, Lavoie EG, Sevigny J, Finger TE. 2006. Nucleoside triphosphate diphosphohydrolase-2 is the ecto-ATPase of type I cells in taste buds. *The Journal of comparative neurology* 497: 1-12

Bartoshuk LM. 2000. Comparing sensory experiences across individuals: recent psychophysical advances illuminate genetic variation in taste perception. *Chemical senses* 25: 447-60

Bartoshuk LM, Duffy VB, Lucchina LA, Prutkin J, Fast K. 1998. PROP (6-n-propylthiouracil) supertasters and the saltiness of NaCl. *Annals of the New York Academy of Sciences* 855: 793-6

Bartoshuk LM, Duffy VB, Miller IJ. 1994. PTC/PROP tasting: anatomy, psychophysics, and sex effects. *Physiology & behavior* 56: 1165-71.

Baryshnikov SG, Rogachevskaja OA, Kolesnikov SS. 2003. Calcium signaling mediated by P2Y receptors in mouse taste cells. *Journal of neurophysiology* 90: 3283-94

Berkemeier LR, Winslow JW, Kaplan DR, Nikolics K, Goeddel DV, Rosenthal A. 1991. Neurotrophin-5: a novel neurotrophic factor that activates trk and trkB. *Neuron* 7: 857-66

Birren SJ, Lo L, Anderson DJ. 1993. Sympathetic neuroblasts undergo a developmental switch in trophic dependence. *Development* 119: 597-610

Bo X, Alavi A, Xiang Z, Oglesby I, Ford A, Burnstock G. 1999. Localization of ATP-gated P2X2 and P2X3 receptor immunoreactive nerves in rat taste buds. *Neuroreport* 10: 1107-11

Boughter Jr JD, Pumplin DW, Yu C, Christy RC, Smith DV. 1997. Differential expression of alpha-gustducin in taste bud populations of the rat and hamster. *Journal of Neuroscience* 17: 2852-8

Bucci C, Alifano P, Cogli L. 2014. The role of rab proteins in neuronal cells and in the trafficking of neurotrophin receptors. *Membranes* 4: 642-77

Bufe B, Breslin PA, Kuhn C, Reed DR, Tharp CD, Slack JP, Kim UK, Drayna D, Meyerhof W. 2005. The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. *Current biology : CB* 15: 322-7

Burnstock G. 2006. Purinergic P2 receptors as targets for novel analgesics. *Pharmacology & therapeutics* 110: 433-54

Bushman JD, Ye W, Liman ER. 2015. A proton current associated with sour taste: distribution and functional properties. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 29: 3014-26

Byers MR. 1980. Development of sensory innervation in dentin. *Journal of Comparative Neurology* 191: 413-27

Byers MR, O´Neill PC, Nishimura MC, Philips HS, Albers KM. 1997. Dependence of dental innervation on NGF: comparison of hetero- and homozygous mice lacking NGF with k14NGF transgenic or hybrid rescued TKO mice. *Soc. Neurosci. Abstr.* 23: 1704

Byers MR, Wheeler EF, Bothwell M. 1992. Altered expression of NGF and P75 NGF-receptor by fibroblasts of injured teeth precedes sensory nerve sprouting. *Growth Factors* 6: 41-52

Castillo D, Seidel K, Salcedo E, Ahn C, de Sauvage FJ, Klein OD, Barlow LA. 2014. Induction of ectopic taste buds by SHH reveals the competency and plasticity of adult lingual epithelium. *Development* 141: 2993-3002

Chandrashekar J, Kuhn C, Oka Y, Yarmolinsky DA, Hummler E, Ryba NJ, Zuker CS. 2010. The cells and peripheral representation of sodium taste in mice. *Nature* 464: 297-301

Chandrashekar J, Yarmolinsky D, von Buchholtz L, Oka Y, Sly W, Ryba NJ, Zuker CS. 2009. The taste of carbonation. *Science (New York, N.Y.)* 326: 443-5

Chao MV, Bothwell MA, Ross AH, Koprowski H, Lanahan AA, Buck CR, Sehgal A. 1986. Gene transfer and molecular cloning of the human NGF receptor. *Science (New York, N.Y.)* 232: 518-21

Chaudhari N, Pereira E, Roper SD. 2009. Taste receptors for umami: the case for multiple receptors. *The American journal of clinical nutrition* 90: 738s-42s

Chaudhari N, Roper SD. 2010. The cell biology of taste. *The Journal of cell biology* 190: 285-96

Chaudhari N, Yang H, Lamp C, Delay E, Cartford C, Than T, Roper S. 1996. The taste of monosodium glutamate: membrane receptors in taste buds. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 16: 3817-26

Chen Y, Bei M, Woo I, Satokata I, Maas R. 1996. Msx1 controls inductive signaling in mammalian tooth morphogenesis. *Development* 122: 3035-44

Chibuzo GA, Cummings JF, Evans HE. 1980. Autonomic innervation of the tongue: a horseradish peroxidase study in the dog. *Journal of the autonomic nervous system* 2: 117-29

Chmilewsky F, About I, Chung SH. 2016a. Pulp Fibroblasts Control Nerve Regeneration through Complement Activation. *Journal of dental research* 95: 913-22

Chmilewsky F, Ayaz W, Appiah J, About I, Chung SH. 2016b. Nerve Growth Factor Secretion From Pulp Fibroblasts is Modulated by Complement C5a Receptor and Implied in Neurite Outgrowth. *Scientific reports* 6: 31799

Christensen LR, Janas MS, Mollgard K, Kjaer I. 1993. An immunocytochemical study of the innervation of developing human fetal teeth using protein gene product 9.5 (PGP 9.5). *Archives of oral biology* 38: 1113-20

Chung G, Jung SJ, Oh SB. 2013. Cellular and molecular mechanisms of dental nociception. *Journal of dental research* 92: 948-55

Clapp TR, Stone LM, Margolskee RF, Kinnamon SC. 2001. Immunocytochemical evidence for co-expression of Type III IP3 receptor with signaling components of bitter taste transduction. *BMC neuroscience* 2: 6

Coons A. 1958. Fluorescent antibody methods In *General Cytochemical Methods*, ed. J Danielli, pp. 399-422. New York: Academic Press

Corrodi H, Jonsson G. 1967. The formaldehyde fluorescence method for the histochemical demonstration of biogenic monoamines. A review of the methodology. *J. Histochem. Cytochem.* 15: 65-78

D'Souza RN, Cavender A, Sunavala G, Alvarez J, Ohshima T, Kulkami AB, MacDougall M. 1997. Gene expression patterns of murine dentin matrix protein 1 (Dmp1) and dentin sialophosphoprotein (DSPP) suggest distinct developmental functions in vivo. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* 12: 2040-9

Dagerlind Å, Friberg K, Bean A, Hökfelt T. 1992. Sensitive mRNA detection using unfixed tissue: combined radioactive and non-radioactive in situ hybridization histochemistry. *Histochemistry* 98: 39-49

de Vicente JC, Cabo R, Ciriaco E, Laura R, Naves FJ, Silos-Santiago I, Vega JA. 2002. Impaired dental cytodifferentiation in glial cell-line derived growth factor (GDNF) deficient mice. *Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft* 184: 85-92.

Dechant G, Rodriguez-Tebar A, Kolbeck R, Barde YA. 1993. Specific high-affinity receptors for neurotrophin-3 on sympathetic neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 13: 2610-6

Donato R, Cannon BR, Sorci G, Riuzzi F, Hsu K, Weber DJ, Geczy CL. 2013. Functions of S100 proteins. *Current molecular medicine* 13: 24-57

Donovan PJ, Gearhart J. 2001. The end of the beginning for pluripotent stem cells. *Nature* 414: 92-7

Doty R. 2015. *Handbook of Olfaction and Gustation, 3rd Edition*.

Downs AM, Jalloh HB, Prater KJ, Fregoso SP, Bond CE, Hampton TG, Hoover DB. 2016. Deletion of neurturin impairs development of cholinergic nerves and heart rate control in postnatal mouse hearts. *Physiological reports* 4

Drewnowski A, Gomez-Carneros C. 2000. Bitter taste, phytonutrients, and the consumer: a review. *The American journal of clinical nutrition* 72: 1424-35

Duffy VB, Davidson AC, Kidd JR, Kidd KK, Speed WC, Pakstis AJ, Reed DR, Snyder DJ, Bartoshuck LM. 2004. Bitter receptor gene (TAS2R38), 6-n-propylthiouracil (PROP) bitterness and alcohol intake. *Alcoholism, clinical and experimental research* 28: 1629-37

Dunnett SB, Björklund A. 1992. Staging and dissection of rat embryos In *Neural transplantation, a practical approach*, ed. SB Dunnett, A Bjorklund, pp. 1-20. New York: Oxford University Press

Durbec P, Marcosgutierrez CV, Kilkenny C, Grigoriou M, Wartiowaara K, Suvanto P, Smith D, Ponder B, Constantini F, Saarma M, Sariola H, Pachnis V. 1996. Gdnf Signalling Through the Ret Receptor Tyrosine Kinase. *Nature* 381: 789-93

Edery P, Pelet A, Mulligan LM, Abel L, Attie T, Dow E, Bonneau D, David A, Flintoff W, Jan D. 1994. Long segment and short segment familial Hirschsprung's disease: variable clinical expression at the RET locus. *Journal of medical genetics* 31: 602-6

Ehrmann EH. 2002. The diagnosis of referred orofacial dental pain. *Australian endodontic journal : the journal of the Australian Society of Endodontology Inc* 28: 75-81

El Karim IA, Linden GJ, Curtis TM, About I, McGahon MK, Irwin CR, Lundy FT. 2011. Human odontoblasts express functional thermo-sensitive TRP channels: implications for dentin sensitivity. *Pain* 152: 2211-23

Elliott Donaghue I, Tator CH, Shoichet MS. 2016. Local Delivery of Neurotrophin-3 and Anti-NogoA Promotes Repair After Spinal Cord Injury. *Tissue engineering. Part A* 22: 733-41

Elson AE, Dotson CD, Egan JM, Munger SD. 2010. Glucagon signaling modulates sweet taste responsiveness. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 24: 3960-9

England MA. 1990. ***A colour atlas of life before birth***. Aylesbury, England: Hazell.

Enokido Y, de Sauvage F, Hongo JA, Ninkina N, Rosenthal A, Buchman VL, Davies AM. 1998. GFR alpha-4 and the tyrosine kinase Ret form a functional receptor complex for persephin. *Current Biology* 8: 1019-22

Enomoto H, Heuckeroth RO, Golden JP, Johnson EM, Milbrandt J. 2000. Development of cranial parasympathetic ganglia requires sequential actions of GDNF and neurturin. *Development* 127: 4877-89

Eriksdotter-Jonhagen M, Linderoth B, Lind G, Aladellie L, Almkvist O, Andreasen N, Blennow K, Bogdanovic N, Jelic V, Kadir A, Nordberg A, Sundstrom E, Wahlund LO, Wall A, Wiberg M, Winblad B, Seiger A, Almquist P, Wahlberg L. 2012. Encapsulated cell biodelivery of nerve growth factor to the Basal forebrain in patients with Alzheimer's disease. *Dementia and geriatric cognitive disorders* 33: 18-28

Ernfors P, Ibanez CF, Ebendal T, Olson L, Persson H. 1990a. Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: developmental and topographical expression in the brain. *Proc. Natl. Acad. Sci. USA* 87: 5454-58

Ernfors P, Lee KF, Kucera J, Jaenisch R. 1994. Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. *Cell* 77: 503-12

Ernfors P, Merlio J, Persson H. 1992. Cells expressing mRNA for neurotrophins and their receptors during embryonic rat development. *Eur. J. Neurosci.* 4: 1140-58

Ernfors P, Wetmore C, Olson L, Persson H. 1990b. Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. *Neuron* 5: 511-26

Falci S, Holtz A, Akesson E, Azizi M, Ertzgaard P, Hultling C, Kjaeldgaard A, Levi R, Ringden O, Westgren M, Lammertse D, Seiger A. 1997. Obliteration of a posttraumatic spinal cord cyst with solid human embryonic spinal cord grafts: first clinical attempt. *J. Neurotrauma* 14: 875-84

Falck B, Hillarp N-Å, Thieme G, Torp A. 1962. Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J. Histochem. Cytochem.* 10: 348-54

Farbman AI. 1965. Electron microscope study of the developing taste bud in rat fungiform papilla. *Dev. Biol.* 11: 110-35

Farbman AI. 1972. Differentiation of taste buds in organ culture. *J. Cell Biol.* 52: 489-93

Farbman AI, Mbiene JP. 1991. Early development and innervation of taste bud-bearing papillae on the rat tongue. *J. Comp. Neurol.* 304: 172-86

Ferreira D, Westman E, Eyjolfsdottir H, Almqvist P, Lind G, Linderoth B, Seiger A, Blennow K, Karami A, Darreh-Shori T, Wiberg M, Simmons A, Wahlund LO, Wahlberg L, Eriksdotter M. 2015. Brain changes in Alzheimer's disease patients with implanted encapsulated cells releasing nerve growth factor. *Journal of Alzheimer's disease : JAD* 43: 1059-72

Finger TE. 1986. Peptide immunohistochemistry demonstrates multiple classes of perigemmal nerve fibers in the circumvallate papillae of the rat. *Cem. Senses* 11: 135-44

Finger TE, Danilova V, Barrows J, Bartel DL, Vigers AJ, Stone L, Hellekant G, Kinnamon SC. 2005. ATP signaling is crucial for communication from taste buds to gustatory nerves. *Science (New York, N.Y.)* 310: 1495-9

Fitch SR, Kimber GM, Wilson NK, Parker A, Mirshekar-Syahkal B, Gottgens B, Medvinsky A, Dzierzak E, Ottersbach K. 2012. Signaling from the sympathetic nervous system regulates hematopoietic stem cell emergence during embryogenesis. *Cell stem cell* 11: 554-66

Fleming HS. 1952. Homologous and heterologous intraocular growth of transplanted tooth germs. *Journal of dental research* 31: 166-88

Fleming HS. 1953. The osteogenic tendency of dentine after formation in tooth germ transplants. *Oral surgery, oral medicine, and oral pathology* 6: 1315-24

Forman MS, Trojanowski JQ, Lee VM. 2004. Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. *Nature medicine* 10: 1055-63

Frings S. 2010. The sour taste of a proton current. *Proceedings of the National Academy of Sciences of the United States of America* 107: 21955-6

Fu XM, Lee JK, Miwa K, Shimizu T, Takagishi Y, Hirabayashi M, Watabe K, Usui A, Kodama I, Ueda Y. 2013. Sympathetic innervation induced in engrafted engineered cardiomyocyte sheets by glial cell line derived neurotrophic factor in vivo. *BioMed research international* 2013: 532720

Fundin BT, Silos-Santiago I, Ernfors P, Fagan AM, Aldskogius H, DeChiara TM, Phillips HS, Barbacid M, Yancopoulos GD, Rice FL. 1997. Differential dependency of cutaneous mechanoreceptors on neurotrophins, trk receptors, and P75 LNGFR. *Developmental biology* 190: 94-116

Gaillard D, Barlow LA. 2011. Taste bud cells of adult mice are responsive to Wnt/beta-catenin signaling: implications for the renewal of mature taste cells. *Genesis (New York, N.Y. : 2000)* 49: 295-306

Gao Y, Toska E, Denmon D, Roberts SG, Medler KF. 2014. WT1 regulates the development of the posterior taste field. *Development* 141: 2271-8

Goodfellow PJ, Wells SA, Jr. 1995. RET gene and its implications for cancer. *Journal of the National Cancer Institute* 87: 1515-23

Granholm AC. 1984. Histology, innervation and radiographic appearance of fetal rat tooth germs developing in oculo. *Scandinavian Journal of Dental Research* 92: 381-90

Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S. 2002. Stem cell properties of human dental pulp stem cells. *Journal of dental research* 81: 531-5.

Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. 2000. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America* 97: 13625-30.

Hall JM, Hooper JE, Finger TE. 1999. Expression of sonic hedgehog, patched and gli1 in developing taste papillae of the mouse. *J. Comp. Neurol* 406: 143-55

Hallböök F, Ibanez CF, Persson H. 1991. Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. *Neuron* 6: 845-58

Handa K, Saito M, Tsunoda A, Yamauchi M, Hattori S, Sato S, Toyoda M, Teranaka T, Narayanan AS. 2002. Progenitor cells from dental follicle are able to form cementum matrix in vivo. *Connective tissue research* 43: 406-8

Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I. 1999. Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. *The Journal of cell biology* 147: 105-20.

Hardcastle Z, Mo R, Hui CC, Sharpe PT. 1998. The Shh signalling pathway in tooth development: defects in Gli2 and Gli3 mutants. *Development* 125: 2803-11

Hayano S, Kurosaka H, Yanagita T, Kalus I, Milz F, Ishihara Y, Islam MN, Kawanabe N, Saito M, Kamioka H, Adachi T, Dierks T, Yamashiro T. 2012. Roles of heparan sulfate sulfation in dentinogenesis. *The Journal of biological chemistry* 287: 12217-29

He H, Han D, Feng H, Qu H, Song S, Bai B, Zhang Z. 2013. Involvement of and interaction between WNT10A and EDA mutations in tooth agenesis cases in the Chinese population. *PloS one* 8: e80393

Heck GL, Mierson S, DeSimone JA. 1984. Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. *Science (New York, N.Y.)* 223: 403-5

Hefti F, Denton TL, Knusel B, Lapchak PA. 1993. Neurotrophic factors: what are they and what are they doing? In *Neurotrophic factors*, ed. SE Loughlin, JH Fallon, pp. 25-50. San Diego: Academic Press, Inc.

Henderson CE, Camu W, Mettling C, Gouin A, Poulsen K, Karihaloo M, Ruilamas J, Evans T, McMahon SB, Armanini MP, Berkemeier K, Phillips HS, Rosenthal A. 1993. Neurotrophins promote motor neuron survival and are present in embryonic limb bud. *Nature* 363: 266-70

Henderson CE, Phillips HS, Pollock RA, Davies AM, Lemeulle C, Armanini M, Simmons L, Moffet B, Vandlen RA, Simpson L, Koliatsos VE, Rosenthal A. 1994. GDNF: a potent survival factor for motoneurons present in peripheral nerve and muscle. *Science (New York, N.Y.)* 266: 1062-64

Herness S, Zhao FL, Lu SG, Kaya N, Shen T. 2002. Expression and physiological actions of cholecystokinin in rat taste receptor cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 22: 10018-29

Heuckeroth RO, Enomoto H, Grider JR, Golden JP, Hanke JA, Jackman A, Molliver DC, Bargett ME, Snider WD, Johnson EM Jr, Milbrandt J. 1999. Gene targeting reveals a critical role for neurturin in the development and maintenance of enteric, sensory, and parasympathetic neurons. *Neuron* 22: 253-63

Heyeraas KJ, Berggreen E. 1999. Interstitial fluid pressure in normal and inflamed pulp. *Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists* 10: 328-36

Hildebrand C, Fried K, Tuisku F, Johansson CS. 1995. Teeth and tooth nerves. *Progr. Neurobiol.* 145: 122-65

Hohn A, Leibrock J, Bailey K, Barde YA. 1990. Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. *Nature* 344: 339-41

Hökfelt T, Fuxe K, Goldstein M, Joh TH. 1973. Immunohistochemical localization of three catecholamine synthesizing enzymes: aspects on methodology. *Histochemie* 33: 231-54

Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJ, Zuker CS. 1999. Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell* 96: 541-51

Hu X, Zhang S, Chen G, Lin C, Huang Z, Chen Y, Zhang Y. 2013. Expression of SHH signaling molecules in the developing human primary dentition. *BMC developmental biology* 13: 11

Huang T, Ma L, Krimm RF. 2015. Postnatal reduction of BDNF regulates the developmental remodeling of taste bud innervation. *Developmental biology* 405: 225-36

Huang YA, Dando R, Roper SD. 2009. Autocrine and paracrine roles for ATP and serotonin in mouse taste buds. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 29: 13909-18

Huang YA, Pereira E, Roper SD. 2011a. Acid stimulation (sour taste) elicits GABA and serotonin release from mouse taste cells. *PloS one* 6: e25471

Huang YA, Stone LM, Pereira E, Yang R, Kinnamon JC, Dvoryanchikov G, Chaudhari N, Finger TE, Kinnamon SC, Roper SD. 2011b. Knocking out P2X receptors reduces transmitter secretion in taste buds. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 31: 13654-61

Hummel T, Landis BN, Huttenbrink KB. 2011. Smell and taste disorders. *GMS current topics in otorhinolaryngology, head and neck surgery* 10: Doc04

Hutchin ME, Kariapper MS, Grachtchouk M, Wang A, Wei L, Cummings D, Liu J, Michael LE, Glick A, Dlugosz AA . 2005. Sustained Hedgehog signaling is required for basal cell carcinoma proliferation and survival: conditional skin tumorigenesis recapitulates the hair growth cycle. *Genes & development* 19: 214-23

Ibanez CF, Ebendal T, Barbany G, Murray RJ, Blundell TL, Persson H. 1992. Disruption of the low affinity receptor-binding site in NGF allows neuronal survival and differentiation by binding to the trk gene product. *Cell* 69: 329-41

Ibáñez CF, Ernfors P, Timmusk T, Ip NY, Arenas E, Yancopoulos GD, Persson H. 1993. Neurotrophin-4 is a target-derived neurotrophic factor for neurons of the trigeminal ganglion. *Development* 117: 1345-53

Ingham PW, McMahon AP. 2001. Hedgehog signaling in animal development: paradigms and principles. *Genes & development* 15: 3059-87

Ip NY, Ibanez CF, Nye SH, McClain J, Jones PF, Gies DR, Belluscio L, Le BM, Espinosa R, Squinto SP, Persson H, Yancopoulos GD. 1992. Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity. *Proc. Natl. Acad. Sci. USA* 89: 3060-64

Irala D, Bonafina A, Fontanet PA, Alsina FC, Paratcha G, Ledda F. 2016. GDNF/GFRalpha1 complex promotes development of hippocampal dendritic arbors and spines via NCAM. *Development*

Ito A, Nosrat CA. 2009. Gustatory papillae and taste bud development and maintenance in the absence of TrkB ligands BDNF and NT-4. *Cell and tissue research* 337: 349-59

Ito A, Nosrat IV, Nosrat CA. 2010. Taste cell formation does not require gustatory and somatosensory innervation. *Neuroscience letters* 471: 189-94

Itoh K, Chiang CY, Li Z, Lee JC, Dostrovsky JO, Sessle BJ. 2011. Central sensitization of nociceptive neurons in rat medullary dorsal horn involves purinergic P2X7 receptors. *Neuroscience* 192: 721-31

Iwatsuki K, Liu HX, Gronder A, Singer MA, Lane TF, Grosschedl R, Mistretta CM, Margolskee RF . 2007. Wnt signaling interacts with Shh to regulate taste papilla development. *Proceedings of the National Academy of Sciences of the United States of America* 104: 2253-8

Jaber L, Zhao FL, Kolli T, Herness S. 2014. A physiologic role for serotonergic transmission in adult rat taste buds. *PloS one* 9: e112152

Jernvall J, Thesleff I. 2012. Tooth shape formation and tooth renewal: evolving with the same signals. *Development* 139: 3487-97

Jing SQ, Wen DZ, Yu YB, Holst PL, Luo Y, Fang M, Tamir R, Antonio L, Hu Z, Cupples R Louis JC, Hu S, Altrock BW, Fox GM. 1996. GDNF-induced activation of the Ret protein tyrosine kinase is mediated by GDNFR-Alpha, a novel receptor for GDNF. *Cell* 85: 1113-24

Johnson D, Lanahan A, Buck CR, Sehgal A, Morgan C, Mercer E, Bothwell M, Chao M. 1986. Expression and structure of the human NGF receptor. *Cell* 47: 545-54

Jones KR, Reichardt LF. 1990. Molecular cloning of a human gene that is a member of the nerve growth factor family. *Proc. Natl. Acad. Sci. U.S.A.* 87: 8060-64

Jung H-S, Oropeza V, Thesleff I. 1999. Shh, Bmp-2, Bmp-4 and Fgf-8 are associated with initiation and patterning of mouse tongue papillae. *Mech. Develop.* 81: 203-06

Juuri E, Saito K, Ahtiainen L, Seidel K, Tummers M, Hochedlinger K, Klein OD, Thesleff I, Michon F. 2012. Sox2+ stem cells contribute to all epithelial lineages of the tooth via Sfrp5+ progenitors. *Developmental cell* 23: 317-28

Kahane N, Kalcheim C. 1994. Expression of trkC receptor mRNA during development of the avian nervous system. *Journal of neurobiology* 25: 571-84

Kanai-Azuma M, Kanai Y, Gad JM, Tajima Y, Taya C, Kurohmaru M, Sanai Y, Yonekawa H, Yazaki K, Hayashi Y. 2002. Depletion of definitive gut endoderm in Sox17-null mutant mice. *Development* 129: 2367-79

Kandel E, Schwartz J, Jessell T, Siegelbaum S, Hudspeth A. 2013. *Principles of Neural Science, Fifth Edition*.

Kaplan DR, Miller FD. 2000. Neurotrophin signal transduction in the nervous system. *Current opinion in neurobiology* 10: 381-91

Karami A, Eyjolfsdottir H, Vijayaraghavan S, Lind G, Almqvist P, Kadir A, Linderoth B, Andreasen N, Blennow K, Wall A, Westman E, Ferreira D, Kristoffersen Wiberg M, Wahlund LO, Seiger A, Nordberg A, Wahlberg L, Darreh-Shori T, Eriksdotter M. 2015. Changes in CSF cholinergic biomarkers in response to

cell therapy with NGF in patients with Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 11: 1316-28
Kataoka S, Baquero A, Yang D, Shultz N, Vandenbeuch A, Ravid K, Kinnamon SC, Finger TE. 2012. A2BR adenosine receptor modulates sweet taste in circumvallate taste buds. *PloS one* 7: e30032
Kataoka S, Toyono T, Seta Y, Ogura T, Toyoshima K. 2004. Expression of P2Y1 receptors in rat taste buds. *Histochemistry and cell biology* 121: 419-26
Kataoka S, Toyono T, Seta Y, Toyoshima K. 2006. Expression of ATP-gated P2X3 receptors in rat gustatory papillae and taste buds. *Archives of histology and cytology* 69: 281-8
Kaukua N, Shahidi MK, Konstantinidou C, Dyachuk V, Kaucka M, Furlan A, An Z, Wang L, Hultman I, Ahrlund-Richter L, Blom H, Brismar H, Lopes NA, Pachnis V, Suter U, Clevers H, Thesleff I, Sharpe P, Ernfors P, Fried K, Adameyko I . 2014. Glial origin of mesenchymal stem cells in a tooth model system. *Nature* 513: 551-4
Kawaja MD, Fisher LJ, Shinstine M, Jinnah HA, Ray J, Chen LS, Gage FH. 1992. Grafting genetically modified cells within the rat central nervous system: methodological conciderations In *Neural transplantation, a practical approach*, ed. SB Dunnett, A Björklund, pp. 21-55. New York: Oxford University Press
Kettunen P, Thesleff I. 1998. Expression and function of FGFs-4, -8, and -9 suggest functional redundancy and repetitive use as epithelial signals during tooth morphogenesis. *Developmental dynamics : an official publication of the American Association of Anatomists* 211: 256-68
Kim DJ, Roper SD. 1995. Localization of serotonin in taste buds - a comparative study in four vertebrates. *Journal of Comparative Neurology* 353: 364-70
Kinnamon JC. 1987. Organization and innervation of taste buds In *Neurobiology of taste and smell*, ed. TE Finger, WL Silver, pp. 277-97. New York: John Wiley & sons.
Kinnamon JC, Dunlap M, Yang R. 2005. Synaptic connections in developing and adult rat taste buds. *Chemical senses* 30 Suppl 1: i60-1
Kishimoto Y, Yabuta C, Shearer TR, Azuma M. 2012. FK962 promotes neurite elongation and regeneration of cultured rat trigeminal ganglion cells: possible involvement of GDNF. *Investigative ophthalmology & visual science* 53: 5312-9
Kiso H, Takahashi K, Saito K, Togo Y, Tsukamoto H, Huang B, Sugai M, Shimizu A, Tabata Y, Economides AN, Slavkin HC, Bessho K. 2014. Interactions between BMP-7 and USAG-1 (uterine sensitization-associated gene-1) regulate supernumerary organ formations. *PloS one* 9: e96938
Kist R, Watson M, Crosier M, Robinson M, Fuchs J, Reichelt J, Peters H. 2014. The formation of endoderm-derived taste sensory organs requires a Pax9-dependent expansion of embryonic taste bud progenitor cells. *PLoS genetics* 10: e1004709
Klein RD, Sherman D, Ho WH, Stone D, Bennett GL, Moffat B, Vandlen R, Simmons L, Gu QM, Hongo JA, Devaux B, Poulsen K, Armanini M, Nozaki C, Asai N, Goddarrd A, Phillips H, Henderson CE, Takahashi M, Rosenthal A. 1997. A

Gpi-Linked Protein That Interacts With Ret to Form a Candidate Neurturin Receptor. *Nature* 387: 717-21

Knox SM, Lombaert IM, Haddox CL, Abrams SR, Cotrim A, Wilson AJ, Hoffman MP. 2013. Parasympathetic stimulation improves epithelial organ regeneration. *Nature communications* 4: 1494

Knox SM, Lombaert IM, Reed X, Vitale-Cross L, Gutkind JS, Hoffman MP. 2010. Parasympathetic innervation maintains epithelial progenitor cells during salivary organogenesis. *Science (New York, N.Y.)* 329: 1645-7

Kobayashi NR, Fan DP, Giehl KM, Bedard AM, Wiegand SJ, Tetzlaff W. 1997. BDNF and NT-4/5 prevent atrophy of rat rubrospinal neurons after cervical axotomy, stimulate GAP-43 and Talpha1-tubulin mRNA expression, and promote axonal regeneration. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 17: 9583-95

Koh S, Loy R. 1989. Localization and development of nerve growth factor-sensitive rat basal forebrain neurons and their afferent projections to hippocampus and neocortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 9: 2999-0318

Kollar EJ, Baird GR. 1970a. Tissue interactions in embryonic mouse tooth germs. I. Reorganization of the dental epithelium during tooth-germ reconstruction. *Journal of embryology and experimental morphology* 24: 159-71

Kollar EJ, Baird GR. 1970b. Tissue interactions in embryonic mouse tooth germs. II. The inductive role of the dental papilla. *Journal of embryology and experimental morphology* 24: 173-86

Kollar EJ, Lumsden AG. 1979. Tooth morphogenesis: the role of the innervation during induction and pattern formation. *Journal de biologie buccale* 7: 49-60

Kondo M, Kondo H, Miyazawa K, Goto S, Togari A. 2013. Experimental tooth movement-induced osteoclast activation is regulated by sympathetic signaling. *Bone* 52: 39-47

Kotzbauer PT, Lampe PA, Heuckeroth RO, Golden JP, Creedon DJ, Johnson EM, Millbrandt J. 1996. Neurturin, a relative of glial-cell-line-derived neurotrophic factor. *Nature* 384: 467-70

Kratochwil K, Galceran J, Tontsch S, Roth W, Grosschedl R. 2002. FGF4, a direct target of LEF1 and Wnt signaling, can rescue the arrest of tooth organogenesis in Lef1($^{-/-}$) mice. *Genes & development* 16: 3173-85

Krimm RF. 2006. Mice lacking the p75 receptor fail to acquire a normal complement of taste buds and geniculate ganglion neurons by adulthood. *The anatomical record. Part A, Discoveries in molecular, cellular, and evolutionary biology* 288: 1294-302

Krimm RF, Miller KK, Kitzman PH, Davis BM, Albers KM. 2001. Epithelial overexpression of BDNF or NT4 disrupts targeting of taste neurons that innervate the anterior tongue. *Developmental biology* 232: 508-21.

Laffitte A, Neiers F, Briand L. 2014. Functional roles of the sweet taste receptor in oral and extraoral tissues. *Current opinion in clinical nutrition and metabolic care* 17: 379-85

Lawton DM, Furness DN, Lindemann B, Hackney CM. 2000. Localization of the glutamate-aspartate transporter, GLAST, in rat taste buds. *The European journal of neuroscience* 12: 3163-71
Lee R, Kermani P, Teng KK, Hempstead BL. 2001. Regulation of cell survival by secreted proneurotrophins. *Science (New York, N.Y.)* 294: 1945-8
Lefkowitz W, Bodecker CF, Mardfin DF. 1953. Odontogenesis of the rat molar; prenatal stage. *Journal of dental research* 32: 749-72
Leibrock J, Lottspeich F, Hohn A, Hofer M, Hengerer B, Masiakowski P, Thoenen H, Barde YA. 1989. Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* 341: 149-52
Lesot H, Brook AH. 2009. Epithelial histogenesis during tooth development. *Archives of oral biology* 54 Suppl 1: S25-33
Levi-Montalcini R. 1987. The nerve growth factor 35 years later. *Science (New York, N.Y.)* 237: 1154-62
Levi-Montalcini R, Caramia F, Angeletti PU. 1969. Alterations in the fine structure of nucleoli in sympathetic neurons following NGF-antiserum treatment. *Brain research* 12: 54-73
Levi-Montalcini R, Cohen S. 1960. Effects of the extract of the mouse submaxillary salivary glands on the sympathetic system of mammals. *Annals of the New York Academy of Sciences* 85: 324-41
Lewin GR, Barde YA. 1996. Physiology of the neurotrophins. *Ann. Rev. Neurosci.* 19: 289-317
Lewin GR, Mendell LM. 1993. Nerve growth factor and nociception. *Trends in neurosciences* 16: 353-9
Lewin GR, Mendell LM. 1994. Regulation of cutaneous C-fiber heat nociceptors by nerve growth factor in the developing rat. *Journal of neurophysiology* 71: 941-9
Li JY, Hu B, Wang XJ, Wang SL. 2008. Temporal and spatial expression of TGF-beta2 in tooth crown development in mouse first lower molar. *European journal of histochemistry : EJH* 52: 243-50
Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E. 2002. Human receptors for sweet and umami taste. *Proceedings of the National Academy of Sciences of the United States of America* 99: 4692-6.
Liebl DJ, Mbiene JP, Parada LF. 1999. NT4/5 mutant mice have deficiency in gustatory papillae and taste bud formation. *Developmental biology* 213: 378-89
Lillesaar C, Eriksson C, Fried K. 2001. Rat tooth pulp cells elicit neurite growth from trigeminal neurones and express mRNAs for neurotrophic factors in vitro. *Neuroscience letters* 308: 161-4.
Lin L, Zhang T, Collins F, Armes L. 1994. Purification and initial characterization of rat B49 glial cell line-derived neurotrophic factor. *J. Neurochem* 63: 758-68
Lin L-F, Doherty D, Lile J, Bektesh S, Collins F. 1993. GDNF: A glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science (New York, N.Y.)* 260: 1130-32
Lindahl M, Poteryaev D, Yu L, Arumae U, Timmusk T, Bongarzone I, Aiello A, Pierotti MA, Airaksinen MS, Saarma M . 2001. Human glial cell line-derived

neurotrophic factor receptor alpha 4 is the receptor for persephin and is predominantly expressed in normal and malignant thyroid medullary cells. *The Journal of biological chemistry* 276: 9344-51

Lindsay RM, Barde YA, Davies AM, Rohrer H. 1985. Differences and similarities in the neurotrophic growth factor requirements of sensory neurons derived from neural crest and neural placode. *Journal of Cell Science Supplement* 3: 115-29

Liot G, Zala D, Pla P, Mottet G, Piel M, Saudou F. 2013. Mutant Huntingtin alters retrograde transport of TrkB receptors in striatal dendrites. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 33: 6298-309

Liu HX, Ermilov A, Grachtchouk M, Li L, Gumucio DL, Dlugosz AA, Mistretta CM. 2013a. Multiple Shh signaling centers participate in fungiform papilla and taste bud formation and maintenance. *Developmental biology* 382: 82-97

Liu TW, Ma ZG, Zhou Y, Xie JX. 2013b. Transplantation of mouse CGR8 embryonic stem cells producing GDNF and TH protects against 6-hydroxydopamine neurotoxicity in the rat. *The international journal of biochemistry & cell biology* 45: 1265-73

Lumsden AG. 1979. Pattern formation in the molar dentition of the mouse. *Journal de biologie buccale* 7: 77-103

Lumsden AGS. 1988. Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development* 103: 155-69

Luukko K. 1998. Neuronal cells and neurotrophins in odontogenesis. *European journal of oral sciences* 106: 80-93.

Luukko K, Arumae U, Karavanov A, Moshnyakov M, Sainio K, Sariola H, Saarma M, Thesleff I. 1997a. Neurotrophin mRNA expression in the developing tooth suggests multiple roles in innervation and organogenesis. *Developmental Dynamics* 210: 117-29

Luukko K, Sainio K, Sariola H, Saarma M, Thesleff I. 1997b. Localization of nerve cells in the developing rat tooth. *Journal of dental research* 76: 1350-56

Luukko K, Suvanto P, Saarma M, Thesleff I. 1997c. Expression of GDNF and its receptors in developing tooth is developmentally regulated and suggests multiple roles in innervation and organogenesis. *Devel. Dynam.* 210: 463-71

Maisonpierre PC, Belluscio L, Squinto S, Ip NY, Furth ME, Lindsay RM, Yancopoulos GD. 1990. Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science (New York, N.Y.)* 247: 1446-51

Mammoto T, Mammoto A, Torisawa YS, Tat T, Gibbs A, Derda R, Mannix R, de Bruijin M, Yung CW, Huh D, Ingber DE. 2011. Mechanochemical control of mesenchymal condensation and embryonic tooth organ formation. *Developmental cell* 21: 758-69

Margolskee RF. 2002. Molecular mechanisms of bitter and sweet taste transduction. *The Journal of biological chemistry* 277: 1-4

Matsuo S, Ichikawa H, Henderson TA, Silos-Santiago I, Barbacid M, Arends JJ, Jacquin MF. 2001. trkA modulation of developing somatosensory neurons in oro-facial tissues: tooth pulp fibers are absent in trkA knockout mice. *Neuroscience* 105: 747-60.

Mbiene JP, Roberts JD. 2003. Distribution of keratin 8-containing cell clusters in mouse embryonic tongue: evidence for a prepattern for taste bud development. *The Journal of comparative neurology* 457: 111-22.
Milbrandt J, de Sauvage FJ, Fahrner TJ, Baloh RH, Leitner ML, Tansey MG, Lampe PA, Heuckeroth RO, Kotzbauer PT, Simburger KS, Golden JP, Davies JA, Vejsada R, Kato AC, Hynes M, Sherman D, Nishimura M, Wang LC, Vandlen R, Moffat B, Klein RD, Poulsen K, Gray C, Garces A, Henderson CE, Phillips HS, Johnson EM. 1998. Persephin, a novel neurotrophic factor related to GDNF and neurturin. *Neuron* 20: 245-53
Miletich I, Sharpe PT. 2004. Neural crest contribution to mammalian tooth formation. *Birth defects research. Part C, Embryo today : reviews* 72: 200-12
Mina M, Kollar EJ. 1987. The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. *Archs. Oral Biol.* 32: 123-12
Mistretta CM, Liu HX, Gaffield W, MacCallum DK. 2003. Cyclopamine and jervine in embryonic rat tongue cultures demonstrate a role for Shh signaling in taste papilla development and patterning: fungiform papillae double in number and form in novel locations in dorsal lingual epithelium. *Developmental biology* 254: 1-18.
Mitsiadis TA, Luukko K. 1995. Neurotrophins in odontogenesis. *International Journal of Developmental Biology* 39: 195-202
Mitsiadis TA, Pagella P. 2016. Expression of Nerve Growth Factor (NGF), TrkA, and p75(NTR) in Developing Human Fetal Teeth. *Frontiers in physiology* 7: 338
Miura H, Barlow LA. 2010. Taste bud regeneration and the search for taste progenitor cells. *Archives italiennes de biologie* 148: 107-18
Miura H, Kusakabe Y, Harada S. 2006. Cell lineage and differentiation in taste buds. *Archives of histology and cytology* 69: 209-25
Miura H, Kusakabe Y, Sugiyama C, Kawamatsu M, Ninomiya Y, Motoyama J, Hino A . 2001. Shh and Ptc are associated with taste bud maintenance in the adult mouse. *Mech Dev* 106: 143-5.
Miura H, Nakayama A, Shindo Y, Kusakabe Y, Tomonari H, Harada S. 2007. Expression of gustducin overlaps with that of type III IP3 receptor in taste buds of the rat soft palate. *Chemical senses* 32: 689-96
Miura H, Scott JK, Harada S, Barlow LA. 2014. Sonic hedgehog-expressing basal cells are general post-mitotic precursors of functional taste receptor cells. *Developmental dynamics : an official publication of the American Association of Anatomists* 243: 1286-97
Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. 2003. SHED: Stem cells from human exfoliated deciduous teeth. *Proceedings of the National Academy of Sciences of the United States of America* 25: 25
Mizuno N, Shiba H, Xu WP, Inui T, Fujita T, Kajiya M, Takeda K, Hasegawa N, Kawaguchi H, Kurihara H. 2007. Effect of neurotrophins on differentiation, calcification and proliferation in cultures of human pulp cells. *Cell biology international* 31: 1462-9

Moe K, Kettunen P, Kvinnsland IH, Luukko K. 2008. Development of the pioneer sympathetic innervation into the dental pulp of the mouse mandibular first molar. *Archives of oral biology* 53: 865-73

Moe K, Sijaona A, Shrestha A, Kettunen P, Taniguchi M, Luukko K. 2012. Semaphorin 3A controls timing and patterning of the dental pulp innervation. *Differentiation; research in biological diversity* 84: 371-9

Moeton M, Stassen OM, Sluijs JA, van der Meer VW, Kluivers LJ, van Hoorn H, Schmidt T, Reits EA, van Strien ME, Hol EM. 2016. GFAP isoforms control intermediate filament network dynamics, cell morphology, and focal adhesions. *Cellular and molecular life sciences : CMLS* 73: 4101-20

Mohamed SS, Atkinson ME. 1983. A histological study of the innervation of developing mouse teeth. *J. Anat.* 136: 735-49

Moore MW, Klein RD, I, Sauer H, Armanini M, Phillips H, Reichardt LF, Ryan AM, Carvermoore K, Rosenthal A. 1996. Renal and neuronal abnormalities In mice lacking GDNF. *Nature* 382: 76-79

Nakagawa E, Zhang L, Shin JO, Kim EJ, Cho SW, Ohshima H, Chen Z, Jung HS. 2012. The novel expression of Oct3/4 and Bmi1 in the root development of mouse molars. *Cell and tissue research* 347: 479-84

Nam H, Kim J, Park J, Park JC, Kim JW, Seo BM, Lee JC, Lee G. 2011. Expression profile of the stem cell markers in human Hertwig's epithelial root sheath/Epithelial rests of Malassez cells. *Molecules and cells* 31: 355-60

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS. 2001. Mammalian sweet taste receptors. *Cell* 106: 381-90.

Nelson GM, Finger TE. 1993. Immunolocalization of different forms of neural cell adhesion molecule (NCAM) in rat taste buds. *Journal of Comparative Neurology* 336: 507-16

Neupane S, Sohn WJ, Rijal G, Lee YJ, Lee S, Yamamoto H, An CH, Cho SW, Lee Y, Shin HI, Kwon TY, Kim JY. 2014. Developmental regulations of Perp in mice molar morphogenesis. *Cell and tissue research* 358: 109-21

Nguyen HM, Barlow LA. 2010. Differential expression of a BMP4 reporter allele in anterior fungiform versus posterior circumvallate taste buds of mice. *BMC neuroscience* 11: 129

Nosrat CA. 1998. Neurotrophic factors in the tongue; expression patterns, biological activity, relation to innervation and studies of neurotrophin knockout mice. *Annals N. Y. Acad. Sci.* 855: 28-50

Nosrat CA, Blomlöf J, Elshamy WM, Ernfors P, Olson L. 1997a. Lingual deficits in BDNF and NT3 mutant mice leading to gustatory and somatosensory disturbances, respectively. *Development* 124: 1333-42

Nosrat CA, Ebendal T, Olson L. 1996a. Differential expression of brain-derived neurotrophic factor and neurotrophin 3 mRNA in lingual papillae and taste buds indicates roles in gustatory and somatosensory innervation. *The Journal of comparative neurology* 376: 587-602

Nosrat CA, Fried K, Ebendal T, Olson L. 1998. NGF, BDNF, NT3, NT4 and GDNF in tooth development. *European journal of oral sciences* 106 Suppl 1: 94-9

Nosrat CA, Fried K, Lindskog S, Olson L. 1997b. Cellular expression of neurotrophin mRNAs during tooth development. *Cell Tissue Res.* 290: 569-80

Nosrat CA, MacCallum DK, Mistretta CM. 2001a. Distinctive spatiotemporal expression patterns for neurotrophins develop in gustatory papillae and lingual tissues in embryonic tongue organ cultures. *Cell and tissue research* 303: 35-45.

Nosrat CA, Olson L. 1995. Brain-derived neurotrophic factor mRNA is expressed in the developing taste bud-bearing tongue papillae of rat. *J. Comp. Neurol.* 360: 698-704

Nosrat CA, Palacios L, Vu K, Kezian S, Henson BS, Vukmanovic Nosrat I. 2016. Innate Overexpression of BDNF Improves Taste Dysfunction Following Chemotherapy *Soc. Neurosci. Abstr.*

Nosrat CA, Tomac A, Lindqvist E, Lindskog S, Humpel C, Stromberg I, Ebendal T, Hoffer BJ, Olson L. 1996b. Cellular expression of GDNF mRNA suggests multiple functions inside and outside the nervous system. *Cell Tiss. Res.* 286: 191-207

Nosrat I, Seiger A, Olson L, Nosrat CA. 2002. Expression patterns of neurotrophic factor mRNAs in developing human teeth. *Cell and tissue research* 310: 177-87

Nosrat IV, Agerman K, Marinescu A, Ernfors P, Nosrat CA. 2004a. Lingual deficits in neurotrophin double knockout mice. *Journal of neurocytology* 33: 607-15

Nosrat IV, Lindskog S, Seiger Å, Nosrat CA. 2000. Lingual BDNF and NT-3 mRNA expression patterns and their relation to innervation in the human tongue: similarities and differences compared with rodents. *J. Comp. Neurol.* 417: 133-52

Nosrat IV, Margolskee RF, Nosrat CA. 2012. Targeted taste cell-specific overexpression of brain-derived neurotrophic factor in adult taste buds elevates phosphorylated TrkB protein levels in taste cells, increases taste bud size, and promotes gustatory innervation. *The Journal of biological chemistry* 287: 16791-800

Nosrat IV, Smith CA, Mullally P, Olson L, Nosrat CA. 2004b. Dental pulp cells provide neurotrophic support for dopaminergic neurons and differentiate into neurons in vitro; implications for tissue engineering and repair in the nervous system. *The European journal of neuroscience* 19: 2388-98.

Nosrat IV, Widenfalk J, Olson L, Nosrat CA. 2001b. Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons in vitro and rescue motoneurons after spinal cord injury. *Devel. Biol.* 238: 120-32

Novikova L, Novikov L, Kellerth JO. 1996. Brain-derived neurotrophic factor reduces necrotic zone and supports neuronal survival after spinal cord hemisection in adult rats. *Neuroscience letters* 220: 203-06

Oakley B. 1991. Neuronal-epithelial interactions in mammalian gustatory epithelium. *Ciba Foundation Symposium* 160: 277-87

Okubo T, Clark C, Hogan BL. 2009. Cell lineage mapping of taste bud cells and keratinocytes in the mouse tongue and soft palate. *Stem cells (Dayton, Ohio)* 27: 442-50

Okubo T, Pevny LH, Hogan BL. 2006. Sox2 is required for development of taste bud sensory cells. *Genes & development* 20: 2654-9

Olgart L. 1996. Neural control of pulpal blood flow. *Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists* 7: 159-71

Olson L. 1967. Outgrowth of sympathetic adrenergic neurons in mice treated with a nerve-growth factor (NGF). *Zeitschrift fur Zellforschung und mikroskopische Anatomie (Vienna, Austria : 1948)* 81: 155-73

Olson L. 1997. Regeneration in the adult central nervous system: experimental repair strategies. *Nature medicine* 3: 1329-35

Olson L, Ayer-LeLievre C, Björklund H, Granholm AC, Hedlund KO, Hokfelt T, Melander T, Seiger A, Stromberg I. 1988. The innervation apparatus of the rodent iris In *Handbook of chemical neuroanatomy*, ed. A Björklund, T Hökfelt, C Owman, pp. 545-97. Amsterdam: Elsevier

Olson L, Malmfors T. 1970. Growth characteristics of adrenergic nerves in the adult rat. Fluorescence histochemical and 3H-noradrenaline uptake studies using tissue transplantations to the anterior chamber of the eye. *Acta Physiol Scand Suppl* 348: 1-112

Olson L, Seiger Å, Strömberg I. 1983. Intraocular transplantation in rodents. A detailed account of the procedure and examples of its use in neurobiology with special reference to brain tissue grafting In *Advances in Cellular Neurobiology, Vol 4*, ed. S Federoff, L Hertz, pp. 407-42. New York: Academic

Oppenheim RW, Houenou LJ, Johnson JE, Lin LF, Li L, Lo AC, Newsome AL, Prevette DM, Wang S. 1995. Developing motor neurons rescued from programmed and axotomy-induced cell death by GDNF. *Nature* 373: 344-6.

Palacios LG, Kezian SA, Luong G, Vu KH, Chung AW, Arwood M, Rajendran L, Henson BS, Nosrat CA, Vukmanovic Nosrat I . 2017. Overexpression of BDNF Prevents Dysgeusia Following Chemotherapy. *Internationl Association for Dental Research*

Park H, Poo MM. 2013. Neurotrophin regulation of neural circuit development and function. *Nature reviews. Neuroscience* 14: 7-23

Patel AV, Krimm RF. 2010. BDNF is required for the survival of differentiated geniculate ganglion neurons. *Developmental biology* 340: 419-29

Pawson T, Nash P. 2000. Protein-protein interactions define specificity in signal transduction. *Genes & development* 14: 1027-47

Pearson AA. 1977. The early innervation of developing deciduous teeth. *J. Anat.* 123: 563-77

Pearson J, Johnson EM, Brandeis L. 1983. Effects of antibodies to nerve growth factor on intrauterine development of derivatives of cranial neural crest and placode in the guinea pig. *Developmental biology* 96: 32-6

Perlson E, Maday S, Fu MM, Moughamian AJ, Holzbaur EL. 2010. Retrograde axonal transport: pathways to cell death? *Trends in neurosciences* 33: 335-44

Peters H, Neubuser A, Kratochwil K, Balling R. 1998. Pax9-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. *Genes & development* 12: 2735-47

Petersen CI, Jheon AH, Mostowfi P, Charles C, Ching S, Thirumangalathu S, Barlow LA, Klein OD. 2011. FGF signaling regulates the number of posterior taste papillae by controlling progenitor field size. *PLoS genetics* 7: e1002098

Pichel JG, Shen LY, Sheng HZ, Granholm AC, Drago J, Grinberg A, Lee EJ, Huang SP, Saarma M, Hoffer BJ, Sariola H, Westphal H. 1996. Defects in enteric innervation and kidney development in mice lacking GDNF. *Nature* 382: 73-76

Pirvola U, Ylikoski J, Palgi J, Lehtonen E, Arumae U, Saarma M. 1992. Brain-derived neurotrophic factor and neurotrophin 3 mRNAs in the peripheral target fields of developing inner ear ganglia. *Proc. Natl. Acad. Sci. USA* 89: 9915-19

Poo MM. 2001. Neurotrophins as synaptic modulators. *Nature Rev. Neurosci.* 2: 24-32

Poon WW, Blurton-Jones M, Tu CH, Feinberg LM, Chabrier MA, Harris JW, Jeon NL, Cotman CW. 2011. beta-Amyloid impairs axonal BDNF retrograde trafficking. *Neurobiology of aging* 32: 821-33

Poon WW, Carlos AJ, Aguilar BL, Berchtold NC, Kawano CK, Zograbyan V, Yaopruke T, Shelanski M, Cotman CW. 2013. beta-Amyloid (Abeta) oligomers impair brain-derived neurotrophic factor retrograde trafficking by down-regulating ubiquitin C-terminal hydrolase, UCH-L1. *The Journal of biological chemistry* 288: 16937-48

Porntaveetus T, Otsuka-Tanaka Y, Basson MA, Moon AM, Sharpe PT, Ohazama A. 2011. Expression of fibroblast growth factors (Fgfs) in murine tooth development. *Journal of anatomy* 218: 534-43

Purves D, Augustine G. 2011. *Neuroscience, Fifth Edition*.

Qian XB, Naftel JP. 1996. Effects of neonatal exposure to anti-nerve growth factor on the number and size distribution of trigeminal neurones projecting to the molar dental pulp in rats. *Archives of oral biology* 41: 359-67

Radeke MJ, Misko TP, Hsu C, Herzenberg LA, Shooter EM. 1987. Gene transfer and molecular cloning of the rat nerve growth factor receptor. *Nature* 325: 593-7

Riethmacher D, Sonnenberg-Riethmacher E, Brinkmann V, Yamaai T, Lewin GR, Birchmeier C. 1997. Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. *Nature* 389: 725-30.

Ringstedt T, Ibáñez CF, Nosrat CA. 1999. Role of BDNF in target invasion in the gustatory system. *J. Neurosci.* 19: 3507-18

Roberts CD, Dvoryanchikov G, Roper SD, Chaudhari N. 2009. Interaction between the second messengers cAMP and Ca2+ in mouse presynaptic taste cells. *The Journal of physiology* 587: 1657-68

Robino A, Mezzavilla M, Pirastu N, Dognini M, Tepper BJ, Gasparini P. 2014. A population-based approach to study the impact of PROP perception on food liking in populations along the Silk Road. *PloS one* 9: e91716

Rodriguez-Serrano LM, Ramirez-Leon B, Rodriguez-Duran LF, Escobar ML. 2014. Acute infusion of brain-derived neurotrophic factor in the insular cortex promotes conditioned taste aversion extinction. *Neurobiology of learning and memory* 116: 139-44

Rodriguez-Tebar A, Dechant G, Gotz R, Barde YA. 1992. Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. *EMBO Journal* 11: 917-22

Roper SD. 2013. Taste buds as peripheral chemosensory processors. *Seminars in cell & developmental biology* 24: 71-9

Rosenthal A, Goeddel DV, Nguyen T, Lewis M, Shih A, Laramee GR, Nikolics K, Winslow JW . 1990. Primary structure and biological activity of a novel human neurotrophic factor. *Neuron* 4: 767-73

Rothova M, Thompson H, Lickert H, Tucker AS. 2012. Lineage tracing of the endoderm during oral development. *Developmental dynamics : an official publication of the American Association of Anatomists* 241: 1183-91

Ryden M, Murray RJ, Glass D, Ilag LL, Trupp M, Yancopoulos GD, McDonald NQ, Ibanez CF. 1995. Functional analysis of mutant neurotrophins deficient in low-affinity binding reveals a role for p75LNGFR in NT-4 signalling. *Embo Journal* 14: 1979-90

Saarma M. 2000. GDNF - a stranger in the TGF-beta superfamily? *European journal of biochemistry / FEBS* 267: 6968-71

Saha K, Schaffer DV. 2006. Signal dynamics in Sonic hedgehog tissue patterning. *Development* 133: 889-900

San Gabriel A, Uneyama H, Yoshie S, Torii K. 2005. Cloning and characterization of a novel mGluR1 variant from vallate papillae that functions as a receptor for L-glutamate stimuli. *Chemical senses* 30 Suppl 1: i25-6

Sarkar L, Sharpe PT. 1999. Expression of Wnt signalling pathway genes during tooth development. *Mech Dev* 85: 197-200

Satokata I, Maas R. 1994. Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nature genetics* 6: 348-56

Sawai H, Clarke DB, Kittlerova P, Bray GM, Aguayo AJ. 1996. Brain-derived neurotrophic factor and neurotrophin-4/5 stimulate growth of axonal branches from regenerating retinal ganglion cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 16: 3887-94

Saxen L, Sariola H. 1987. Early organogenesis of the kidney. *Pediatric nephrology (Berlin, Germany)* 1: 385-92

Schlessinger J, Ullrich A. 1992. Growth factor signaling by receptor tyrosine kinases. *Neuron* 9: 383-91

Segade LA, Suarez-Quintanilla D. 1988. Otic ganglion parasympathetic neurons innervate the pulp of the mandibular incisor of the guinea pig. *Neuroscience letters* 90: 33-8

Sekulic A, Migden MR, Oro AE, Dirix L, Lewis KD, Hainsworth JD, Solomon JA, Yoo S, Arron ST, Friedlander PA, Marmur E, Rudin CM, Chang AL, Low JA, Mackey HM, Yauch RL, Graham RA, Reddy JC, Hauschild A . 2012. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. *The New England journal of medicine* 366: 2171-9

Sendtner M, Holtmann B, Kolbeck R, Thoenen H, Barde YA. 1992. Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. *Nature* 360: 757-59.

Seta Y, Kataoka S, Toyono T, Toyoshima K. 2006. Expression of galanin and the galanin receptor in rat taste buds. *Archives of histology and cytology* 69: 273-80

Sharpe PT. 2001. Neural crest and tooth morphogenesis. *Advances in dental research* 15: 4-7

Shen T, Kaya N, Zhao FL, Lu SG, Cao Y, Herness S. 2005. Co-expression patterns of the neuropeptides vasoactive intestinal peptide and cholecystokinin with the transduction molecules alpha-gustducin and T1R2 in rat taste receptor cells. *Neuroscience* 130: 229-38

Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S. 2005. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. *Orthod Craniofac Res* 8: 191-9

Shim J, Son HJ, Kim Y, Kim KH, Kim JT, Moon H, Kim MJ, Misaka T, Rhyu MR. 2015. Modulation of Sweet Taste by Umami Compounds via Sweet Taste Receptor Subunit hT1R2. *PloS one* 10: e0124030

Shin JO, Kim EJ, Cho KW, Nakagawa E, Kwon HJ, Cho SW, Jung HS. 2012. BMP4 signaling mediates Zeb family in developing mouse tooth. *Histochemistry and cell biology* 137: 791-800

Shin YK, Martin B, Golden E, Dotson CD, Maudsley S, Kim W, Jang HJ, Mattson MP, Drucker DJ, Egan JM, Munger SD. 2008. Modulation of taste sensitivity by GLP-1 signaling. *Journal of neurochemistry* 106: 455-63

Shinmura Y, Tsuchiya S, Hata K, Honda MJ. 2008. Quiescent epithelial cell rests of Malassez can differentiate into ameloblast-like cells. *Journal of cellular physiology* 217: 728-38

Sire JY, Delgado SC, Girondot M. 2008. Hen's teeth with enamel cap: from dream to impossibility. *BMC evolutionary biology* 8: 246

Skaper SD. 2012. The neurotrophin family of neurotrophic factors: an overview. *Methods in molecular biology (Clifton, N.J.)* 846: 1-12

Slavkin HC. 1991. Molecular determinants during dental morphogenesis and cytodifferentiation: a review [see comments]. *Journal of Craniofacial Genetics & Developmental Biology* 11: 338-49

Sollars SI, Hill DL. 2000. Lack of functional and morphological susceptibility of the greater superficial petrosal nerve to developmental dietary sodium restriction. *Chemical senses* 25: 719-27

Son AR, Yang YM, Hong JH, Lee SI, Shibukawa Y, Shin DM. 2009. Odontoblast TRP channels and thermo/mechanical transmission. *Journal of dental research* 88: 1014-9

Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Wang S, Shi S. 2006. Mesenchymal stem cell-mediated functional tooth regeneration in swine. *PloS one* 1: e79

Spradling A, Drummond-Barbosa D, Kai T. 2001. Stem cells find their niche. *Nature* 414: 98-104

Springer JE, Koh S, Tayrien MW, Loy R. 1987. Basal forebrain magnocellular neurons stain for nerve growth factor receptor: correlation with cholinergic cell bodies and effects of axotomy. *Journal of neuroscience research* 17: 111-8

Sternini C. 2013. In search of a role for carbonation: is this a good or bad taste? *Gastroenterology* 145: 500-3

Swett JE, Wikholm RP, Blanks RH, Swett AL, Conley LC. 1986. Motoneurons of the rat sciatic nerve. *Exp Neurol* 93: 227-52

Szalay FS, Seligsohn D. 1977. Why did the strepsirhine tooth comb evolve. *Folia primatologica; international journal of primatology* 27: 75-82

Takai S, Yasumatsu K, Inoue M, Iwata S, Yoshida R, Shigemura N, Yanagawa Y, Drucker DJ, Margolskee RF, Ninomiya Y. 2015. Glucagon-like peptide-1 is specifically involved in sweet taste transmission. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 29: 2268-80

Tarsa L, Balkowiec-Iskra E, Kratochvil FJ, 3rd, Jenkins VK, McLean A, Brown AL, Smith JA, Baumgartner JC, Balkowiec A. 2010. Tooth pulp inflammation increases brain-derived neurotrophic factor expression in rodent trigeminal ganglion neurons. *Neuroscience* 167: 1205-15

Tessarollo L, Vogel KS, Palko ME, Reid SW, Parada LF. 1994. Targeted mutation in the neurotrophin-3 gene results in loss of muscle sensory neurons. *Proceedings of the National Academy of Sciences of the United States of America* 91: 11844-8

Thesleff I, Tummers M. 2008. Tooth organogenesis and regeneration In *StemBook*. Cambridge (MA): Harvard Stem Cell Institute

Copyright: (c) 2008 Irma Thesleff and Mark Tummers.

Thesleff I, Tummers M. 2009. Tooth organogenesis and regeneration. Cambridge (MA): Harvard Stem Cell Institute: Bookshelf

Thoenen H, Barde YA. 1980. Physiology of nerve growth factor. *Physiol. Rev.* 60: 1284-335

Thoenen H, Barde YA, Davies AM, Johnson JE. 1987. Neurotrophic factors and neuronal death. [Review]. *Ciba Foundation Symposium* 126: 82-95

Tomac A, Lindqvist E, Lin L, Ögren S, Young D, Hoffer BJ, Olson L. 1995a. Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo. *Nature* 373: 335-39

Tomac A, Widenfalk J, Lin L, Kohno T, Ebendal T, Hoffer BJ, Olson L. 1995b. Retrograde axonal transport of glial cell line-derived neurotrohic factor in the adult nigrostriatal system suggests a trophic role in the adult. *Proc Natl Acad Sci USA* 92: 8274-78

Tominaga M, Caterina MJ. 2004. Thermosensation and pain. *Journal of neurobiology* 61: 3-12

Treanor JJ, Goodman L, de Sauvage F, Stone DM, Poulsen KT, Beck CD, Gray C, Armanini MP, Pollock RA, Hefti F, Phillips HS, Goddard A, Moore MW, Buj-Bello A, Davies AM, Asai N, Takahashi M, Vandlen R, Henderson CE, Rosenthal A. 1996. Characterization of a multicomponent receptor for GDNF. *Nature* 382: 80-3

Treanor JJ, Schmelzer C, Knusel B, Winslow JW, Shelton DL, Hefti F, Nikolics K, Burton LE. 1995. Heterodimeric neurotrophins induce phosphorylation of Trk receptors and promote neuronal differentiation in PC12 cells. *The Journal of biological chemistry* 270: 23104-10

Treffy RW, Collins D, Hoshino N, Ton S, Katsevman GA, Oleksiak M, Runge EM, Cho D, Russo M, Spec A, Gomulka J, Henkemeyer M, Rochlin MW. 2016. Ephrin-B/EphB Signaling Is Required for Normal Innervation of Lingual Gustatory Papillae. *Developmental neuroscience* 38: 124-38

Trupp M, Rydén M, Jörnvall H, Funakoshi H, Timmusk T, Arenas E, Ibanez CF. 1995. Peripheral expression and biological activities of GDNF, a new neurotrophic

factor for avian and mammalian peripheral neurons. *The Journal of cell biology* 130: 137-48
Tucker AS, Headon DJ, Schneider P, Ferguson BM, Overbeek P, Tschopp J, Sharpe PT . 2000. Edar/Eda interactions regulate enamel knot formation in tooth morphogenesis. *Development* 127: 4691-700
Tucker AS, Sharpe PT. 1999. Molecular genetics of tooth morphogenesis and patterning: the right shape in the right place. *Journal of dental research* 78: 826-34
Tucker KL, Meyer M, Barde YA. 2001. Neurotrophins are required for nerve growth during development. *Nat Neurosci* 4: 29-37.
Tucker RP, Tran H, Gong Q. 2008. Neurogenesis and neurite outgrowth in the spinal cord of chicken embryos and in primary cultures of spinal neurons following knockdown of Class III beta tubulin with antisense morpholinos. *Protoplasma* 234: 97-101
Tummers M, Thesleff I. 2009. The importance of signal pathway modulation in all aspects of tooth development. *Journal of experimental zoology. Part B, Molecular and developmental evolution* 312b: 309-19
Tziafas D, Papadimitriou S. 1998. Role of exogenous TGF-beta in induction of reparative dentinogenesis in vivo. *European journal of oral sciences* 106 Suppl 1: 192-6
Ullrich A, Gray A, Berman C, Dull TJ. 1983. Human beta-nerve growth factor gene sequence highly homologous to that of mouse. *Nature* 303: 821-5
Ulupinar E, Jacquin MF, Erzurumlu RS. 2000. Differential effects of NGF and NT-3 on embryonic trigeminal axon growth patterns. *The Journal of comparative neurology* 425: 202-18
Uttara B, Singh AV, Zamboni P, Mahajan RT. 2009. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current neuropharmacology* 7: 65-74
Vaahtokari A, Åberg T, Thesleff I. 1996. Apoptosis in the developing tooth: association with an embryonic signaling center and suppression by EGF and FGF-4. *Development* 122: 121-29
Vandenbeuch A, Clapp TR, Kinnamon SC. 2008. Amiloride-sensitive channels in type I fungiform taste cells in mouse. *BMC neuroscience* 9: 1
Velagapudi C, Nilsson RP, Lee MJ, Burns HS, Ricono JM, Arar M, Barnes VL, Abboud HE, Barnes JL. 2012. Reciprocal induction of simple organogenesis by mouse kidney progenitor cells in three-dimensional co-culture. *The American journal of pathology* 180: 819-30
Verge VM, Riopelle RJ, Richardson PM. 1989. Nerve growth factor receptors on normal and injured sensory neurons. *Journal of Neuroscience* 9: 914-22
Vilbig R, Cosmano J, Giger R, Rochlin MW. 2004. Distinct roles for Sema3A, Sema3F, and an unidentified trophic factor in controlling the advance of geniculate axons to gustatory lingual epithelium. *Journal of neurocytology* 33: 591-606
von Bartheld CS, Byers MR, Williams R, Bothwell M. 1996a. Anterograde transport of neurotrophins and axodendritic transfer in the developing visual system. *Nature* 379: 830-3

von Bartheld CS, Williams R, Lefcort F, Clary DO, Reichardt LF, Bothwell M. 1996b. Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the p75NTR and trkB receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 16: 2995-3008

Wang D, Chan CC, Cherry S, Hiesinger PR. 2013. Membrane trafficking in neuronal maintenance and degeneration. *Cellular and molecular life sciences : CMLS* 70: 2919-34

Wang HW, Chiou WY. 2004. Sympathetic innervation of the tongue in rats. *ORL; journal for oto-rhino-laryngology and its related specialties* 66: 16-20

Whitlock JA, Richman JM. 2013. Biology of tooth replacement in amniotes. *International journal of oral science* 5: 66-70

Widenfalk J, Nosrat CA, Tomac A, Westfal H, Hoffer BJ, Olson L. 1997. Neurturin and GDNFR-b, novel proteins related to GDNF and GDNFR-a with specific cellular patterns of expression suggesting roles in the developing and adult nervous system and in peripheral organs. *J. Neurosci.* 17: 8506-19

Williams DL. 1993. A comparative approach to anterior segment dysgenesis. *Eye (London, England)* 7 (Pt 5): 607-16

Witt M, Kasper M. 1999. Distribution of cytokeratin filaments and vimentin in developing human taste buds. *Anatomy and embryology* 199: 291-9

Witt M, Reutter K. 1996. Embryonic and early fetal development of human taste buds: a transmission electron microscopical study. *Anat. Rec.* 246: 507-23

Witt M, Reutter K. 1997. Scanning electron microscopical studies of developing gustatory papillae in humans. *Chem. Senses* 22: 601-12

Wright DE, Zhou L, Kucera J, Snider WD. 1997. Introduction of a neurotrophin-3 transgene into muscle selectively rescues proprioceptive neurons in mice lacking endogenous neurotrophin-3. *Neuron* 19: 503-17

Wu P, Wu X, Jiang TX, Elsey RM, Temple BL, Divers SJ, Glenn TC, Yuan K, Chen MH, Widelitz RB, Chuong CM. 2013. Specialized stem cell niche enables repetitive renewal of alligator teeth. *Proceedings of the National Academy of Sciences of the United States of America* 110: E2009-18

Xiang L, Chen M, He L, Cai B, Du Y, Zhang X, Zhou C, Wang C, Mao JJ, Ling J. 2014. Wnt5a regulates dental follicle stem/progenitor cells of the periodontium. *Stem cell research & therapy* 5: 135

Yamasaki H, Kubota Y, Takagi H, Tohyama M. 1984. Immunoelectron-microscopic study on the fine structure of substance-P-containing fibers in the taste buds of the rat. *J. Comp. Neurol.* 227: 380-92

Yan Q, Elliott J, Snider WD. 1992. Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. *Nature* 360: 753-5

Yang H, Cong WN, Yoon JS, Egan JM. 2014. Vismodegib, an antagonist of hedgehog signaling, directly alters taste molecular signaling in taste buds. *Cancer medicine*

Yang J, Wang SK, Choi M, Reid BM, Hu Y, Lee YL, Herzog CR, Kim-Berman H, Lee M, Benke PJ, Lloyd KC, Simmer JP, Hu JC. 2015. Taurodontism, variations in tooth number, and misshapened crowns in Wnt10a null mice and human kindreds. *Molecular genetics & genomic medicine* 3: 40-58

Yang R, Crowley HH, Rock ME, Kinnamon JC. 2000. Taste cells with synapses in rat circumvallate papillae display SNAP-25-like immunoreactivity. *The Journal of comparative neurology* 424: 205-15.
Yang R, Ma H, Thomas SM, Kinnamon JC. 2007. Immunocytochemical analysis of syntaxin-1 in rat circumvallate taste buds. *The Journal of comparative neurology* 502: 883-93
Yang R, Stoick CL, Kinnamon JC. 2004. Synaptobrevin-2-like immunoreactivity is associated with vesicles at synapses in rat circumvallate taste buds. *The Journal of comparative neurology* 471: 59-71.
Yee CL, Yang R, Bottger B, Finger TE, Kinnamon JC. 2001. "Type III" cells of rat taste buds: immunohistochemical and ultrastructural studies of neuron-specific enolase, protein gene product 9.5, and serotonin. *The Journal of comparative neurology* 440: 97-108.
Yen AH, Sharpe PT. 2008. Stem cells and tooth tissue engineering. *Cell and tissue research* 331: 359-72
Yokohama-Tamaki T, Ohshima H, Fujiwara N, Takada Y, Ichimori Y, Wakisaka S, Ohuchi H, Harada H. 2006. Cessation of Fgf10 signaling, resulting in a defective dental epithelial stem cell compartment, leads to the transition from crown to root formation. *Development* 133: 1359-66
Yongchaitrakul T, Pavasant P. 2007. Transforming growth factor-beta1 up-regulates the expression of nerve growth factor through mitogen-activated protein kinase signaling pathways in dental pulp cells. *European journal of oral sciences* 115: 57-63
Yoshikawa DK, Kollar EJ. 1981. Recombination experiments on the odontogenic roles of mouse dental papilla and dental sac tissues in ocular grafts. *Archives of oral biology* 26: 303-7
Yoshizaki K, Yamamoto S, Yamada A, Yuasa K, Iwamoto T, Fukumoto E, Harada H, Saito M, Nakasima A, Nonaka K, Yamada Y, Fukumoto S. 2008. Neurotrophic factor neurotrophin-4 regulates ameloblastin expression via full-length TrkB. *The Journal of biological chemistry* 283: 3385-91
Zetterstrom RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T. 1997. Dopamine neuron agenesis in Nurr1-deficient mice. *Science (New York, N.Y.)* 276: 248-50
Zhang C, Oakley B. 1996. The distribution and origin of keratin 20-containing taste buds in rat and human. *Differentiation; research in biological diversity* 61: 121-7
Zhang J, Geula C, Lu C, Koziel H, Hatcher LM, Roisen FJ. 2003a. Neurotrophins regulate proliferation and survival of two microglial cell lines in vitro. *Exp Neurol* 183: 469-81.
Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJ. 2003b. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell* 112: 293-301
Zhao FL, Shen T, Kaya N, Lu SG, Cao Y, Herness S. 2005. Expression, physiological action, and coexpression patterns of neuropeptide Y in rat taste-bud cells. *Proceedings of the National Academy of Sciences of the United States of America* 102: 11100-5

Zhao H, Feng J, Seidel K, Shi S, Klein O, Sharpe P, Chai Y. 2014. Secretion of shh by a neurovascular bundle niche supports mesenchymal stem cell homeostasis in the adult mouse incisor. *Cell stem cell* 14: 160-73