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1. PREIS GRUNDLAGENFORSCHUNG

# Odontoblast TRPC5 channels signal cold pain in teeth

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## Introduction

Teeth are composed of many tissues, and they become extremely cold sensitive when inflamed. However, the mechanisms of this cold sensation are not understood.

In the skin, TRPM8 and TRPA1 represent the key sensors of environmental cooling as well as painful cold. TRPC5 is cold sensitive, but no such function has yet been ascribed in native cells.

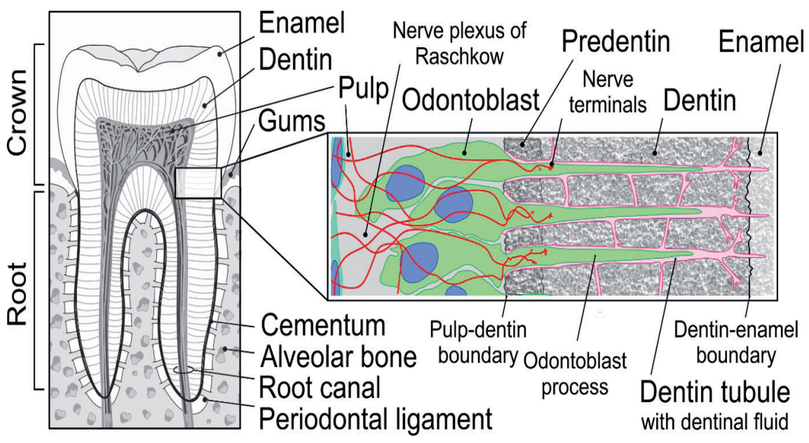
Here, we set out to understand the roles of TRPA1, TRPM8, and TRPC5 ion channels in cold sensing in teeth.

## Conclusions

- The restricted TRPC5 expression pattern in the odontoblastic layer, block of responses in genetically modified mice, and extracellularly recorded TRPC5 cold sensing indicate an essential sensory receptor function for the odontoblasts in tooth cold sensing.
- TRPC5 is a cold sensor in healthy teeth and, with TRPA1, is sufficient for cold sensing. Under human inflammatory conditions with injured or patent tooth pulp, TRPC5's expression increases in sensory axons.

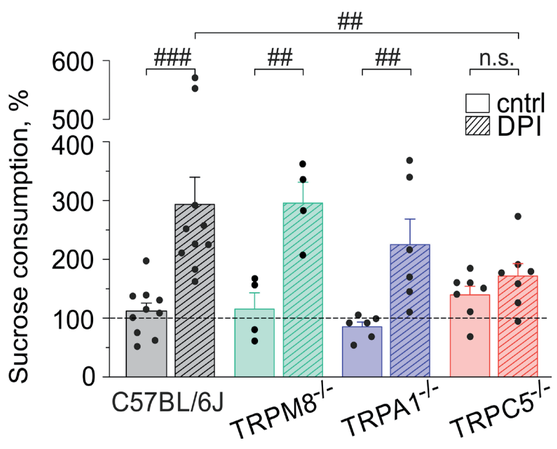
## Results

### *Figure 1. Teeth are complex organs.*



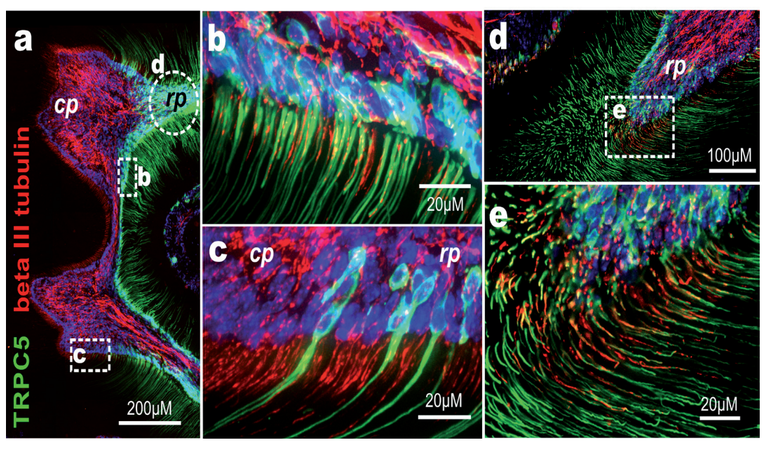

The highly mineralized surface enamel that acts as a barrier. The layer underneath the enamel is called dentin, which is a dense extracellular calcium matrix formed and maintained by odontoblasts, a post-mitotic cell layer located at the outer surface of the soft tooth pulp.

### *Figure 2. TRPC5 is essential for inflammatory tooth pain.*



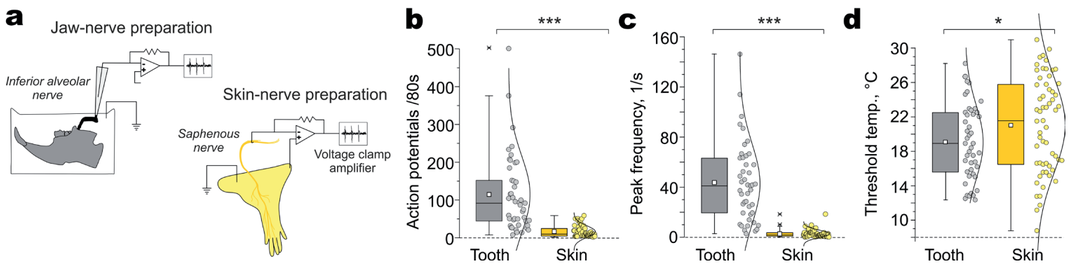

An increase in sucrose consumption was previously described as a major sign of painful dental pulp injury (DPI) in mice (Gibbs et al 2013). We studied percent change in sucrose consumption relative to baseline (dashed line) after DPI. Percent change in sucrose consumption relative to baseline (dashed line) after dental pulp injury (DPI). DPI enhanced the consumption of 5% room temperature sucrose water to 293 ± 46% above baseline. Lack of TRPC5 (171 ± 21%), but not TRPM8 (299 ± 34%) or TRPA1 (225 ± 43%), reverts sucrose consumption. Sucrose consumption in TRPC5$^{-/-}$, but not TRPA1$^{-/-}$ or TRPM8$^{-/-}$, was not different from the respective controls without DPI.

### *Figure 3. TRPC5 channels are located in the odontoblast layer.*



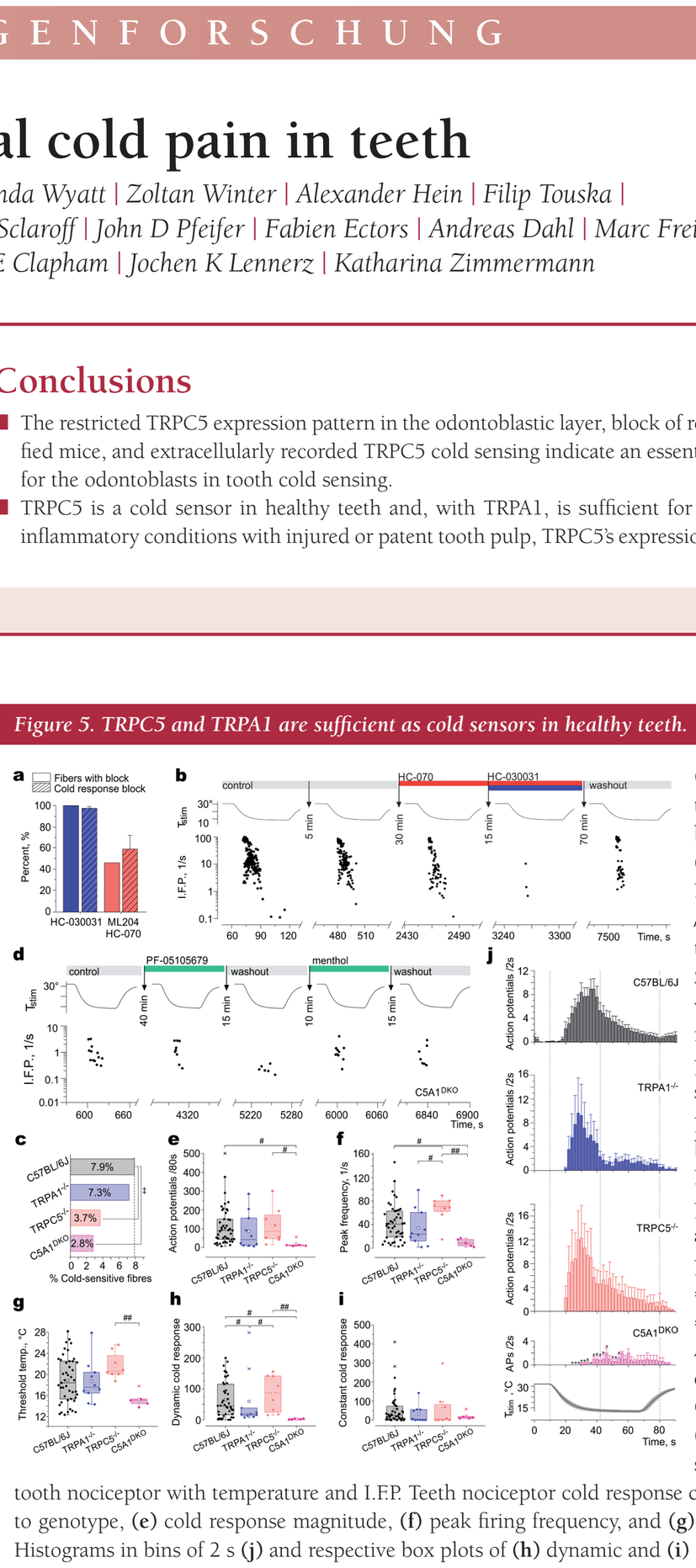

**(a)** TRPC5 reporter mouse molar tooth whole mount with densely packed TRPC5+ odontoblasts at the pulp-dentin boundary. **(b and c)** In tight association with sensory nerves. Green, TRPC5; red, β III-tubulin; circle indicates area shown in **(d)** and **(e)** from a subsequent section. cp, coronal pulp; rp, radicular pulp.

### *Figure 4. Tooth nociceptor cold responses are much larger than skin cold nociceptor responses.*



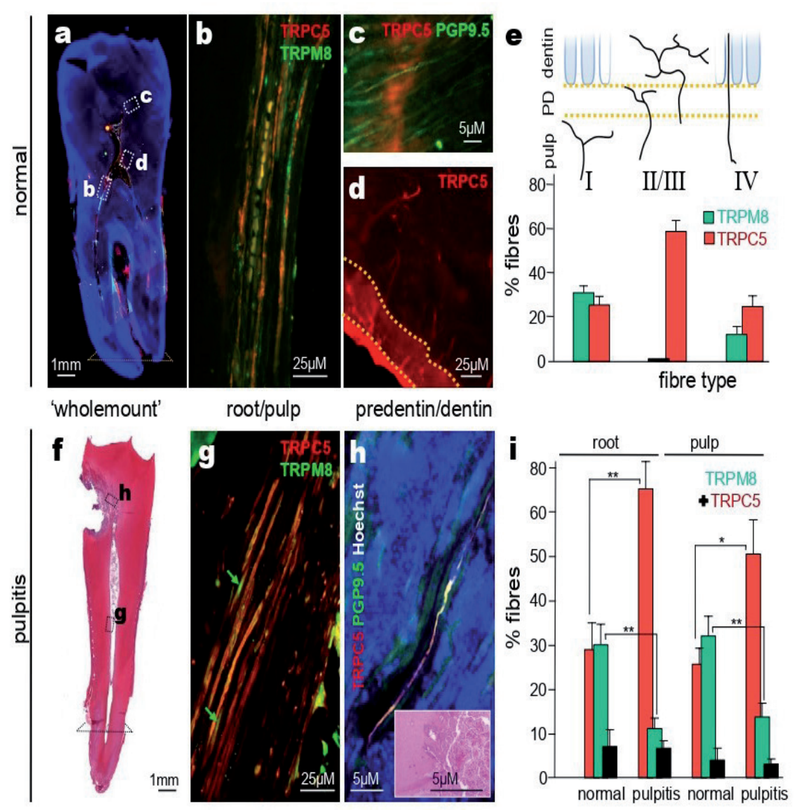

**(a)** Schematic illustration of extracellular recordings from jaw-nerve as compared to skin-nerve preparations. Comparison of the **(b)** cold response magnitude, **(c)** peak frequency, and **(d)** threshold temperature of C57BL/6J teeth (n = 45) and skin (n = 59) nociceptors. Statistical significance by a two-sided Student's t test. Skin nociceptor cold responses are from (Zoltan et al. 2017; Veter et al. 2013) and refer to the same background strain at equivalent stimulus conditions.

### *Figure 5. TRPC5 and TRPA1 are sufficient as cold sensors in healthy teeth.*




**(a)** Percent cold-sensitive tooth nociceptors blocked by HC-030031 (n = 9/9 fibers) and ML204/HC-070 (n = 6 /13) and respective fraction of block (means ± SEM). **(b)** C57BL/6J wt tooth nociceptor recording with temperature (top) and instantaneous frequency pattern (I.F.P.; bottom) blocked by HC-070 and HC-030031. Circles represent action potentials. **(c)** Tooth cold responses in TRPA1$^{-/-}$ (n = 10/138), TRPC5$^{-/-}$ (n = 8/217), and TRPC5/A1-DKO (n = 5/177), chi-square tests versus C57BL/6J (n = 45/570). **(d)** Typical cold response of a TRPC5/A1-DKO tooth nociceptor with temperature and I.F.P. Teeth nociceptor cold response characteristics according to genotype, **(e)** cold response magnitude, **(f)** peak firing frequency, and **(g)** temperature threshold. Histograms in bins of 2 s **(j)** and respective box plots of **(h)** dynamic and **(i)** constant cold responses (one-way ANOVA).

### *Figure 6. TRPC5 is expressed in normal human teeth and increases with pulpitis.*




**(a)** Whole-mount panography of a human tooth; regions evaluated for TRPC5 expression are indicated; dotted line, level used in root measurement. **(b)** TRPC5+ nerve fibers in the radicular pulp double labeled with TRPM8. **(c)** TRPC5+ nerve fibers double labeled with PGP9.5 within the inner third of the dentinal tubules (type IV) ~40 µm above the odontoblast-predentinal border. **(d)** TRPC5+ branched fibers (type II/III) in the predentin/dentin layer. **(e)** Quantification of TRPC5+ and TRPM8+ fiber types (four teeth). **(f)** Hematoxylin and eosin (H&E)–stained human tooth whole mount with degenerated dentin (caries) and pulpitis. **(g)** Abundant TRPC5+ and decreased TRPM8+ (arrows) nerve fibers in the pulpitic tooth root. **(h)** TRPC5+ nerve fibers (type IV) in degenerating dentin (inset, H&E). **(i)** Increased TRPC5+, decreased TRPM8+, and similar proportions of colabeled fibers (black) in normal versus pulpitic tooth roots and in the tooth pulp.

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