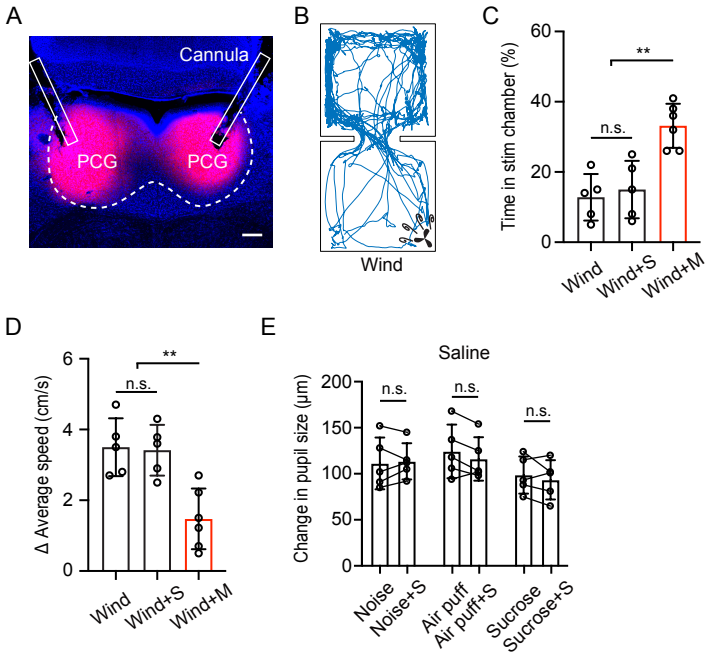

Figure S1. Related to Figure 1.

PCG mediates wind blow induced place aversion and saline control experiments for pupil size changes to various sensory stimuli.

(A) Confocal image showing spread of muscimol in the PCG region. Scale, 200 μm.

(B) Movement tracking for an example animal in the two-chamber place preference test. Wind blow was applied in the predesignated stimulation chamber.

(C) Summary of percentage time spent in the stimulation chamber in wind alone, wind plus saline infusion, and wind plus muscimol infusion groups. N = 5, 5, 6 respectively. **P < 0.01, one-way ANOVA.

(D) Summary of increase in average speed in the stimulation chamber in wind alone, wind plus saline infusion, and wind plus muscimol infusion groups. N = 5, 5, 6 respectively. **P < 0.01, one-way ANOVA.

(E) Summary of pupil size changes responding to different sensory stimuli (noise, air puffs, sucrose water) before and after saline infusion. N = 5, 5, 5 respectively. P > 0.05, paired t-test.

Error Bar = SD in all plots.

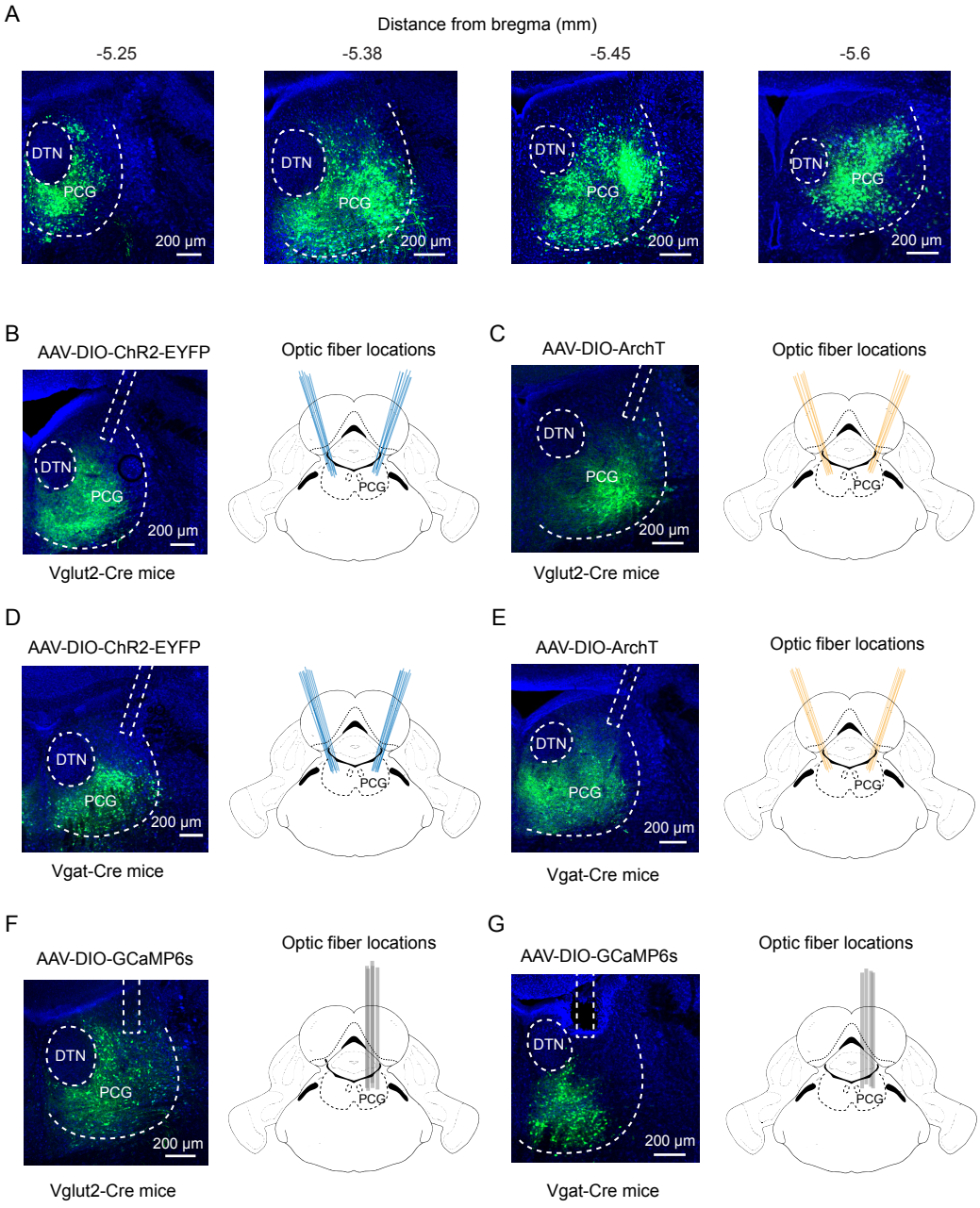

Figure S2. Related to Figure 2, 3, 4.

Post hoc histological verifications of viral expression and placements of optic fibers.

(A) Confocal images of viral expression across different coronal sections for a representative Vglut2-Cre animal with AAV1-CAG-FLEX-GFP injected into PCG. The images suggest a potential physical boundary that likely isolates DTN from PCG.

(B) Left, an example coronal section showing the track of the optic fiber implanted in a Vglut2-Cre mouse expressing ChR2-EYFP in PCG. Right, superimposed tracks of optic fibers across experiments (n = 8).

(C) Left, track of the optic fiber implanted in a Vglut2-Cre mouse with ArchT-GFP expression in PCG. Right, superimposed tracks of optical fibers across experiments (n = 6).
(D) Left, an example coronal section showing track of the optic fiber implanted in a Vgat-Cre mouse expressing ChR2-EYFP in PCG. Right, superimposed tracks of optic fibers across experiments (n = 9).
(E) Left, an example coronal section showing track of the optic fiber implanted in a Vglut2-Cre mouse expressing ArchT-GFP. Right, superimposed tracks of the optic fibers across experiments (n = 7).
(F) Left, an example image showing expression of GCaMP6s in PCG of a Vglut2-Cre mouse. Right, superimposed tracks of optical fibers across experiments (n = 5).
(G) Left, an example image showing expression of GCaMP6s in PCG of a Vgat-Cre mouse Right, tracks of the optic fibers implanted in Vgat-Cre mice expressing GCaMP6s in PCG (n = 4).

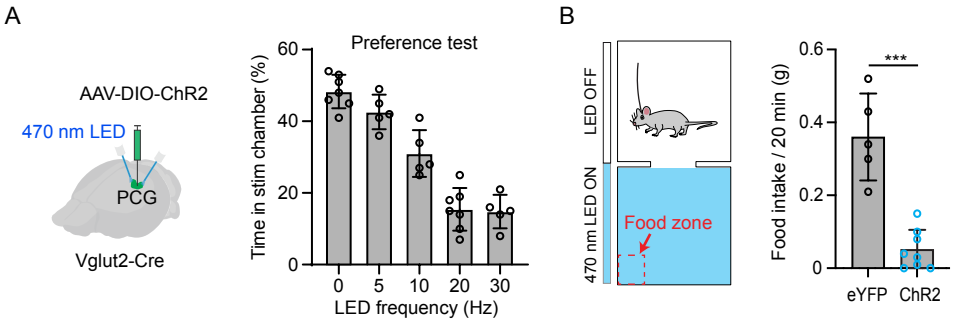

Figure S3. Related to Figure 2.

Dependency on the optogenetic stimulation frequency in the RTPP test and quantification of the food intake test.

(A) Left, experimental condition: AAV-DIO-ChR2 was injected into PCG of Vglut2-Cre mice, and optic fibers were bilaterally implanted above PCG. Right, percentage time spent in the stimulation chamber at different optical stimulation frequencies during the RTPP test for different groups of mice.

(B) Left, schematic food intake test. Whenever the animal (food-deprived for 24 hours) entered the LED-On chamber (marked by blue shade), PCG glutamatergic neurons were photo-stimulated continuously until it exited. Right, food intake within a 20-min test session. ***P < 0.001, t-test, n = 5 and 8 animals for the eYFP control and ChR2 groups, respectively.

Error Bar = SD in all plots.

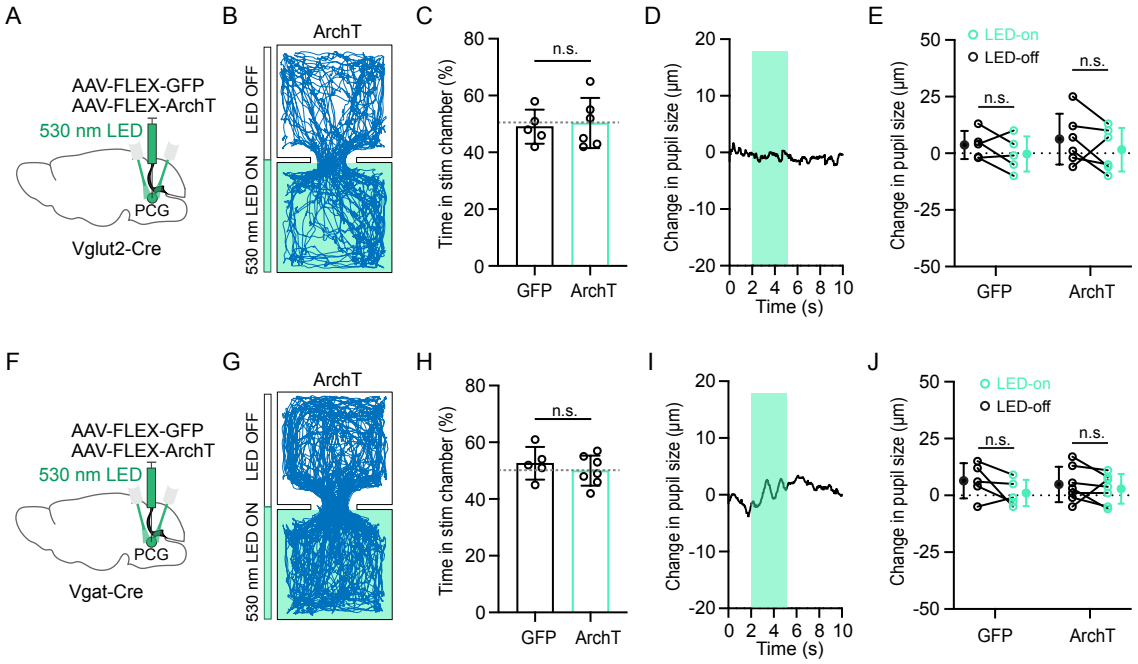

Figure S4. Related to Figure 4.

Inhibition of PCG glutamate and GABA neurons alone had no effect on place preference/avoidance or pupil size.

(A) Left, schematic viral injection for silencing PCG Vglut2+ neurons.

(B) Movement tracks for an example mouse with inhibition of PCG Vglut2+ neurons alone (no sensory stimulation) in the RTPP test.

(C) Percentage time spent in the LED-On chamber in GFP control (n = 5) and ArchT (n = 6) groups. Grey dash line marks 50% level. P > 0.05, t-test.

(D) Plot of pupil size changes for an example animal responding to the optical inhibition of PCG Vglut2+ neurons (marked by green shade).

(E) Summary of peak pupil size changes in GFP control (n = 5) and ArchT (n = 6) groups. P > 0.05, paired t-test.

(F) Left, schematic viral injection for silencing PCG Vgat+ neurons.

(G) Example movement tracks for an example animal in the RTPP test with inhibition of PCG Vgat+ neurons alone.

(H) Percentage time spent in the LED-On chamber in GFP control (n = 5) and ArchT (n = 7) groups. P > 0.05, t-test.

(I) Plot of pupil size changes for an example animal responding to the optical inhibition of PCG Vgat+ neurons alone.

(J) Summary of peak pupil size changes in GFP control (n = 5) and ArchT (n = 7) groups. P > 0.05, paired t-test.

Error Bar = SD in all plots.

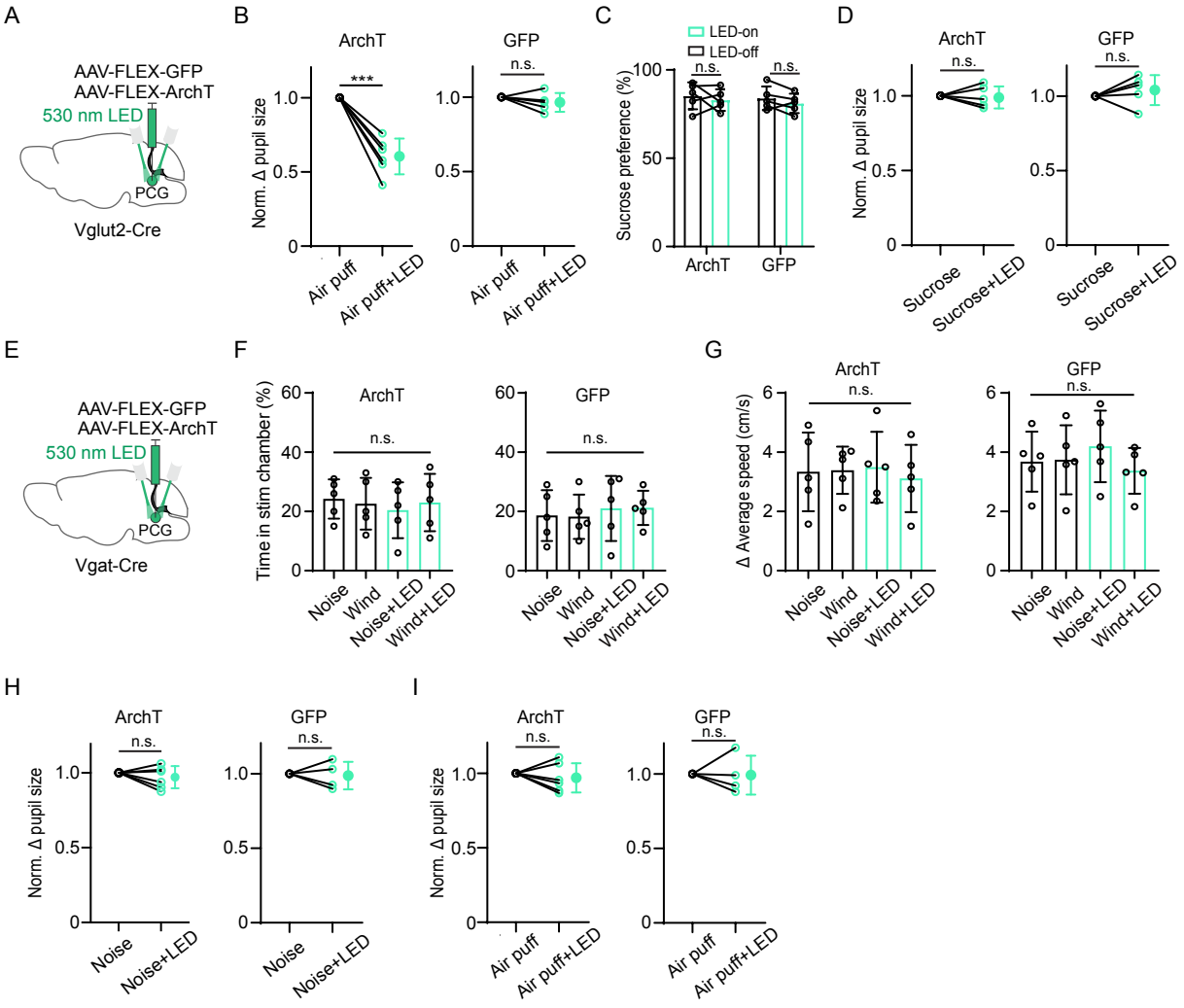

Figure S5. Related to Figure 4.

Effects of optogenetic inhibition PCG glutamate/GABA neurons on the reward/aversion-related behaviors.

(A) Schematic viral injection for silencing PCG Vglut2+ neurons.

(B) Comparison of Δ pupil size (normalized) between air puffs alone and air puffs plus LED-On conditions in ArchT (n = 6) and GFP control (n = 5) groups. *** P < 0.001, paired t-test.

(C) Summary of sucrose preference without and with LED illumination in ArchT (n = 5) and GFP (n = 5) groups. P > 0.05, paired t-test.

(D) Normalized Δ pupil size in sucrose alone and sucrose plus LED-On conditions in ArchT (n = 5) and GFP (n = 5) groups. P > 0.05, paired t-test.

(E) Schematic viral injection for silencing PCG Vgat+ neurons.

(F) Summary of percentage time spent in the stimulation chamber in ArchT (n = 5 for each subgroup) and GFP (n = 5 for each subgroup) groups under different conditions. P > 0.05, one-way ANOVA.
(G) Summary of increase in average speed in the stimulation chamber in ArchT (n = 5 for each subgroup) and GFP (n = 5 for each subgroup) groups under different conditions. P > 0.05, one-way ANOVA.
(H) Normalized Δ pupil size in noise alone and noise plus LED-On conditions in ArchT (n = 6) and GFP (n = 4) groups. P > 0.05, paired t-test.
(I) Normalized Δ pupil size in air puff alone and air puff plus LED-On conditions in ArchT (n = 6) and GFP (n = 4) groups. P > 0.05, paired t-test.
Error Bar = SD in all plots.

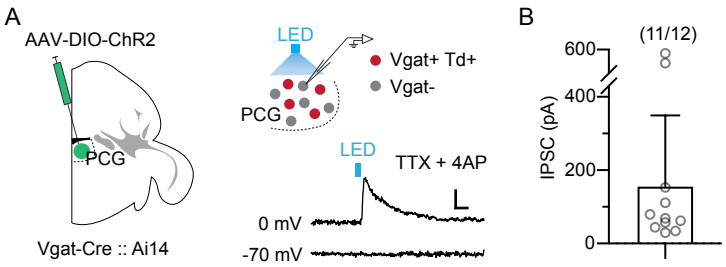

Figure S6. Related to Figure 5.

PCG Vgat+ neurons locally suppress glutamatergic neurons.

(A) Left, viral injection. Right upper, recording from PCG Vgat- neurons while stimulating Vgat+ neurons in the slice preparation. Right lower, current traces recorded from a PCG glutamatergic neuron under two different holding potentials. Blue tick indicates the onset of light stimulation. Scale, 30 pA, 10 ms.

(B) Average IPSC amplitudes in 11 responsive PCG glutamatergic neurons. Bar = SD.

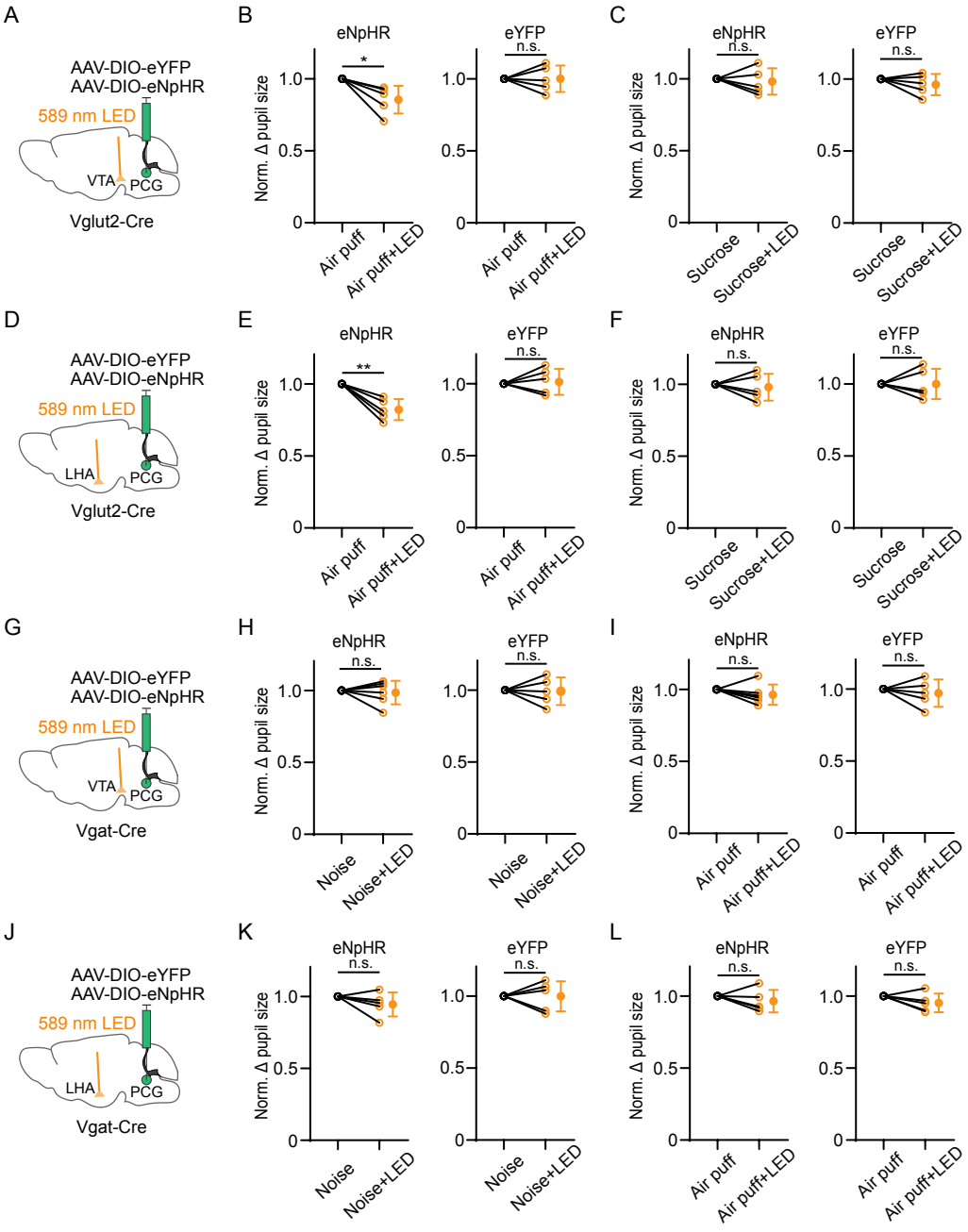

Figure S7. Related to Figure 7.

Effects of silencing PCG glutamatergic and GABAergic axon terminals on sensory-indued arousal.

(A) Schematic of viral injection and optogenetic silencing of PCG–VTA terminals in Vglut2-Cre mice.

(B) Normalized Δ pupil size in air puff alone and air puff plus LED-On conditions in eNpHR (n = 5) and eYFP (n = 5) groups. * P < 0.05; n.s., P > 0.05, paired t-test.

(C) Normalized Δ pupil size in sucrose alone and sucrose plus LED-On conditions in eNpHR (n = 5) and eYFP (n = 5) groups.

(D-F) Similar to (A-C) but for optogenetic silencing of PCG–LHA terminals in Vglut2-Cre mice.
** $P < 0.01$, paired t-test, n = 5 and 5 for eNpHR and eYFP groups respectively.
(G-I) Similar to (A-C) but for optogenetic silencing of PCG–VTA terminals in Vgat-Cre mice. $P > 0.05$, paired t-test, n = 6 and 5 for eNpHR and eYFP groups respectively.
(J-L) Similar to (A-C) but for optogenetic silencing of PCG–LHA terminals in Vgat-Cre mice. $P > 0.05$, paired t-test, n = 5 and 5 for eNpHR and eYFP groups respectively.
Error Bar = SD in all plots.

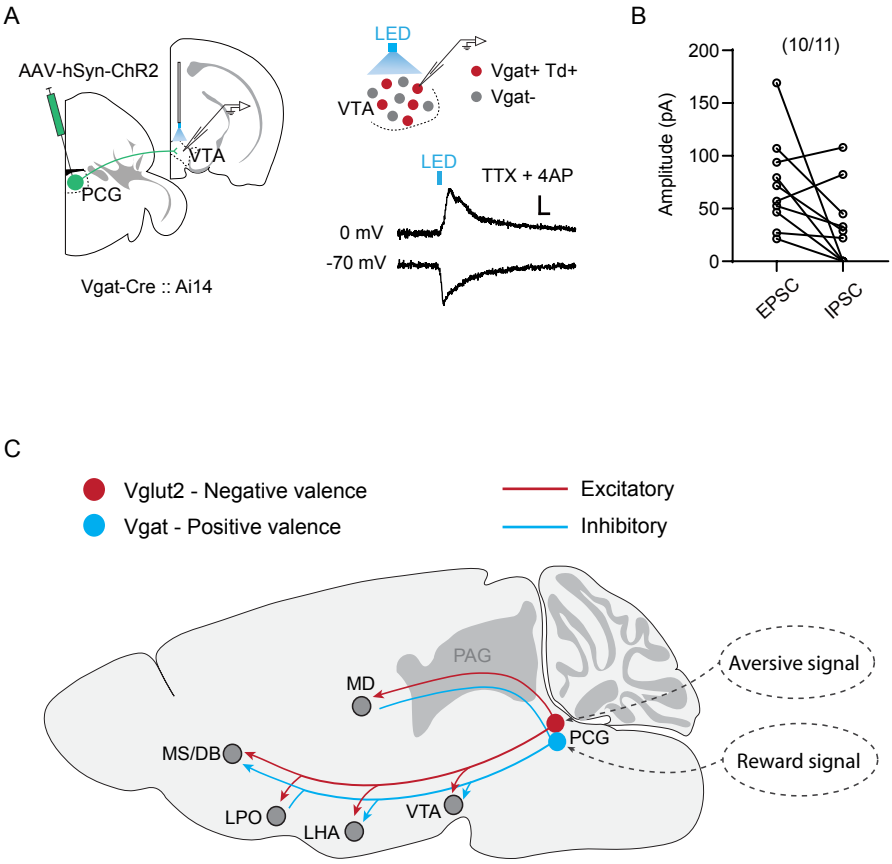

Figure S8. Related to Figure 5, 6, 7.

PCG to VTA functional connectivity and illustration of our proposed circuit model.

(A) Left, schematic experimental scheme. Right upper, recording from VTA GABAergic neurons (tdTomato+) and optical activation of PCG axons (both glutamatergic and GABAergic). Right lower, LED-evoked IPSC (recorded at 0 mV) and EPSC (at −70 mV) in an example VTA GABAergic neuron. Scale, 40 pA, 10 ms.

(B) Comparison of EPSC and IPSC amplitudes in the same VTA GABAergic cells (n = 10).

(C) A proposed circuit model. PCG Vglut2+ neurons encode negative valence, while its Vgat+ neurons encode positive valence. The glutamatergic neurons respond to aversive but not reward sensory signals, while the GABAergic neurons to reward but not aversive sensory signals. These two cell populations project proximately in parallel to similar targets (dorsally to MD, and ventrally to VTA, LHA, LPO and MS/DB) yet with distinguishable downstream targets for their roles in mediating aversion/preference behavior (arrowheads indicates contributing targets). Thus, PCG may serves as a critical node to process valence of incoming sensory signals and relay the valence-specific information into an extended brain network to enhance arousal and to drive valence-specific behaviors.