Supporting Information

Dimitrov et al. 10.1073/pnas.1306342110

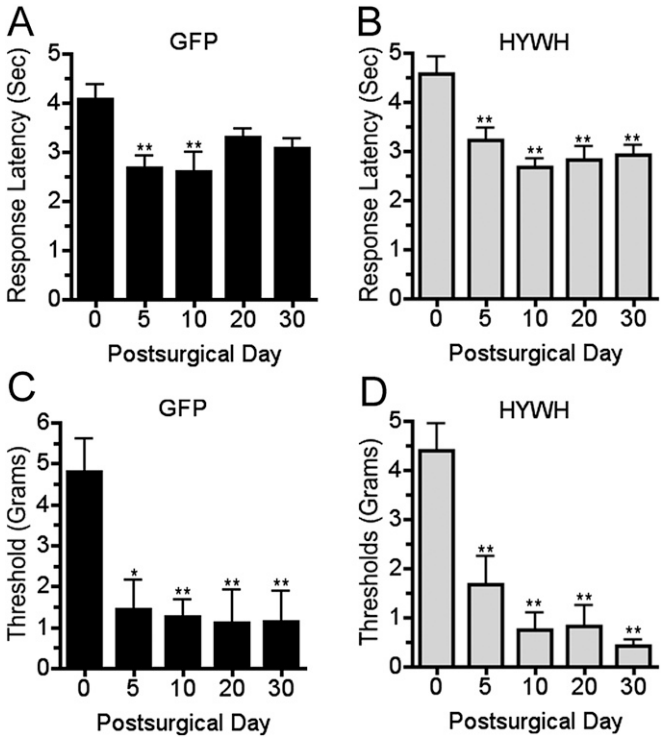

Fig. S1. Amygdalar injection of virus encoding a secreted PTH2-R antagonist does not affect development of hypersensitivity in mice with PNL. The central amygdaloid nucleus was targeted with a lentivirus encoding the secreted PTH2-R antagonist (HYWH-lenti). PNL was performed 3 wk after virus injection and thermal (*A* and *B*) and tactile (*C* and *D*) sensitivity assessed over time. $*P < 0.05$, $**P < 0.01$.

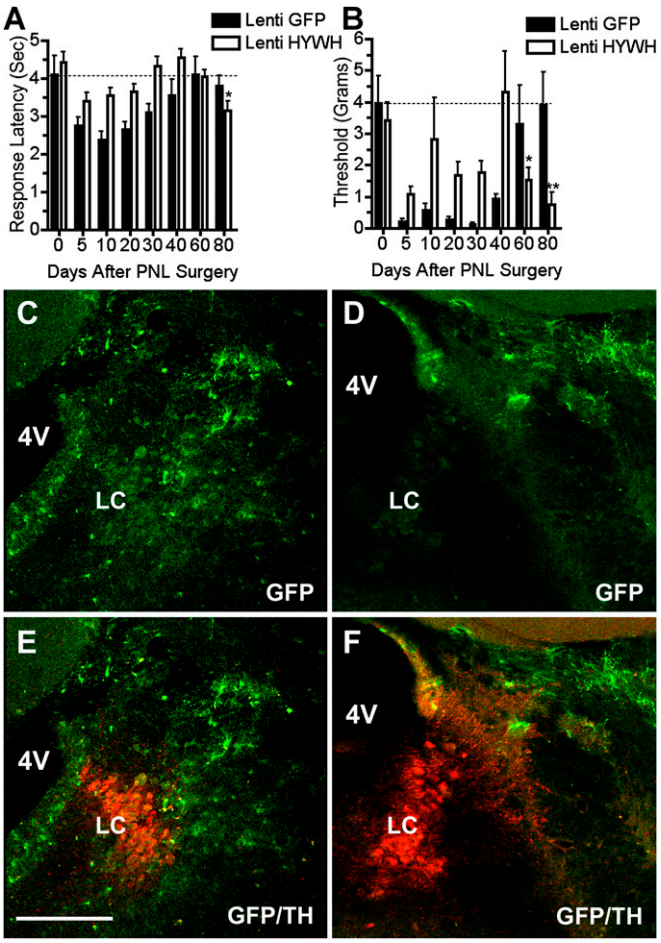

Fig. S2. Hypoalgesia of mice with brainstem injection of PTH2-R antagonist encoding virus is reversed at later time points. Full-time courses for the data in Fig. 5, and GFP expression in the area of HYWH-lenti injection, are shown. HYWH-lenti–injected mice developed significant thermal hypersensitivity (*A*) and mechanical allodynia (*B*) between the 60th and 80th postsurgical days, the last time point examined. The expression of GFP, a marker for ongoing lentiviral-encoded protein synthesis, was decreased after 80th postsurgical day (over 100 d after the viral injection into the LC area; *D* and *F*). Lentiviral GFP expression was greater 15 d after injection into locus ceruleus (LC) area (*C* and *E*). Noradrenergic LC neurons are labeled with anti-tyrosine hydroxylase antibody and red secondary antibody, GFP-ir is green. 4V, forth ventricle. $*P < 0.05$, $**P < 0.01$. (Scale bar: 200 μm.)

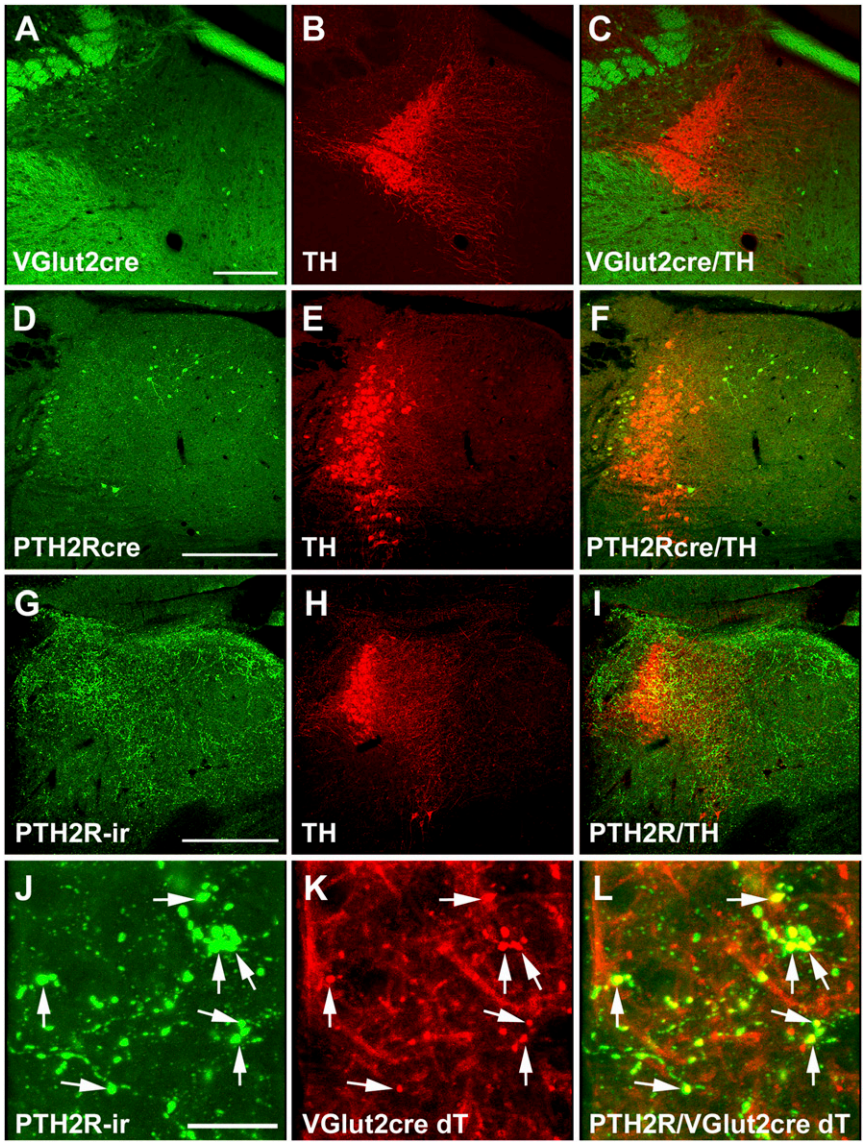

Fig. S3. Colocalization of PTH2R and glutamatergic markers in the LC area. Immunolabeling of tyrosine hydroxylase was performed on sections from mice that contain a fluorescent marker in VGlut2- or PTH2R-expressing neurons, or as double labeling with an antibody to the PTH2R, and PTH2R immunolabeling was performed in sections from mice with a VGlut2 marker. *A–C* show the expression of tdTomato by VGlut2-cre–positive neurons (presented in green) in the LC dendritic areas, as well as a few among the TH-ir cells of the densely populated LC core. *D–F* show PTH2R-cre cells with a distribution similar to the VGlut2-cre cells. *G–I* show the expression of PTH2R-ir fibers overlapping TH-ir cell bodies in the LC core and TH-ir processes in the LC dendritic area. High magnification images in *J–L* show colocalization of PTH2R-ir with tdTomato that marks processes of VGlut2 cells in the LC area. Arrows indicate regions of colocalization between PTH2R-ir and tdTomato expressed by VGlut2 neurons. (Scale bars: *A–I*, 200 μm; *J–L*, 20 μm.)

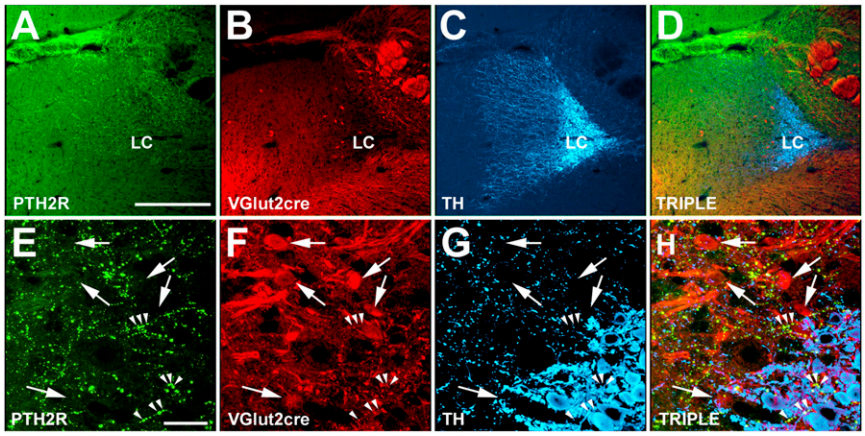

Fig. S4. PTH2R immunoreactive fibers and VGlut2 marker containing fibers appear colocalized in and near the LC. *A* shows PTH2R-ir fibers, *B* shows fluorescent protein expressed by VGlut2 neurons, *C* shows TH-ir, and *D* is a merged image of the PTH2R/VGlut2/TH from the triple labeling experiment. *E–H* are higher magnification images from part of the field above to show the relationship between glutamatergic fibers expressing PTH2R-ir and noradrenergic neurons. Arrows point to VGlut2-expressing cells close to TH neurons, and arrowheads point to PTH2R/VGlut2 fibers penetrating the LC proper. (Scale bars: *A–D*, 200 μm; *E–H*, 20 μm.)

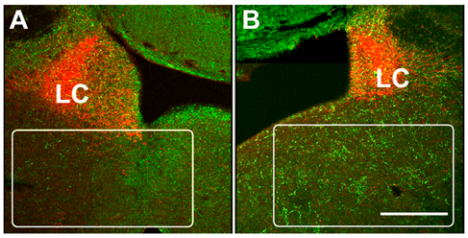

Fig. S5. PTH2-Rs are expressed by glutamatergic neurons near the LC. A Vglut2-ires-Cre mouse was injected near the LC on one side with AAV-FLEX-DTR and then administered diphtheria toxin. Three weeks after diphtheria toxin administration, sections through the LC area were labeled with antibodies to tyrosine hydroxylase (red) and the PTH2-R (green). The white rectangle encloses equivalent areas on the injected (*A*) and noninjected (*B*) sides. Note the decrease in PTH2-R-ir on the virus-injected side. (Scale bar: 100 μm.)

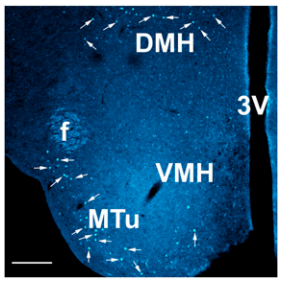

Fig. S6. Barley lectin transfers to hypothalamic cells following expression by LC area glutamatergic neurons. BL-ir in the hypothalamus is shown following LC-targeted injection of virus encoding Cre-dependent lectin. The section is from the animal with the injection shown in Fig. 6. Arrows point to numerous BL-ir–labeled neurons. 3V, third ventricle; DMH, dorsomedial hypothalamic nucleus; f, fornix; MTu, medial tuberal nucleus; VMH, ventromedial hypothalamic nucleus. (Scale bar: 200 μm.)