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Serotonin in stem cell based-dental repair and bone formation: a review

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ABSTRACT

Genetic and pharmacological studies provided evidence that serotonin (5-HT) is an important signaling molecule for the development and the maintenance of mineralized tissues. However, how 5-HT takes part to the homeostasis of teeth and bone remains elusive. In the dental field, a major breakthrough comes from the identification of 5-HT but also dopamine (DA) as "damage" signals necessary for stem cell-based tooth repair. Pulpal stem cells express the overall functions of 5-HT and DA neurons including a definite set of functional 5-HT/DA receptors that render cells responsive for circulating bioamines. Upon tooth injury, activated platelets release bulks of 5-HT/DA that mobilize pulpal stem cells for natural dental repair. The contribution of 5-HT to bone metabolism is more documented with description of both anabolic and resorptive effects. By controlling the tissue-non specific alkaline phosphatase (TNAP), 5-HT_{2B} receptors exert an anabolic function and a pivotal role in mineralization processes. Increasing our understanding of the role of 5-HT receptors in bone metabolism may pave the road for the development of therapeutic strategies towards skeletal-associated pathologies and ectopic calcification.

Keywords: Serotonin, Stem cells, Bone mineralization, Tooth repair

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1. Introduction

Serotonin (5-hydroxytryptamine ; 5-HT) is a tryptophan-derived bioamine synthesized by neurons of the raphe nuclei in the central nervous system and gut enterochromaffin cells in the periphery, this last site accounting for 95% of 5-HT produced in the human body. In the brain, 5-HT acts as a neurotransmitter and mediates a wide variety of behavioral and physiological processes such as sleep, memory, cognition, appetite or mood [1]. Peripheral 5-HT stored in platelet-dense granules is involved in the regulation of cardiovascular, gastrointestinal, smooth muscle and endocrine functions [1]. This monoamine influences such broad physiological processes thanks to the presence at the surface of recipient cells of a serotonin transporter (SERT) and/or serotonergic receptors (5-HTRs). To date, 15 serotonergic receptor subtypes were identified, divided in seven classes (5-HT_{1-7}R) [2]. They all belong to the G-protein coupled receptor (GPCR) superfamily except for the 5-HT_3R, a ligand gated ion channel [2].

At early stages of embryonic mammalian development, 5-HT of maternal origin [3] was shown to exert a trophic role [4-6], notably in craniofacial development [7,8]. Shuey et al. reported craniofacial malformations, including mandibular and maxillary hypoplasia, in mouse embryos exposed to antidepressants such as sertraline or fluoxetine (Prozac®) that target and block the SERT [9]. Similar morphological defects were observed when mouse embryos were treated with the 5-HT_2R inverse agonist ritanserin [7]. Supporting the role of 5-HT in skeleton development and tooth formation, 5-HT would drive the migration and differentiation of neural crest cells which are at the origin of oral facial mineralized tissues. Following migration from the rostral hindbrain (rhombomeres 1 and 2) and caudal midbrain, neural crest cells populate the first branchial arch to give rise to the ectomesenchyme required for the formation of (i) mandibular and maxillary bones and (ii) the central core dentin and root lining cementum in teeth [10,11] (Fig. 1). The ectomesenchyme functionaly interacts

with the dental epithelium to promote formation of the tooth bud preceding cell differentiation into dentin-secreting odontoblasts and enamel-secreting ameloblasts. Interestingly, the presence of 5-HT_{2B}R at day 9 of mouse embryogenesis in the first branchial arch argues for an involvement of this receptor in the migration of neural crest cells and their commitment towards osteo-odontogenic lineages. Consistent with this, by using the alternative vertebrate model *Xenopus laevis*, Reisoli et al. showed that 5-HT_{2B}R contributes to morphogenesis of the first branchial arch and formation of the jaw joint [12], reinforcing the key role of the 5-HT_{2B}R in craniofacial development.

Expression of the SERT and diverse serotonergic receptors has been evidenced at the mRNA or protein levels in osteoblast primary cultures, osteoblastic cell lines or RANKL-induced RAW264.7 osteoclasts as well as in tooth tissues [13-20]. This suggests that bone-forming osteoblasts, bone-resorbing osteoclasts, ameloblasts and odontoblasts are directly influenced by circulating 5-HT. Accordingly, Gustafsson et al. showed that 5-HT stimulates proliferation of human osteoclasts and primary osteoblasts as well as murine preosteoblasts *in vitro* [21]. Two-months old rats treated by daily 5-HT administration for 3 months display higher bone mineral density, altered bone architecture and higher femoral stiffness [22]. Further, clinical studies have reported that the use of selective serotonin reuptake inhibitors (SSRIs) antidepressants such as fluoxetine, for a long time period, is associated with altered bone mineral density, osteoporosis or increased risks of bone fracture [23-25]. Four-weeks old wild-type mice treated with fluoxetine exhibit reduced bone mass accrual during growth [26]. SERT knockout mice also display reduced bone mass, altered skeletal architecture and decreased femur mechanical properties [26]. Yun et al. recently reported that activation of 5-HT_6R by the agonist ST1936 inhibits osteoblast differentiation *in vitro* and calvarial bone regeneration *in vivo* [20]. The 5-HT_{1B}R was also involved in the negative control of bone mass. 5-HT_{1B}R knockout mice display increased osteoblast proliferation and bone formation

resulting in high bone mass [27]. In contrast, pharmacological blockade of the 5-HT_{2A}R with MDL11939 leads to loss of bone mass in mice, due to an impairment of bone formation [28]. The deletion of 5-HT_{2B}R provokes an age-dependent reduction of bone mineral density, but only in females [16]. The osteopenia observed in 5-$HT_{2B}R^{-/-}$ mice can be accounted for by a defect of osteoprogenitor recruitment and a decrease of osteoblast proliferation [16]. The bony phenotype of 5-$HT_{2B}R^{-/-}$ mice is reminiscent to that of $SERT^{-/-}$ mice [26], but the damages on skeleton caused by the absence of 5-HT_{2B}R are less pronounced and occur later compared to $SERT^{-/-}$ mice. Of note, 5-HT_{2A}R is up-regulated in 5-$HT_{2B}R^{-/-}$ calvaria-derived osteoblasts [16]. 5-HT_{2A}R may, at least partly, compensate for the absence of the 5-HT_{2B}R. Besides, we monitored structural alterations of dental tissues in 5-HT_{2B}R knockout mice [17,29]. Electron microscopy analysis revealed that teeth of both male and female 5-$HT_{2B}R^{-/-}$ mice exhibit numerous enamel porosities and reduced thickness of the crystallite, which are worsened in female (Fig. 2).

In this review, we will highlight how the use of stem cell lines, which combine the capacities to maintain a stable immature phenotype and to recapitulate *in vitro* upon induction the spatio-temporal features of odontogenic or osteogenic differentiation, has contributed to our understanding of the role of 5-HT and serotonergic receptors in bone homeostasis and tooth repair.

2. Serotonin in tooth repair: a dialogue between odontogenic stem cells and platelets

To rational the development of stem cell-based tooth regenerative strategies, a prerequisite is to define the intrinsic properties of odontogenic stem cells and to identify the physiological signals leading to the recruitment of those pulpal progenitors for reparative dentin formation upon tooth injury. Since the 2000's, odontogenic stem cells were isolated from the pulp of human permanent third molars that referred to as dental pulp stem cells (DPSCs) [30] and

from exfoliated deciduous teeth called SHED cells [31]. Upon induction, DPSCs and SHEDs can differentiate *in vitro* into odontoblasts, osteoblasts, adipocytes and neuron-like cells. *In vivo*, they give rise to dentin-like structures when transplanted subcutaneously with hydroxyapatite/tricalcium phosphate (HA/TCP) powder into the dorsal surface of immunocompromised mice [30,32]. Other stem cells have been derived from the periodontal ligament of permanent tooth (PDLCs) [33]. PDLCs showed the capacity to differentiate into cementoblasts, adipocytes and osteoblasts *in vitro* and to form cementum and periodontal ligament-like structures after transplantation *in vivo* [33]. SCAP cells, derived from the root apical papilla of human tooth, are able to differentiate into osteoblasts and adipocytes *in vitro* [34]. Upon transplantation with HA/TCP in immunocompromised mice, SCAP cells formed dentin [34]. These dental stem cells are heterogeneous cell populations and the lack of specific markers render their isolation difficult. This limits their use for defining the proper identity of odontogenic stem cells. Establishing homogenous dental stem cell lines will help to challenge this issue.

2.1. Isolation of clonal pulpal stem cell lines

In 2005, our laboratory isolated clonal cell lines from the first molar tooth germs of day 18 mouse embryos [35], which display stem cell properties [36]. They maintain a stable phenotype under long-term standard culture conditions, even in 10% fetal calf serum and exhibit the hallmarks of the odontogenic lineage. They express the transcription factors Lhx6 and Lhx7, two LIM-domain homeobox genes that specify the first branchial arch where the tooth bud is formed, as well as low levels of the odontoblast markers, type 1 collagen, dentin matrix protein (DMP1) and dentin sialoprotein (DSP). After implantation in a mouse incisor or rat molar, two pulpal stem cell lines (A4 and H8) induce spatio-temporally controlled-osteodentin formation and promote efficient tooth repair [36,37]. A4 and H8 cells are

however precursors at different stages of commitment along the odontogenic lineage. The A4 cell line behaves as a multipotent mesoblastic stem cell, which undergo *in vitro* odonto/osteogenic, chondrogenic or adipogenic differentiation, depending on the inducers. H8 cells are monopotent progenitors whose potential is restricted towards the odontogenic fate [36].

2.2. Odontogenic pulpal stem cells display a mixed serotonergic/dopaminergic phenotype

As human DPSCs that secrete diverse neurotrophic factors [38,39], A4 and H8 pulpal cell lines display high contents of Brain-Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF) as well as insulin-like growth factor-1 (IGF1), which are all stored in large dense-core vesicles [40]. Of note, the dental pulp is a tissue highly innervated. BDNF, NGF and IGF-1 are all well known to promote axon guidance and elongation as well as neuronal survival [41,42]. These neurotrophins released by pulpal stem cells might contribute to tooth innervation along root formation and tooth repair.

Surprisingly, we discovered that odontogenic stem cells have a dual serotonergic and dopaminergic identity [40]. Both A4 and H8 pulpal cell lines are able to synthesize 5-HT and dopamine (DA) and express all functional enzymes ensuring complete 5-HT/DA metabolisms. This includes the tryptophane hydroxylase and the tyrosine hydroxylase, the rate-limiting enzymes in 5-HT and DA synthesis, respectively, as well as monoamine oxidases involved in the catabolism of these bioamines. Further, both cell lines express functional SERT and vesicular transporters (VMATs), which confer the ability to co-store 5-HT and DA. Until now, such a mixed 5-HT/DA phenotype is an intriguing finding that has never been described. Finally, the properties of pulpal stem cells to produce neurotrophins as well as overall bioaminergic functions led foundation for their use for therapeutic purposes, including the treatment of neural tissue injury [43] or neurodegenerative diseases [44,45].

2.3. Functional serotonergic 5-HT$_{1D,2B,7}$ and dopaminergic D$_{1,3}$ autoreceptors are expressed by odontogenic stem cells

A striking feature of A4 and H8 pulpal stem cells is the expression at the plasma membrane of the same specific subset of serotonergic and dopaminergic receptors. This includes the 5-HT$_{2B}$, 5HT$_{1D}$ and 5-HT$_7$ receptor subtypes and dopaminergic D$_1$ and D$_3$ receptor subtypes, which are all G-protein coupled receptors (GPCRs). Of note, the three 5-HT receptors selectively expressed by odontogenic stem cells have been shown to mediate trophic effects of 5-HT during early embryogenesis [4,46]. 5-HT$_{1D}$R and 5-HT$_7$R are detected in preimplantation mouse embryos [47]. The 5-HT$_{2B}$R is expressed at embryonic day 9 by neural crest-derived tooth buds [46]. All these bioaminergic receptors are functional in pulpal cell lines since they are coupled to downstream signaling effectors (Fig. 3). For instance, 5-HT$_7$R and D$_1$R positively control adenylate cyclase activity while 5-HT$_{1D}$R and D$_3$R are negatively coupled to cAMP production. The pleiotropic 5-HT$_{2B}$R couplings to phospholipase C-dependent inositol 1, 4, 5-triphosphate (IP3), NO and phospholipase A2 (PLA2)-mediated eicosanoid productions put forward some preferential role of this receptor in dental repair since all these regulatory molecules are known to contribute to mechanical stress, inflammation and vasodilatation mechanisms in other tissues [48-50]. Such specific expression of 5-HT/DA receptors at the cell surface of pulpal stem cells suggests these receptors take part to the fine-tuning of odontogenic stem cell functions in an autocrine and/or paracrine manner. An overexpression of 5-HT$_{1D,2B,7}$R and D$_{1,3}$R has been reported in various types of cancer stem cells [51,52]. In a physiological context, whether 5-HT and DA receptor-dependent signalings act together to maintain pulpal stem cell quiescence and/or influence the balance between self-renewal and differentiation remains to be delineated.

Stem cell behavior is regulated by a local microenvironment referred as to "the stem cell

niche". This anatomic space includes (i) stem cells and (ii) stromal-supporting cells that interact with the stem cells via secreted factors and/or extracellular matrix (ECM), which allows the diffusion of mechanical and chemical signals [53-56]. The distribution of tissue stem cells is not random, and, several distinct stem cell niches (apex, isthmus between the mesial and the central pulp chambers, mesial part below the pulp exposure site,...) potentially exist within the dental pulp [37,40,57]. In these specialized niches, whether the maintenance of the odontogenic stem cell state and/or differentiation of odontogenic progenitors would relate to 5-HT/DA receptors-dependent control of developmental signals such as BMP, FGF, Notch, Hedgehog and Wnt remains to be explored. Finally, the role of 5-HT and DA signaling in the recruitment of pulpal stem cells, their self-renewal, migration and differentiation for tooth repair deserves further *in vitro* and *in vivo* investigations.

2.4. Serotonergic and dopaminergic receptors relay the action of platelets-derived 5-HT and DA to mobilize pulpal stem cells for tooth repair

The presence of a specific repertoire of serotonergic and dopaminergic receptors at the plasma membrane of odontogenic stem cells renders these cells competent to respond to both 5-HT and DA, and in particular to those circulating bioamines. In the periphery, 5-HT and DA are mainly stored in blood platelets. In platelets, 5-HT and DA contents are about 25,000 and 100-300 times higher than the concentration of free circulating 5-HT and DA in plasma, respectively [58]. Of note, the dental pulp is highly vascularized [59]. This raised the question of whether 5-HT and DA released by injury-activated platelets would represent "warning" signals necessary for mobilizing pulpal stem cells for tooth repair. Such issue was tackled using Fawn-hooded and reserpine-treated rats, two rat models whose blood platelets are deficient in bioamine storage. In wild-type rats, after pulp exposure of the mesial part of the first maxillary molar, the formation of a reparative dentin was observed at the lesion site after

30 days and the thickness of the dental bridge increased until 3 months. By contrast, with Fawn-hooded and reserpine-treated rats, natural dentin formation was not detected [40]. Thus, natural dental repair depends on platelet-stored bioamines that recruit genuine stem cells expressing 5-HT/DA receptors (Fig. 4). The pharmacological inhibition of each of the 5-$HT_{2B,7}$ and $D_{1,3}$ receptors upon introduction of selective antagonists within the pulp also altered the reparative process [40]. Upon tooth injury, these receptors are involved in pulpal stem cell-based dental repair by transducing 5-HT and DA co-released by activated platelets. The identification of 5-HT/DA as crucial "damage" signals for stem cell-mediated tooth repair paves the road for novel therapeutic approaches in the dental field.

3. Bone mineralization: a major role of 5-HT_{2B}R signaling

Bone development, growth, remodeling and repair are highly regulated processes ensuring skeleton homeostasis. Despite considerable efforts to dissect the molecular pathways sustaining osteogenesis, the regulatory mechanisms governing collagen matrix mineralization still remain enigmatic. It is now clear that the serotonergic system contributes to bone metabolism [60], but our understanding of the role of serotonergic receptors in bone mineralization is still scarce. Notably, what are the signaling pathways and targets governed by 5-HT receptors to orchestrate bone homeostasis? Tackling this issue *in vivo* or with primary cultures is rendered difficult by the heterogeneity of cell types and the multiplicity of signals at play. Clonal osteogenic cell lines with homogenous phenotypic properties permit to overcome these limitations.

3.1. The C1 mesoblastic cell line selectively expresses 5-HT_{2B}Rs during osteogenic differentiation

The C1 tripotential mesoblastic cell line maintains a stable undifferentiated phenotype even in 10% serum. Upon addition of appropriate inducers, C1 cells engage at high frequency

(>90%), in a mutually exclusive and synchronous manner, into the osteogenic, chondrogenic or adipogenic programs [61]. For osteogenic differentiation, C1 cells must first form three-dimensional nodules mimicking the *in vivo* mesodermal stem cell condensation required for skeleton development and bone repair. After addition of ß-glycerophosphate (ßGP) and ascorbate, C1 cells reach terminal differentiation within 12 days (Fig. 5). Till day 4 of the osteogenic program, close to 100% C1 aggregates direct the deposition of a profuse type 1 collagen extracellular matrix, consistent with what observed during intramembranous bone formation *in vivo*. At day 7, hydroxyapatite crystals start to be formed and deposited on collagen fibrils surrounding C1 osteoblasts leading to progressive mineralization of the extracellular matrix. At day 12, C1 cells convert into mature osteocyte-like cells embedded in a calcified matrix and cease to divide [62].

Pharmacological studies led to show that a functional 5-HT_{2B}R is implemented at day 5 of the osteogenic program, just before matrix mineralization [63,64]. Of note, the mRNAs encoding 5-HT_{2B}R are already present at the stem cell state in condensing C1 mesoblastic cells and their level do not vary during the 12 day differentiation time course. The selective expression of 5-HT_{2B}R is an intrinsic event of the C1 osteogenic program and requires post-transcriptional regulation mechanisms that have yet-to-be identified. 5-HT_{2B}R signaling optimizes matrix mineralization since blockade of 5-HT_{2B}R by ritanserin from day 5 reduces by 40% Ca^{2+} incorporation within the bone matrix [63], in agreement with the osteopenic phenotype of 5-$HT_{2B}R^{-/-}$ mice [16].

3.2. 5-HT_{2B}Rs are coupled to NO and eicosanoid productions along C1 osteogenic program

From day 5 to terminal differentiation, 5-HT_{2B}Rs exhibit an agonist dependent- and independent-coupling to the nitric oxide (NO) signaling pathway (Fig. 5). The critical role of NO radical in the dynamic process of bone remodeling has been described for more than a

decade [65]. In bone, the endothelial isoform of nitric oxide synthase (eNOS) is constitutively expressed while the inductible NOS (iNOS) is only activated in inflammatory conditions. eNOS knockout mice display osteoporosis, which sustains an anabolic role of NO in bone metabolism [66,67]. Resorptive effects of NO on the skeleton have also been reported [68]. The importance of a tight regulation of NO production for bone homeostasis is further supported by bone defects in patients suffering of inflammatory diseases like rheumatoid arthritis, associated with strong NO production [68].

In addition to its coupling to NOS, the 5-HT_{2B}Rs are coupled to the phospholipase A2 (PLA2)/acid arachidonic (AA) signaling pathway. 5-HT_{2B}R/PLA2 coupling contributes to osteogenic differentiation by controlling the cyclooxygenase (COX) and lipoxygenase (LOX) pathways in a stepwise manner. During the initial phase of mineralization process, *i.e.* from day 5 to day 10, 5-HT_{2B}Rs control a COX-dependent prostaglandin PGE2 synthesis (Fig. 5). At day 10, when osteoblasts are converted into osteocytes, COX activity switches off. 5-HT_{2B}R/PLA2-derived AA becomes substrates for LOX enzymes and osteocytes produce the leukotriene LTB4. As for NO, both PGE2 and LTB4 eicosanoids are known to be involved in the regulation of bone metabolism by exercising either bone-forming and/or bone-resorbing actions [69]. Of note, COX knockout mice display reduced bone formation [70]. NO/PGE2/LTB4 can either act as intra- and/or inter-cellular second messengers. This raises the question of the nature of the osteoblast specific-functions governed by these effectors in bone homeostasis.

3.3. 5-HT_{2B}Rs govern bone mineralization through control of TNAP activity via eicosanoids and phosphatidylinositol-specific phospholipase C

Although not an osteoblast specific enzyme, the tissue-non specific alkaline phosphatase (TNAP) is a key actor in bone mineralization that governs the inorganic phosphate

(Pi)/pyrophosphate (PPi) ions ratio. The essential role of this glycosylphosphatidylinositol (GPI)-anchored enzyme in mineral crystal formation has been evidenced in human patients with TNAP loss-of-function mutation [71] or TNAP knockout mice [72], which are defective for bone and teeth mineralization. Furthermore, transgenic mice, which ectopically express TNAP specifically in the dermis enriched in type 1 collagen, develop pathological skin calcification [73]. The dynamics of the C1 cell differentiation allowed to show that TNAP activity is fully controlled by 5-HT_{2B}R-dependent PLA2 signaling pathway (Fig. 5). As for 5-HT_{2B}R transcripts, TNAP mRNAs are dormant at the stem cell stage and their levels remain constant along the osteogenic program. To date, the signals that turn on 5-HT_{2B}R and TNAP translation at day 5 are still missing. While expressed, TNAP enzyme remains inactive until day 7. Its activation depends on post-translational modifications elicited by 5-HT_{2B}R signaling. 5-HT_{2B}R coupling to PLA2 stimulates phosphatidylinositol-specific phospholipase C (PIPLC) activity, which cleaves the GPI anchor of TNAP and thereby unlocks TNAP activity. Such a functional link between 5-HT_{2B}R, PIPLC and TNAP has also been evidenced in primary calvarial cells [64]. 5-$HT_{2B}R^{-/-}$ osteoblasts also revealed a hitherto-unkown interaction between 5-HT_{2B}Rs and the nuclear PPARβ/δ receptors via prostacyclins [74]. While the functional impact of 5-HT in the skeleton is still debated, the 5-HT_{2B}R plays a key role in mineralization processes.

4. Conclusions and future directions

This review reports some recent aspects of the implication of 5-HT and serotonergic receptors in mineralized tissues homeostasis. As concern dental tissues, tooth decay is one of the widest spread infections around the world (almost 100%). Root canal sealers used for endodontic treatments aim to eradicate bacterial infection and protect tooth against re-contaminations. These filling materials are not designed to preserve and/or mobilize resident pulpal stem cells

for tooth repair. The identification of a dialogue mediated by 5-HT and DA between blood platelets and odontogenic stem cells provides a rational for the development of novel therapeutic strategies targeting pulpal stem cells and therefore enhancing natural tooth repair. Regarding the skeleton, the integrity of this dynamic tissue is ensured by a constant remodeling through the coordinated action of bone-forming osteoblasts and bone-resorbing osteoclasts. Although, it is becoming clear that the 5-HT system contributes to bone metabolism, the action of 5-HT on skeleton homeostasis is still controversed. A genetic approach developed by Yadav et al. revealed a low bone mass phenotype in mice depleted for TPH2, the brain TPH isoform. These data suggest that brain-derived 5-HT contributes to skeleton homeostasis through the hypothalamus by suppression of sympathetic tone [75]. Yadav et al. have also shown that gut-specific TPH1 conditional knockout mice display increased bone formation and high bone mass suggesting an inhibiting effect of circulating 5-HT on bone formation through 5-HT_{1B}Rs [27]. However, others studies reported that adult $TPH1^{-/-}$ mice had no change in bone mass density [76,77]. Further analyses indicate altered bone resorption in $TPH1^{-/-}$ mice due to osteoclastic differentiation defect [77]. Additional works are thus required to better characterize the role of 5-HT in bone metabolism taking into account the relative affinity of serotonergic receptors for 5-HT and the diversity of their couplings. Among the 5-HT receptors, the 5-HT_{2B}R exerts a pivotal role in mineralization processes by controlling TNAP activity through its PLA2/eicosanoids/PIPLC coupling. Several lines of evidence support the notion that physiological and pathological mineralizations mobilize similar pathways [78]. Currently, molecular mechanisms involved in ectopic mineralization of soft tissues are unsolved. Of note, deregulations of PGE2 and LTB4 levels have been associated with pathological situations such as bone fracture, rheumatoid arthritis, osteoarthritis, osteoporosis [69]. Whether alteration of 5-HT_{2B}R signaling would drive ectopic pathological calcifications deserves further investigations.

Conflicts of interest

The authors declare no conflict of interest.

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Figure legends

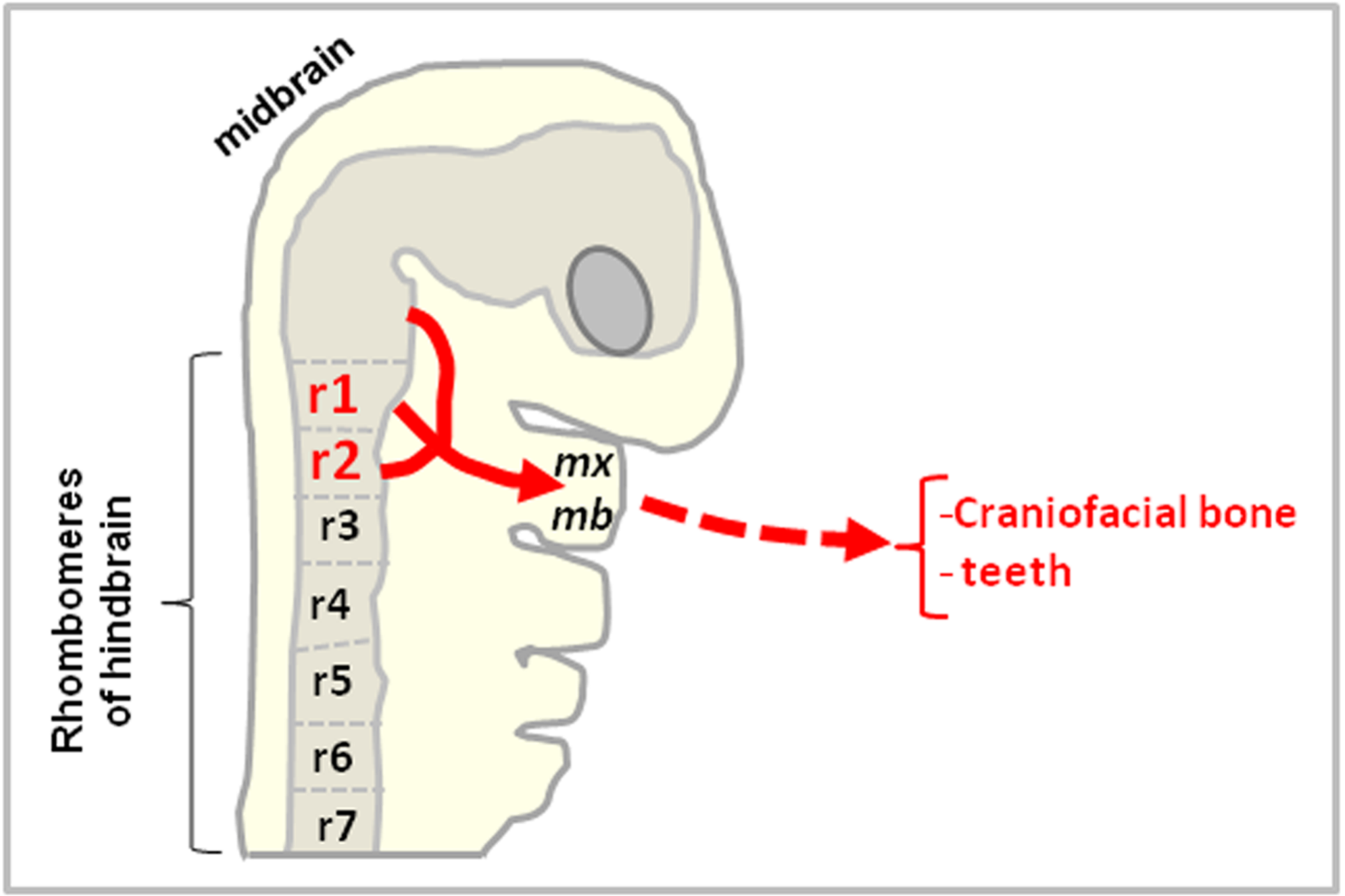
Fig. 1. Schematic representation of the migration of neural crest cells from the rostral hindbrain and caudal midbrain to reach the first branchial arch at the origin of teeth and mandibular (mb) and maxillary (mx) bones.

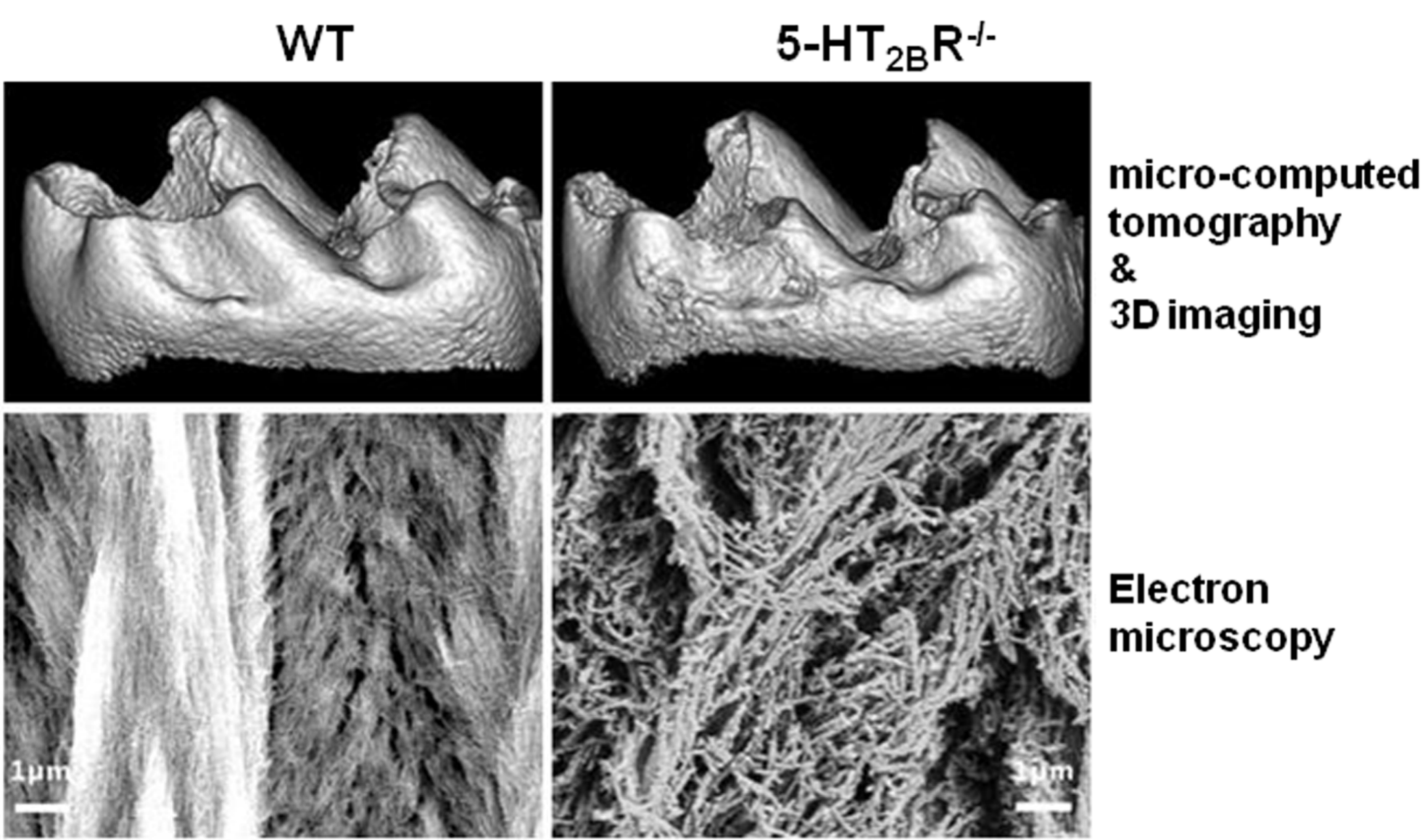
Fig. 2. Enamel structural defects in molar of 5-HT_{2B}R knockout mice observed by micro-computed tomography and electron microscopy analysis.

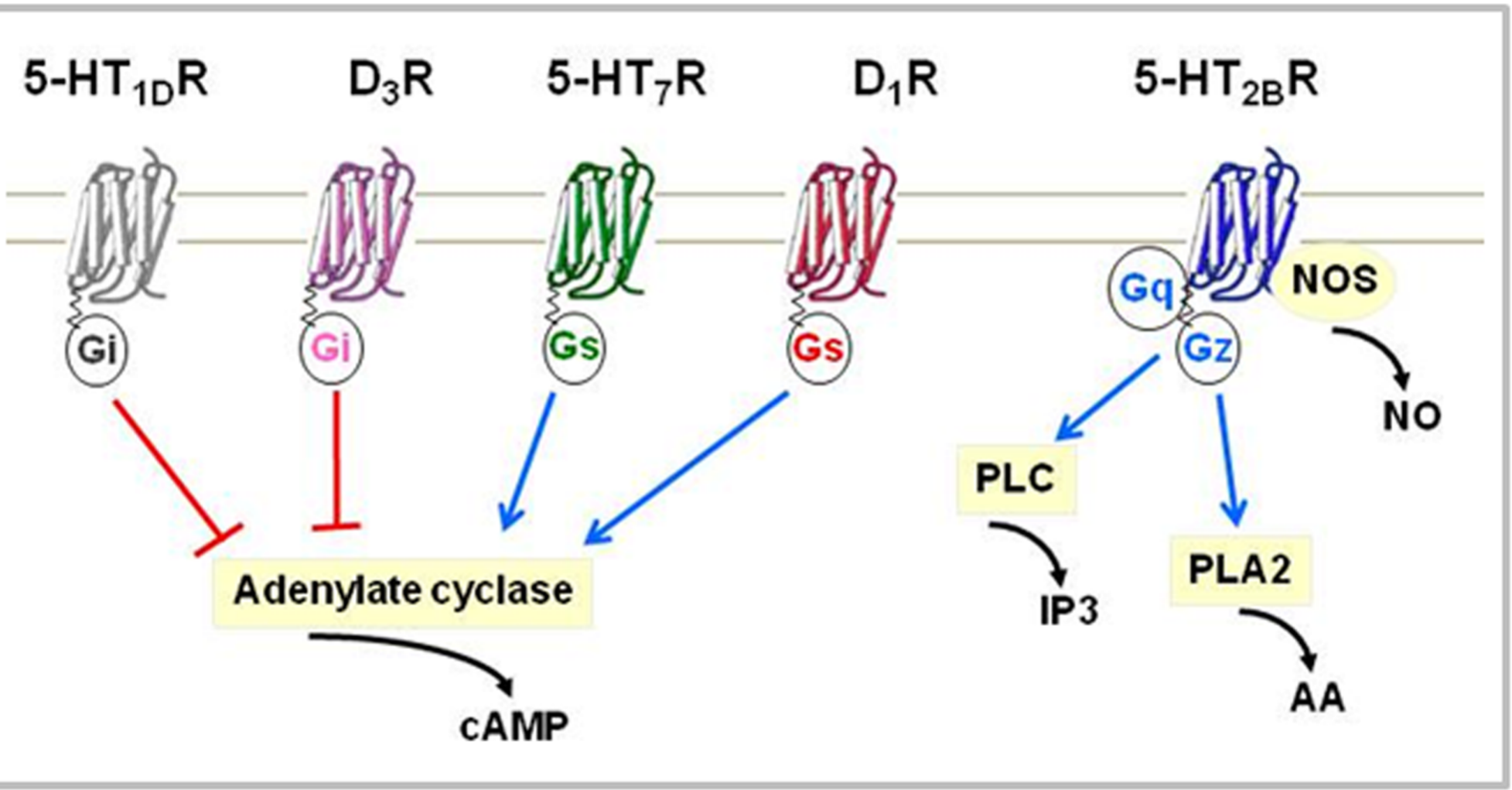
Fig. 3. 5-HT and DA receptors present at the surface of odontogenic stem cells and their couplings.

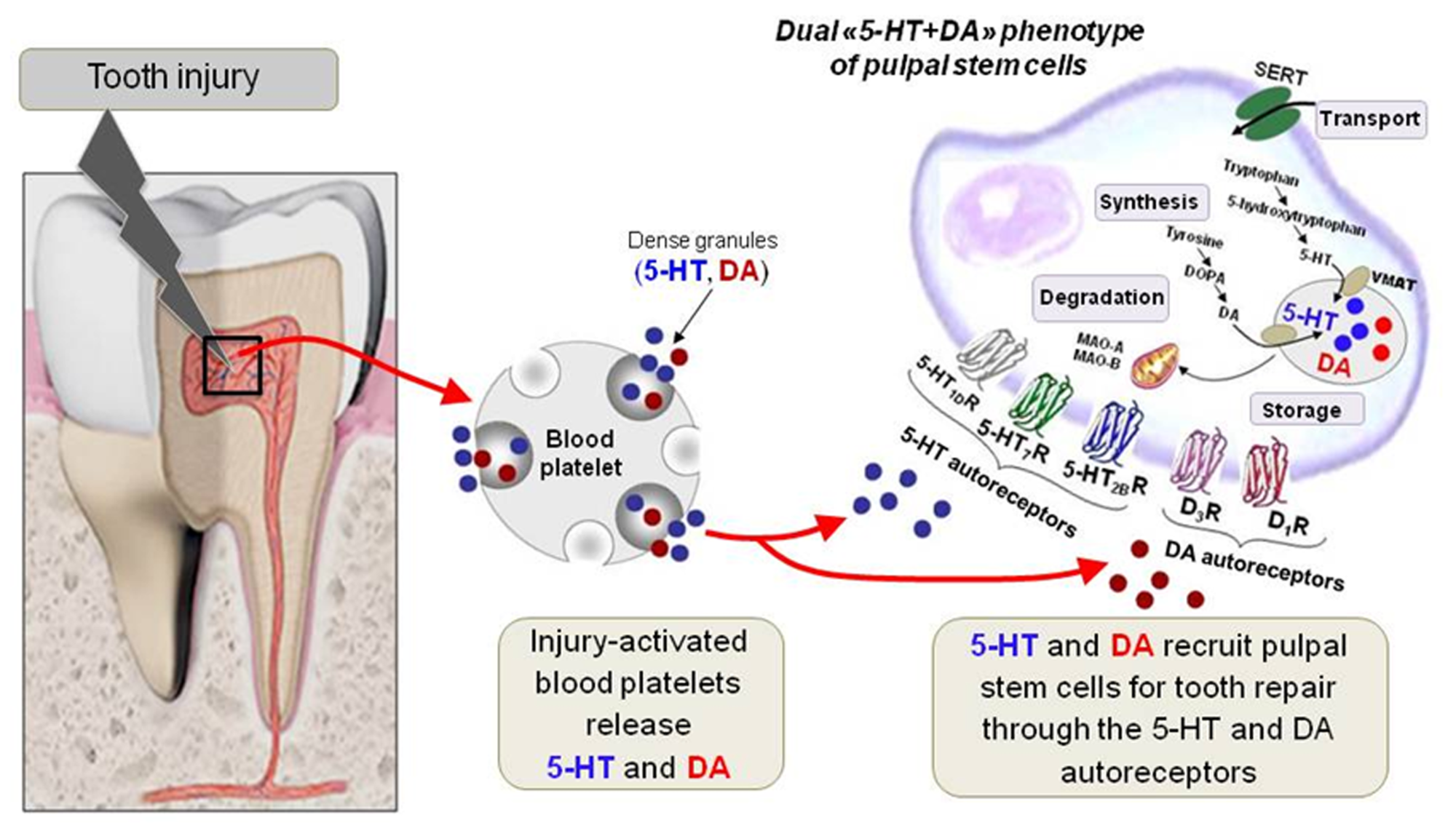
Fig. 4. 5-HT and DA co-released by activated platelets upon tooth injury are "warning" signals that mobilize pulpal stem cells for tooth repair through 5-HT/DA receptors.

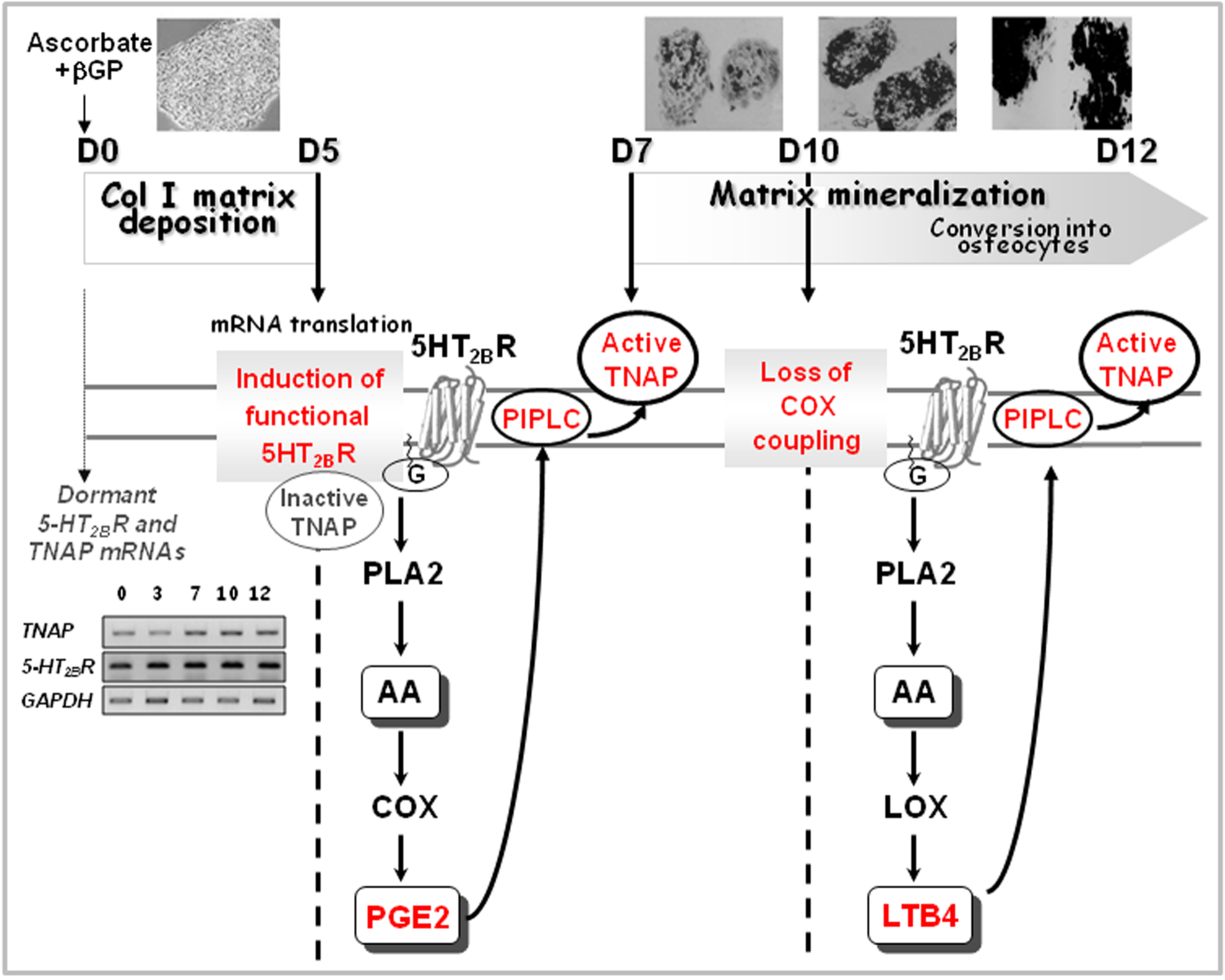
Fig. 5. Diagram summarizing the regulation of TNAP activity by 5-HT_{2B}R through its PLA2/eicosanoids/PIPLC coupling during osteogenic differentiation.


midbrain
r1
r2
r3
r4
r5
r6
r7
Rhombomeres of hindbrain
mx
mb
-Craniofacial bone
- teeth


WT
5-HT2BR-/-
micro-computed tomography & 3D imaging
Electron microscopy
1µm
1µm


5-HT1DR
D3R
5-HT7R
D1R
5-HT2BR
Gi
Gi
Gs
Gs
Gq
Gz
NOS
NO
PLC
IP3
PLA2
AA
Adenylate cyclase
cAMP


Tooth injury
Dense granules
(5-HT, DA)
Blood platelet
Injury-activated blood platelets release 5-HT and DA
Dual «5-HT+DA» phenotype of pulpal stem cells
SERT
Transport
Tryptophan
5-hydroxytryptophan
Synthesis
Tyrosine
DOPA
5-HT
VMAT
Degradation
DA
5-HT
DA
MAO-A
MAO-B
Storage
5-HT1DR
5-HT7R
5-HT2BR
5-HT autoreceptors
D3R
D1R
DA autoreceptors
5-HT and DA recruit pulpal stem cells for tooth repair through the 5-HT and DA autoreceptors


Ascorbate
+βGP
D0
D5
D7
D10
D12
Col I matrix deposition
Matrix mineralization
Conversion into osteocytes
mRNA translation
Induction of functional 5HT2BR
5HT2BR
Active TNAP
PIPLC
Loss of COX coupling
5HT2BR
Active TNAP
PIPLC
G
G
Inactive TNAP
Dormant 5-HT2BR and TNAP mRNAs
0 3 7 10 12
TNAP
5-HT2BR
GAPDH
PLA2
PLA2
AA
AA
COX
LOX
PGE2
LTB4