Molecular, cellular and behavioral changes associated with pathological pain signaling occur after dental pulp injury.

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Supplementary Figures 1-3



Supplementary Figure 1:

Supplementary Figure 1: Histology of tooth and dental pulp after DPI procedure. A) N52 immunostaining of mouse maxilla, seven days after DPI. Two teeth are shown in this image. M1: First molar, which underwent pulp exposure (PE). Anterior-posterior width of tooth is demonstrated with a bracketed line. The size of the PE is shown with the pointed bar. Below the PE is necrotic tissue/debris in the pulp chamber (NP). The most posterior part of the pulp chamber has vital tissue, which is magnified in B. B) Very little N52 stained fibers present here, demonstrating die-back of pulpal afferents in response to injury from the pulp exposure. M2: Second molar, which had no pulp exposure, and shows what a normal density of staining looks like. C)Enlarged image of the pulp space in M2 showing normal afferent density in an uninjured pulp. D) H&E staining of a the first maxillary molar, 7 days after DPI. Most of the pulp tissue in the core pulp chamber is gone, but some tissue is remaining in the anterior root, highlighted with an arrow.

Supplementary Figure 2



Supplementary Fig 2: Effect of i.p. gabapentin or vehicle on DPI and DPI sham mice 21-Days after injury. Mice received i.p. gabapentin (10 mg/kg) the night before and morning of the sucrose consumption test. The percent change from baseline was calculated for each animal, where the baseline included the last 3 sucrose consumption tests before the drug administration test. The mean % change by group is plotted. There was no observable effect of gabapentin relative to vehicle treatment, but rather it appeared that mice with DPI decreased their sucrose consumption regardless of receiving active drug or vehicle (2-way ANOVA: Injury: p=0.02, F=6.1, DOF=1; Drug: p=0.9; Interaction: p=0.4). n=6-8 mice per group, results compiled from 2 experiments.

Supplementary Figure 3:



Supplementary Figure 3: Effect of DPI and MNI on daily changes in body weight. DPI mice differed from DPI sham mice in the daily weight changes over time, while MNI mice did not differ from MNI sham A) The mean daily weight change was calculated for each treatment group at various time points after injury (2-way RM ANOVA Interaction: p=0.04 F=2.2 DOF=8; Injury: p=0.9 Time: p=<0.0001 F=6.3 DOF=8) n=4-5 per group. B) The mean daily weight change of mice receiving MNI did not differ over time from MNI sham (2- way RM ANOVA Interaction: p=0.3; Injury: p=0.7; Time: p=<0.0001) n=5/group. C) The daily weight change was calculated for each mouse and combined over post-injury days 2 and 4 and averaged by treatment group. Overall, a significant effect of group assignment was found (1-way ANOVA p=0.04, F=3.8, DOF=3). With post-hoc analyses, DPI mice were found to differ significantly from DPI sham, but not from MNI or MNI sham mice (Newman-Keuls Multiple Comparison Test, p=<0.05).