

Table S1 - Viral injections for functional studies in the strain of VGat-cre. Related to STAR methods.

| Mouse strain | VGat-Cre | | | | | | | |
|--|--|--------------------------------------|----------------------------|------------|------------|-----------------------------|--------------------------|------------|
| Injection type | Viral construct | Volume (μL/side), (Rate: 0.1 μL/min) | Injection Coordinates (mm) | | | Opto fiber Coordinates (mm) | | |
| | | | AP | ML | DV | AP | ML | DV |
| CeA-ChR2 | AAV5-EF1a-DIO-hChR2(H134R)-EYFP | 0.5 | -1.0 | ±2.5 | -5.0 | -1.0 | ±2.5 | -4.5 |
| CeA-eArch | AAV5-EF1a-DIO-eArch3.0-EYFP | 0.5 | -1.0 | ±2.5 | -5.0 | -1.0 | ±2.5 | -4.5 |
| CeA-EYFP | AAV5-EF1a-DIO-EYFP | 0.5 | -1.0 | ±2.5 | -5.0 | -1.0 | ±2.5 | -4.5 |
| CeA-Gq | AAV5-hSyn-DIO-hM3D(Gq)-mCherry | 0.5 | -1.0 | ±2.5 | -5.0 | N/A | | |
| CeA-Gi | AAV5-hSyn-DIO-hM4D(Gi)-mCherry | 0.5 | -1.0 | ±2.5 | -5.0 | N/A | | |
| Caudal Striatum - ChR2 | AAV5-EF1a-DIO-hChR2(H134R)-EYFP | 0.5 | -0.9 | ±3.0 | -4.2 | -1.0 | ±2.5 | -3.8 |
| Globus pallidus-ChR2 | AAV5-EF1a-DIO-hChR2(H134R)-EYFP | 0.5 | -1.0 | ±2.25 | -4.0 | -1.0 | ±2.25 | -3.5 |
| CeA-ChR2, opto fibers in PCRt | AAV5-EF1a-DIO-hChR2(H134R)-EYFP | 0.5 | -1.0 | ±2.5 | -5.0 | -6.1 | ±1.5 | -4.5 |
| CeA-ChR2, opto fibers in CeA; depolarizing designer receptor in PCRt | AAV5-EF1a-DIO-hChR2(H134R)-EYFP; AAV5-hSyn-DIO-hM3D(Gq)-mCherry | 0.5; 0.5 | -1.0; -6.1 | ±2.5; ±1.5 | -5.0; -5.4 | -1.0 | ±2.5 | -4.5 |
| CeA-ChR2, opto fibers in CeA; Cre-dependent Caspase in PCRt | AAV5-EF1a-DIO-hChR2(H134R)-EYFP; AAV5-flex-taCasp3-TEVp | 0.5; 0.5 | -1.0; -6.1 | ±2.5; ±1.5 | -5.0; -5.4 | -1.0 | ±2.5 | -4.5 |
| PCRt-ChR2 | AAV5-EF1a-DIO-hChR2(H134R)-EYFP | 0.5 | -6.1 | ±1.5 | -5.4 | -6.1 | ±1.5 | -4.5 |
| PCRt-eArch | AAV5-EF1a-DIO-eArch3.0-EYFP | 0.5 | -6.1 | ±1.5 | -5.4 | -6.1 | ±1.5 | -4.5 |
| CeA-ChR2, opto fibers in PAG | AAV5-EF1a-DIO-hChR2(H134R)-EYFP | 0.5 | -1.0 | ±2.5 | -5.0 | -4.4 | ±0.5 | -2.0 |
| CeA-ChR2, opto fibers in CeA; depolarizing designer receptor in PAG | AAV5-hSyn-hChR2(H134R)-EYFP; AAV5-hSyn-DIO-hM3D(Gq)-mCherry | 0.5; 0.3 | -1.0; -4.4 | ±2.5; ±0.5 | -5.0; -5.4 | -1.0 | ±2.5 | -4.5 |
| CeA-ChR2, opto fibers in PAG; depolarizing designer receptor in MLR | AAV5-EF1a-DIO-hChR2(H134R)-EYFP; AAV5-hSyn-HA-hM3D(Gq)-IRES-mCitrine | 0.5; 0.3 | -1.0; -5.0 | ±2.5; ±1.2 | -5.0; -5.4 | -4.4 | ±0.5 | -2.0 |
| CeA-ChR2, opto fibers in PCRt + PAG | AAV5-EF1a-DIO-hChR2(H134R)-EYFP | 0.5 | -1.0 | ±2.5 | -5.0 | -6.1; -4.4 | L/R -1.5/1.9*; -0.5/0.7* | -4.5; -2.0 |

*Right side: tilting to the right 6 °

Table S2 - Viral injections for functional studies in the strain of VGat-floxed. Related to STAR methods.

| Mouse strain | VGat-floxed | | | | | | | |
|---------------------------------|---|--|----------------------------|------|------|-----------------------------|------|------|
| Injection type | Viral construct | Volume (μL/side) (Rate: 0.1 μL/min) | Injection Coordinates (mm) | | | Opto fiber Coordinates (mm) | | |
| | | | AP | ML | DV | AP | ML | DV |
| +CRE (<i>CeA-ChR2+Cre</i>) | AAV5-hSyn-hChR2(H134R)-EYFP: AAV5-CMV-Cre-GFP. Mixed [1:1] | 0.5 | -1.0 | ±2.5 | -5.0 | -1.0 | ±2.5 | -4.5 |
| CONTROL (<i>CeA-ChR2+GFP</i>) | AAV5-hSyn-hChR2(H134R)-EYFP: AAV5-CMV-GFP. Mixed [1:1] | 0.5 | -1.0 | ±2.5 | -5.0 | -1.0 | ±2.5 | -4.5 |

Table S3 - Viral injections for functional studies in the strain of VGlut2-cre. Related to STAR methods.

| Mouse strain | VGlut2-Cre | | | | | | | |
|--|--|--|----------------------------|---------------|---------------|-----------------------------|------|------|
| Injection type | Viral construct | Volume (μL/side) (Rate: 0.1 μL/min) | Injection Coordinates (mm) | | | Opto fiber Coordinates (mm) | | |
| | | | AP | ML | DV | AP | ML | DV |
| <i>CeA-ChR2, opto fibers in CeA; Cre-dependent Caspase in PCRt</i> | AAV5-EF1a-DIO-hChR2(H134R)-EYFP; AAV5-flex-taCasp3-TEVp | 0.5; 0.5 | -1.0; -6.1 | ±2.5; ±1.5 | -5.0; -5.4 | -1.0 | ±2.5 | -4.5 |
| <i>PCRt-ChR2</i> | AAV5-EF1a-DIO-hChR2(H134R)-EYFP | 0.5 | -6.1 | ±1.5 | -5.4 | -6.1 | ±1.5 | -4.5 |
| <i>CeA-ChR2, opto fibers in CeA; depolarizing designer receptor in PAG</i> | AAV5-hSyn-hChR2(H134R)-EYFP; AAV5-hSyn-DIO-hM3D(Gq)-mCherry | 0.5; 0.3 | -1.0; -4.4 | ±2.5; ±0.5 | -5.0; -5.4 | -1.0 | ±2.5 | -4.5 |
| <i>PAG-ChR2, opto fibers in MLR</i> | AAV5-EF1a-DIO-hChR2(H134R)-EYFP | 0.3 | -4.4 | ±0.5 | -2.7 | -5.0 | ±1.2 | -3.0 |

Table S4 - Viral injections for functional studies in the strain of C57BL6/J. Related to STAR methods.

| <u>Mouse strain</u> | C57BL6/J | | | | | | | |
|---|--|--|----------------------------|------------------------|------------------------|-----------------------------|------|------|
| <u>Injection type</u> | <u>Viral construct</u> | Volume (µL/side) (Rate: 0.1 µL/min) | Injection Coordinates (mm) | | | Opto fiber Coordinates (mm) | | |
| | | | AP | ML | DV | AP | ML | DV |
| <i>PCRt CAV2-Cre; Cre-dependent ChR2 in CeA and opto fiber in CeA</i> | CAV2-Cre-GFP; AAV5-EF1a-DIO-hChR2(H134R)-mCherry-WPRA-pA | 0.5; 0.5 | -6.1; -1.0 | ±1.5; ±2.5 | -5.0; -5.4 | -1.0 | ±2.5 | -4.5 |
| <i>PAG CAV2-Cre; Cre-dependent ChR2 in CeA and opto fiber in CeA</i> | CAV2-Cre-GFP; AAV5-EF1a-DIO-hChR2(H134R)-mCherry-WPRA-pA | 0.3; 0.5 | -4.4; -1.0 | ±0.5; ±2.5 | -2.7; -5.0 | -1.0 | ±2.5 | -4.5 |
| <i>PCRt CAV2-Cre; PAG CAV2-Cre; Cre-dependent ChR2 in CeA and opto fiber in CeA</i> | CAV2-Cre-GFP; CAV2-Cre-GFP; AAV5-EF1a-DIO-hChR2(H134R)-mCherry-WPRA-pA | 0.5; 0.3; 0.5 | -6.1; -4.4; -1.0 | ±1.5; ±0.5; ±2.5 | -5.4; -2.7; -5.0 | -1.0 | ±2.5 | -4.5 |
| <i>PCRt CAV2-Cre; Cre-dependent Caspase in CeA</i> | CAV2-Cre-GFP; AAV5-flex-taCasp3-TEVp | 0.5; 0.5 | -6.1; -1.0 | ±1.5; ±2.5 | -5.4; -5.0 | N/A | | |
| <i>PAG CAV2-Cre; Cre-dependent Caspase in CeA</i> | CAV2-Cre-GFP; AAV5-flex-taCasp3-TEVp | 0.3; 0.5 | -4.4; -1.0 | ±0.5; ±2.5 | -2.7; -5.0 | N/A | | |

Table S5 - Caspase efficiency studies for PCRt lesions in the strains of VGat-cre and VGlut2-cre. Related to STAR methods.

| <u>Mouse strains</u> | <i>VGat-Cre and VGlut2-Cre</i> | Volume | Injection Coordinates (mm) | | |
|--------------------------------------|---|--|----------------------------|------|------|
| <u>Injection type</u> | <u>Viral construct</u> | Volume (µL) unilateral (Rate: 0.1 µL/min) | AP | ML | DV |
| <i>PCRt-ChR2; PCR-ChR2 + Caspase</i> | AAV5-EF1a-DIO-hChR2(H134R)-EYFP; AAV5-DIO-ChR2-EYFP and AAV5-flex-taCasp3-TEVp. Mixed [1:1] | 0.5; 0.5 | -6.1 | ±1.5 | -5.4 |

Table S6 - Rabies injections for anatomical studies. Related to STAR methods.

| Retrograde tracing using Cre-dependent pseudo-typed rabies virus expression | | | | | | |
|--|---|---|------|----------------------------|------|--|
| Mouse strains | VGat-Cre and VGlut2-Cre | | | Injection Coordinates (mm) | | |
| Injection type | Viral construct | Volume (µL) unilateral (Rate: 0.1 µL/min) | AP | ML | DV | |
| <i>PCRt retrograde tracing</i> | AAV5-CA-FLEX-RG and AAV5-EF1a-FLEX-TVA-mCherry Mixed [1:1]; SAD-ΔG-GFP* | 0.5; 0.5 | -6.1 | ±1.5 | -4.5 | |
| <i>PAG retrograde tracing</i> | AAV5-CA-FLEX-RG and AAV5-EF1a-FLEX-TVA-mCherry Mixed [1:1]; SAD-ΔG-GFP* | 0.5; 0.5 | -4.4 | ±0.5 | -2.0 | |

* SAD-ΔG-GFP was injected 2 weeks after the first viral injection

Table S7 - AAVs injections for anatomical studies. Related to STAR methods.

| Anatomical tracing studies | | | | | | | | | |
|-----------------------------------|--|---|---|-----------|------------|------------|---|------|------|
| Mouse strain | Injection type | Viral construct | Viral Injection Coordinates (mm) | | | | FluoroGold Injections | | |
| | | | Volume (µL) unilateral (Rate: 0.1 µL/min) | AP | ML | DV | AP | ML | DV |
| VGat-Cre | <i>CeA-SynB; PCRt-mCherry</i> | <i>AAV5-EF1a-DIO-Synb-eGFP; AAV5-hSyn-DIO-mCherry</i> | 0.5; 0.5 | -1.0; 6.1 | ±2.5; ±1.5 | -5.0; -5.4 | N/A | | |
| VGat-Cre/ VGlut2-cre | <i>PCRt-SynB; FluoroGold in muscle</i> | <i>AAV5-EF1a-DIO-Synb-eGFP</i> | 0.5 | -6.1 | ±1.5 | -5.4 | In either Masseter or Trapezius with 1% FG. 10µL unilateral; rate 0.5µL/min | | |
| VGlut2-Cre | <i>VL/LPAG-SynB; FluoroGold in MLR**</i> | <i>AAV5-EF1a-DIO-Synb-eGFP</i> | 0.3 | -4.4 | ±0.5 | -2.7 | -5.0 | ±1.2 | -3.4 |

**1%FG injected at rate of 0.05µL/20min

Table S8 - Retrograde tracer injections for anatomical studies. Related to STAR methods.

| Dual retrograde labeling from PCRt and PAG | | | | | | |
|---|-----------------|------------------------|----------------------------|-------|-------|---------------|
| Mouse strain | C57BL6/J | | Injection Coordinates (mm) | | | |
| Injection type | Retrograde dye | Volume (µL) unilateral | AP | ML | DV | Rate |
| FluoroGold (FG) in PCRt ; Cholera Toxin subunit B (CTb) | 1% FG; | 0.05; | -6.1; | ±1.5; | -5.5; | 0.05µL/20min; |
| | 1% CTb | 0.5 | -4.4 | ±0.5 | -2.7 | 0.5µL/20min. |