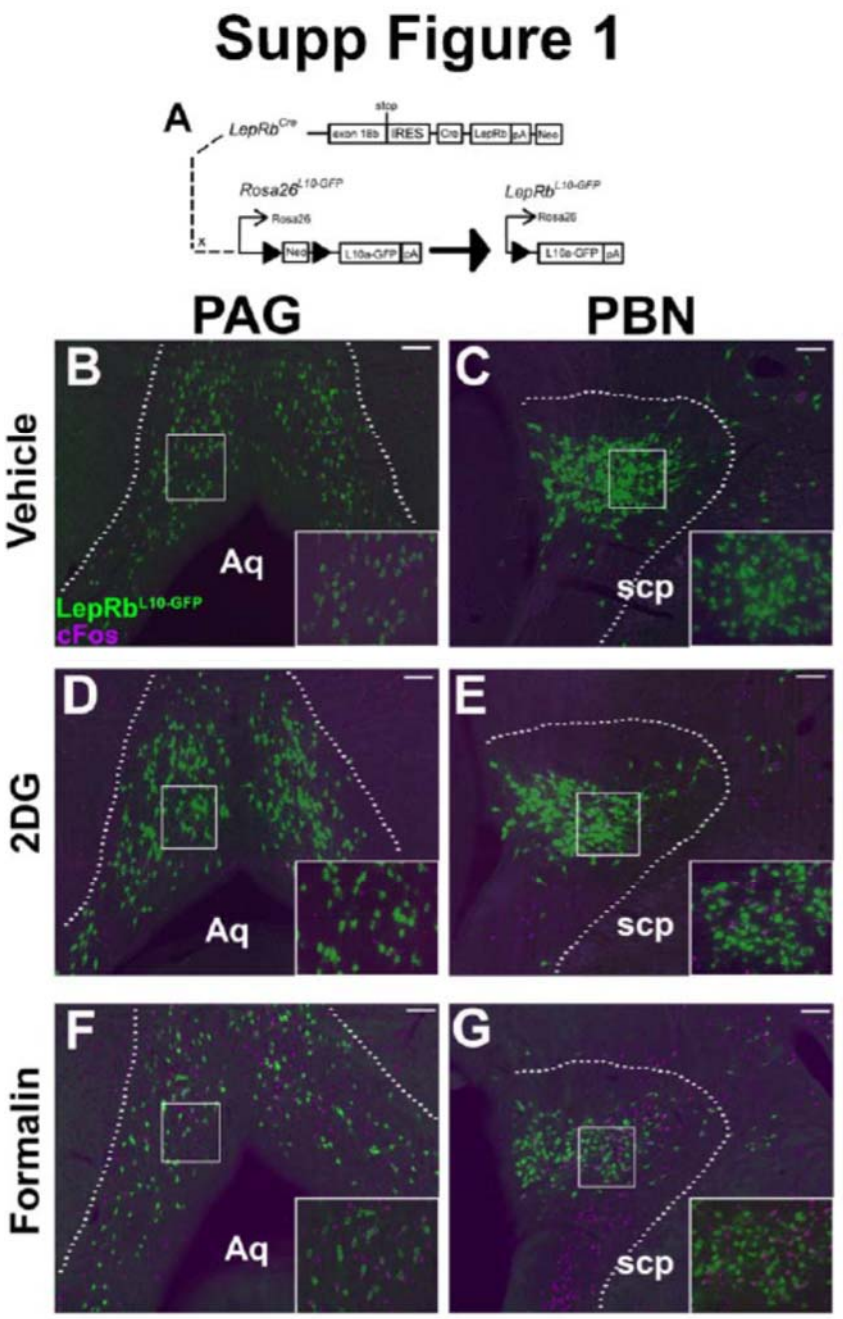
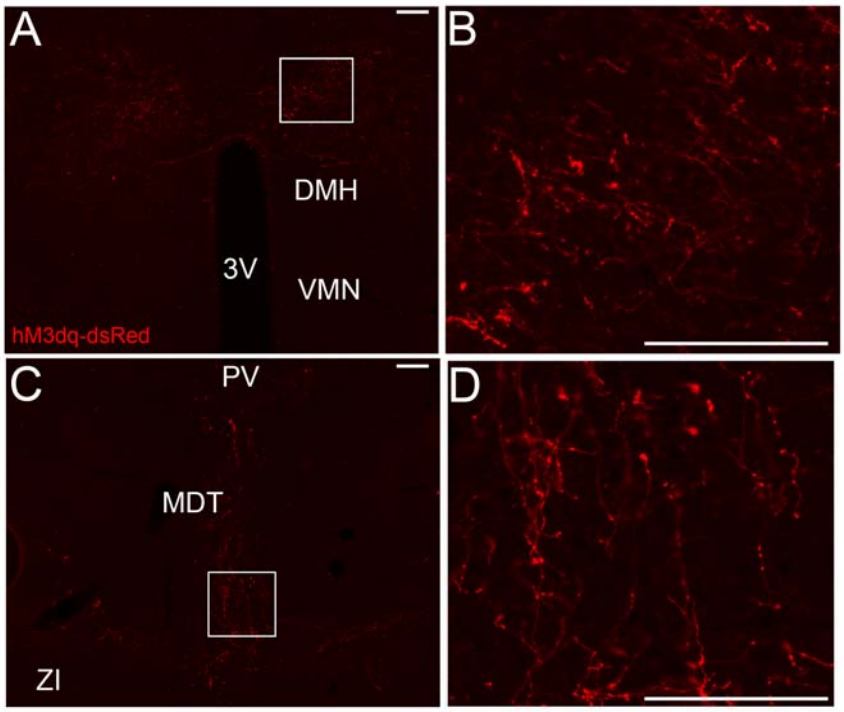
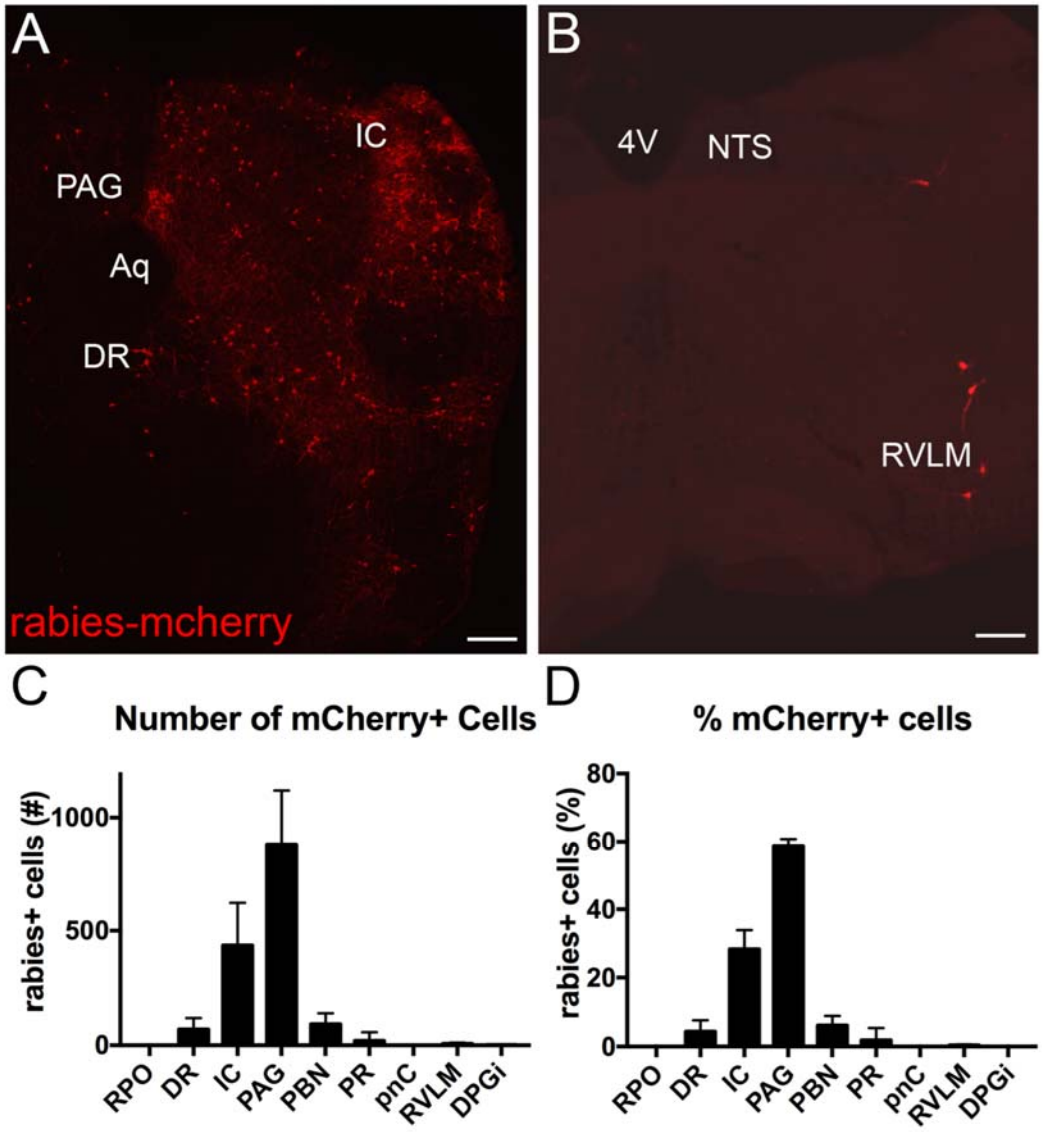


Supplemental Figure 1. Noxious stimuli activate PAG^{LepRb} and PBN^{LepRb} neurons. (A) LepRb$^{eGFP\text{-}L10a}$ mice were treated with vehicle (B,C), intra-hindpaw formalin (D,E) (n=4 (veh) ,5 (form)), or intra-peritoneal 2-dg (F,G) (n=5 (veh), 4(2dg)) and perfused two hours later. Brains were sectioned and stained for cFos (purple) and GFP (green). Images shown (B-F) are representative of 4-5 similar cases for each group. Dashed lines denote the boundaries of each region. The boxes demonstrate the location of the included zoomed image. Scale bar =100 μm. PAG=Periaqueductal grey, Aq=Cerebral Aqueduct, PBN=Parabrachial Nucleus, scp=superior cerebellar peduncle. Images are less magnified versions of those shown in Figure 1 in the main text.



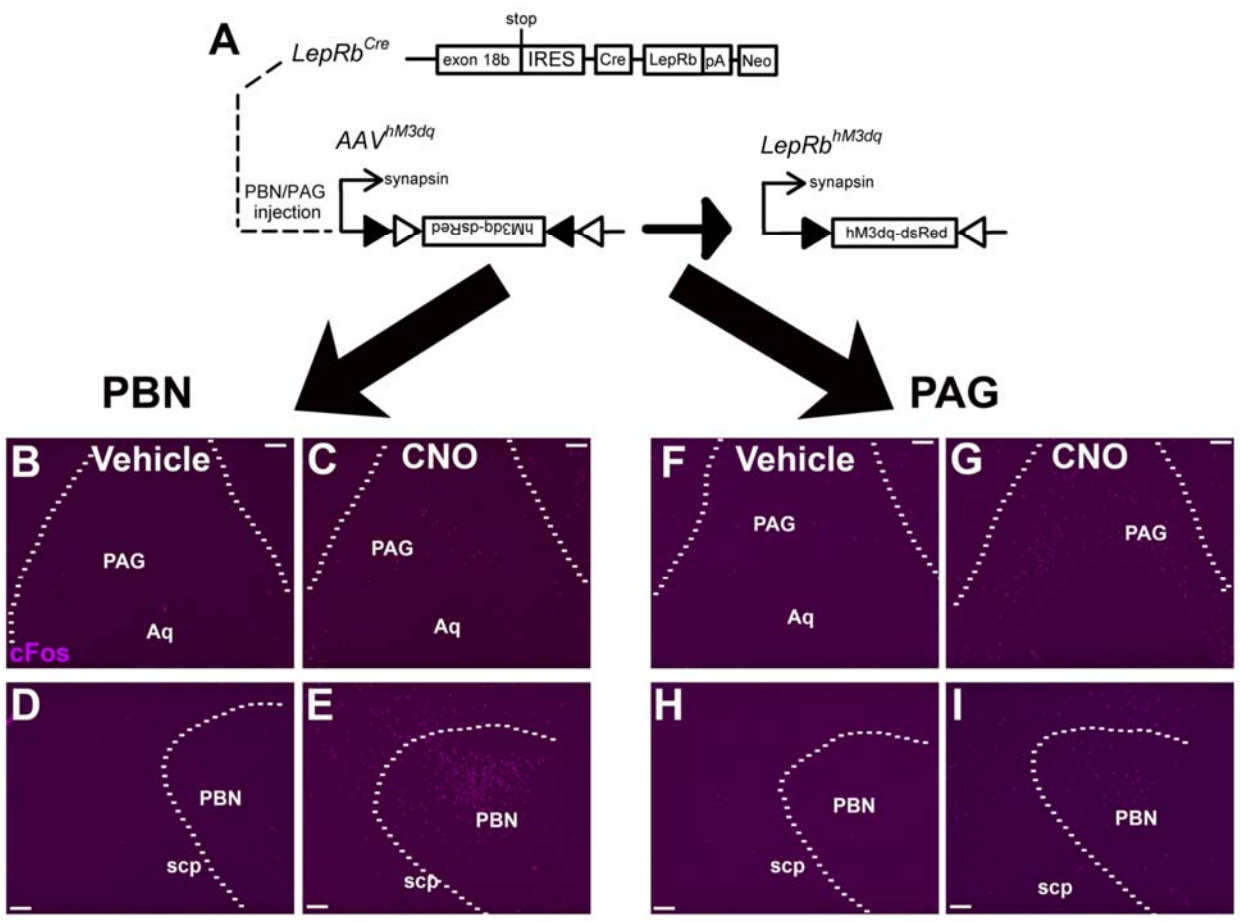
Supplemental Figure 2:. Additional projections from PAG^{LepRb} neurons. (A) In addition to the projections from LepRb^{PAG} cells to the PBN, injecting syn-gfp into the PAG of *Lepr*$^{cre/cre}$ mice revealed GFP (green) fibers in the dorsal component of the dorsomedial hypothalamus (A,B) and mediodorsal thalamus (C,D). Images are representative of four similar cases. Boxes within (A) and (C) denote the locations of the zoomed images in (B) and (D), respectively. Scale bar =100 μm. DMH=dorsomedial hypothalamus, VMN=ventromedial hypothalamic nucleus, ARC=arcuate nucleus of the hypothalamus, MDT=mediodorsal thalamus, PV=paraventricular thalamus, ZI=zona incerta.

Supp Figure 3



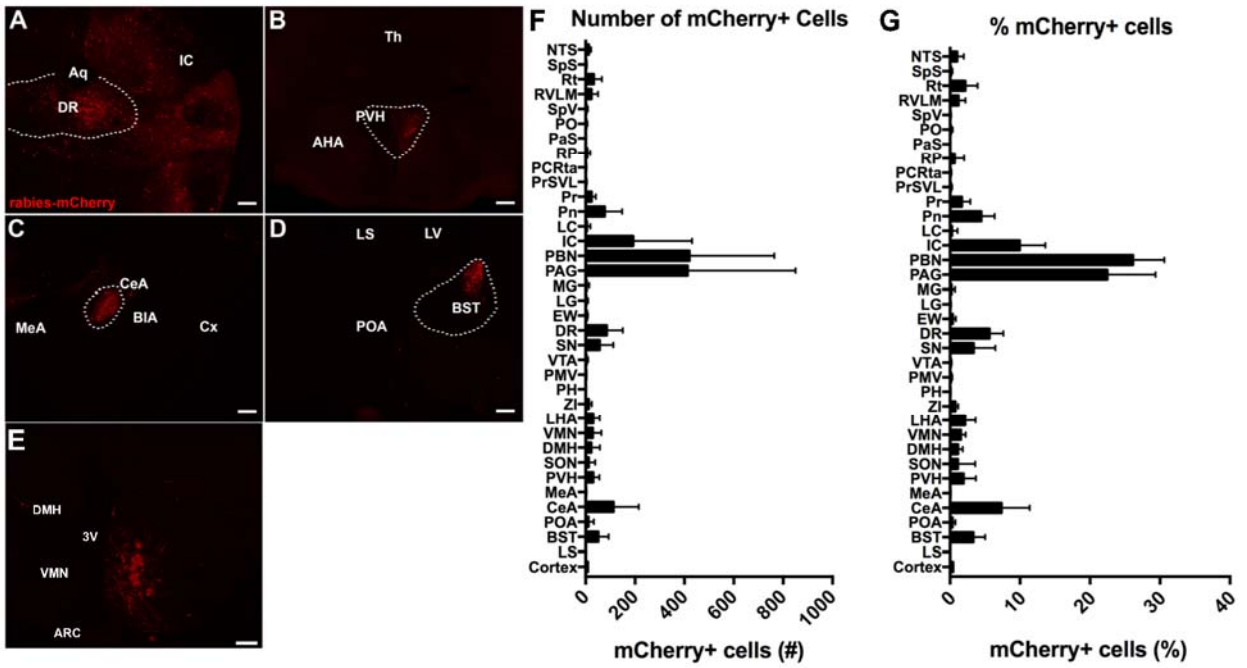
Supplemental Figure 3. PAG^{LepRb} neurons receive projections from neurons within neighboring midbrain sites and the RVLM. (A) After injecting the rabies helper virus in the PAG and the rabies-mCherry virus into the PBN of *Lepr*$^{cre/cre}$ mice, we observed mCherry-positive (red) afferents of PBN-projecting PAG^{LepRb} neurons within neighboring sites (including the dorsal raphe) (A), and also in the RVLM (B). Images are representative of four similar cases. Rabies-mCherry-expressing cells were quantified throughout the brain and are plotted as number of cells per region (C) or percent of total cells counted that lie in each region (D). Scale bar =100 μm. Data are plotted as mean +/-SEM; n= 4. PAG=Periaqueductal grey, IC=Inferior Colliculus, Aq=Cerebral Aqueduct, DR=Dorsal Raphe, NTS=Nucleus of the Solitary Tract, 4V=Fourth Ventricle, RVLM=Rostral Ventrolateral Medulla.

Supp Figure 4



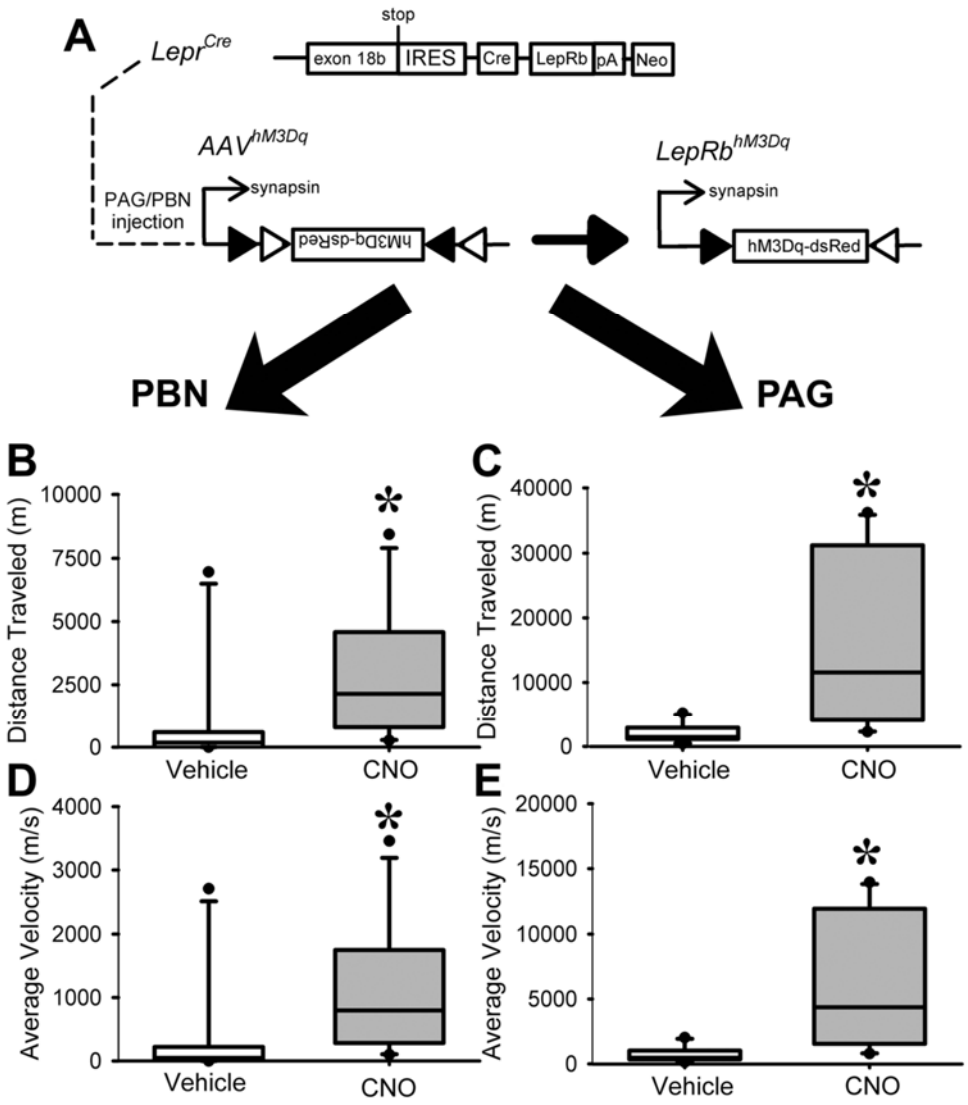
Supplemental Figure 4. hM3Dq-mediated activation of PAG^{LepRb} neurons increases PBN cFos. (A) After injecting AAV-hM3Dq in either the PAG or the PBN of *Lepr^{cre}* mice, we treated these mice with either CNO or vehicle. After two hours, the mice were perfused; brains were sectioned and stained for cFos (purple). Representative images of the PAG and PBN regions are shown (B-I); each image is representative of at least four similar cases. Dashed lines indicate boundaries of each region. Scale bar =100 μm. PAG=Periaqueductal grey, Aq=Cerebral Aqueduct, PBN=Parabrachial Nucleus, scp=superior cerebellar peduncle.

Supp Figure 5



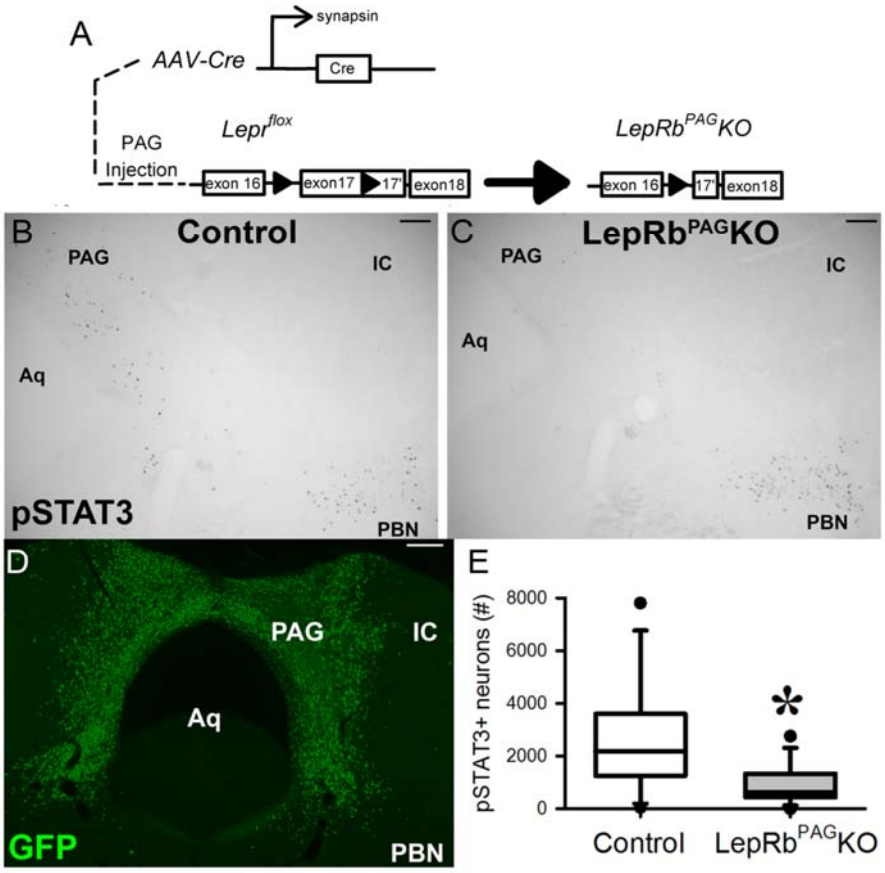
Supplemental Figure 5. VMN-projecting LepRb^{PBN} neurons receive projections from several distal sites. (A) After injecting the rabies helper virus (TVA+G) into the PBN and rabies-mCherry into the VMN of *Lepr*^{cre} mice, the mice were perfused under anesthesia. The brains were sectioned and stained for dsRed (red). Dashed lines demonstrate the boundaries of each area. We observed mCherry cells in the dorsal raphe (A), paraventricular nucleus of the hypothalamus (B), central nucleus of the amygdala (C), and bed nucleus of the stria terminalis (D). Panel E shows a representative VMN injection site, including non-specific fluorescence due to inflammation plus a few mCherry-positive cells. Rabies-mCherry-expressing cells were quantified throughout the brain and are plotted as number of cells per region (F) or percent of total cells counted that lie in each region (G). Data are plotted as mean +/-SEM; n= 10. Scale bar = 100 μm. IC=Inferior Colliculus, Aq=Cerebral Aqueduct, DR=Dorsal Raphe, Th=Thalamus, PVH=Paraventricular Nucleus of the Hypothalamus, AHA=Anterior Hypothalamic Area, MeA=Medial Amygdala, CeA=Central Amygdala, BlA=Basolateral Amygdala, Cx=Cortex, LS=Lateral Septum, LV=Lateral Ventricle, POA=Preoptic Area, BST=Bed Nucleus of the Stria Terminalis.

Supp Figure 6



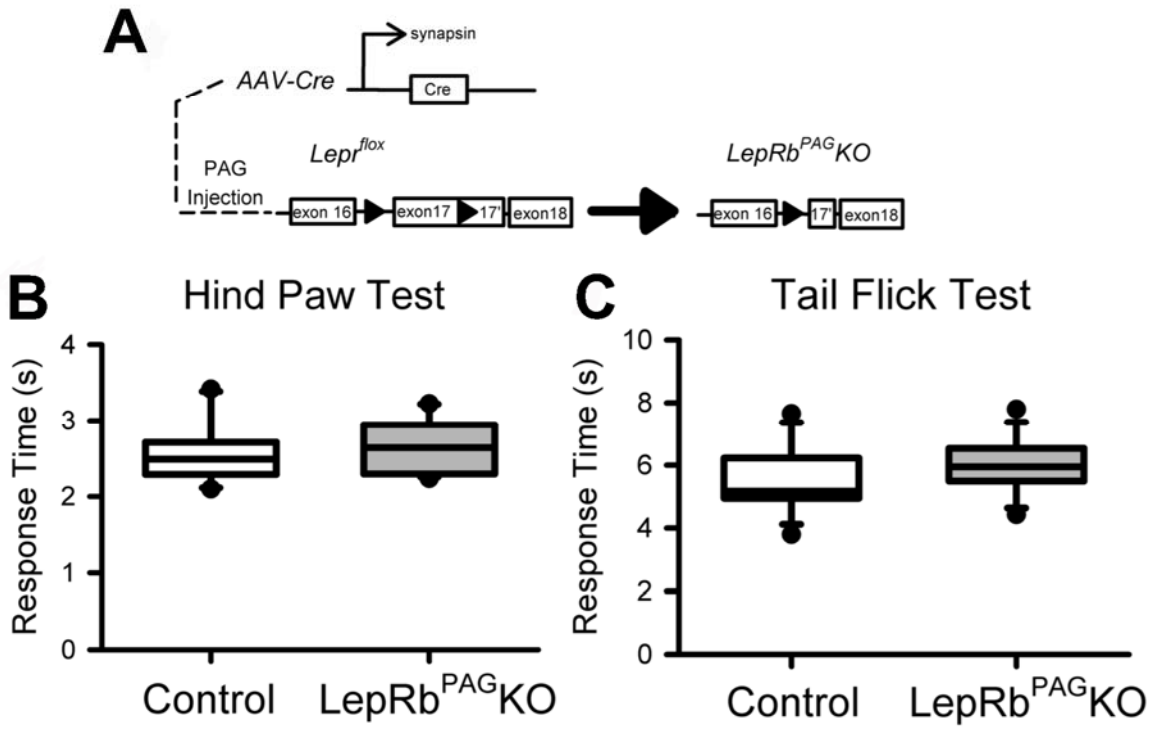
Supplemental Figure 6. hM3Dq-mediated activation of PAG^{LepRb} or PBN^{LepRb} neurons increases locomotor activity. (A) *Lepr^{cre}* mice were injected with AAV-hM3Dq in either the PBN (B,D) (n=10) or PAG (C,E) (n=11). Following recovery, these animals were treated with CNO or vehicle and exposed to the open field for 60 minutes, during which time distance traveled (B,C) and velocity (D,E) were tracked. Data are shown as mean +/-SEM; *p<0.05.

Supp Figure 7



Supplemental Figure 7. AAV-Cre injection in the PAG of Lepr$^{flox/flox}$ ablates PAG leptin action. (A) *Lepr$^{flox/flox}$* mice on the cre-inducible eGFP-L10a background that had been injected with AAV-cre (LepRb^{PAG}KO, B) (n=14) or AAV-mCherry (control, B) (n=13) into the PAG for the analysis in Figure 6 were treated with leptin (5 mg/kg) and perfused 2 hours later. The brains were collected, sectioned, and stained for pSTAT3 (black). Additional series were stained for GFP to reveal the spread of the AAV-cre; a representative image is shown in (D). pSTAT3-immunoreactive neurons were quantified throughout the PAG and the number of cells plotted (E). Scale bar = 100 μm. Data are plotted as mean +/-SEM; n= 13-14; *p<0.05 by one-tailed T-test. PAG=Periaqueductal grey, IC=Inferior Colliculus, Aq=Cerebral Aqueduct, PBN=Parabrachial Nucleus.

Supp Figure 8



Supplemental Figure 8. Normal perception and behavioral responses to noxious stimuli in LepRb^{PAG}KO mice. (a) *Lepr$^{flox/flox}$* mice that had been injected with AAV-cre (LepRb^{PAG}KO) (n=14) or AAV-mCherry (control) (n=13) into the PAG were challenged with the hind paw test (b) and tail flick test (c) to determine behavioral pain responses. Data are plotted as mean response times +/- SEM.