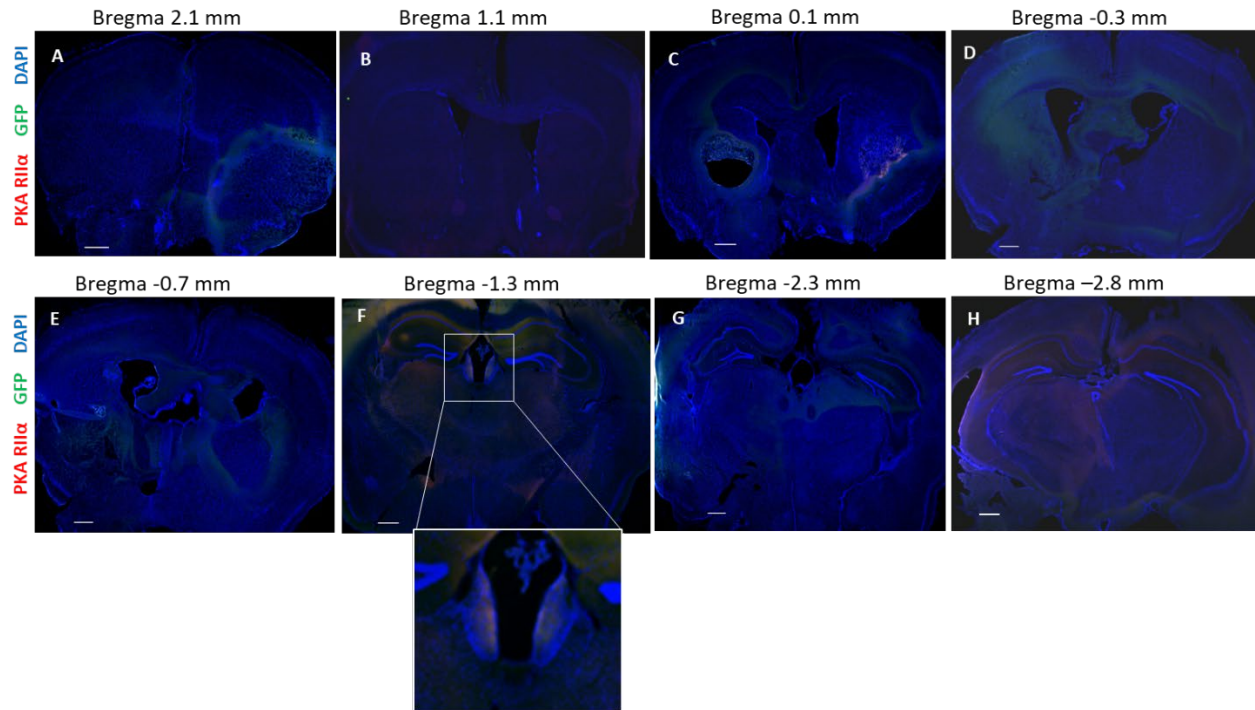


**Supplemental Figure 1.** Characterization of habenular *Prkar2a* expression anterior to posterior in WT C57/BL6 mouse and general MHb cell type categorization. A. Representative ISH mapping images of *Prkar2a* expression in 20 $\mu$ M thick sections spanning the entirety of habenula defined as bregma -0.98 mm—2.06 mm.<sup>54</sup> Sections were also probed for *Slc17a7* as a glutamatergic marker and counterstained with DAPI. B. Representative image of MHb ISH with probes for *Prkar2a* (red), *Syn1* (green), and *Gfap* (purple) showed *Prkar2a* expression in both neurons and glial cells merged (left), *Syn1* (green) and *Prkar2a* (red) only (middle) and *Gfap* (green) and *Prkar2a* (red) only (right), C. ISH using probes for *Slc17a7* and *Gad1* as markers of glutamatergic or GABAergic cells, respectively was performed to confirm general cell type classification of *Prkar2a*-expressing cells in MHb.



**Supplemental Figure 2.** Representative immunofluorescent images show that re-expression of PKA RII $\alpha$  by bilateral stereotaxic injection of rAAV (pAAV-EF1a-mPrkar2a-IRES-eGFP) was successfully achieved in the MHb. A-E. Coronal sections rostral to the injection target show no expression of PKA RII $\alpha$  or GFP, F. Shows re-expression of PKA RII $\alpha$  (and expression of GFP), and G-H. show two show coronal sections caudal to the injection target. Scale bars represent 500  $\mu$ M.

**Supplemental Table 1.** List of antibodies and conditions used for western blot and immunofluorescence experiments. For western blots, antibodies were diluted in 3% bovine serum albumin in 1X Tris-buffered saline with 0.1% Tween-20. For immunofluorescence of floating and mounted sections, antibodies were diluted in 3% donkey serum in 1X phosphate-buffered saline with 0.1% Triton-X 100 and 0.1% glycine.

target	primary antibody	dilution	secondary antibody	dilution
<b>western blot</b>				
DARPP-32	DARPP-32 rabbit monoclonal; Cell Signaling #2302S	1:1000	AffiniPure Donkey Anti-Rabbit IgG (H+L), HRP-conjugated; Jackson ImmunoResearch Laboratories # 711-035-152	1:10,000
DARPP-32 (T <sup>34</sup> )	DARPP-32 (T34) (D27A4) rabbit monoclonal; Cell Signaling #12438	1:800	AffiniPure Donkey Anti-Rabbit IgG (H+L), HRP-conjugated; Jackson ImmunoResearch Laboratories # 711-035-152	1:10,000
DARPP-32 (T <sup>75</sup> )	DARPP-32 (T75) (D27A4) rabbit monoclonal; Cell Signaling #2301S	1:800	AffiniPure Donkey Anti-Rabbit IgG (H+L), HRP-conjugated; Jackson ImmunoResearch Laboratories # 711-035-152	1:10,000
GAPDH	(FL-335) rabbit polyclonal; Santa Cruz # sc-25778	1:1100	AffiniPure Donkey Anti-Rabbit IgG (H+L), HRP-conjugated; Jackson ImmunoResearch Laboratories # 711-035-152	1:10,000
Histone 3	Histone 3, loading control and CHIP grade, mouse monoclonal; Abcam # ab1791	1:2000	AffiniPure Donkey Anti-Mouse IgG (H+L), HRP-conjugated; Jackson ImmunoResearch Laboratories # 711-035-150	1:10,000
HPRT	HPRT (F-1) mouse monoclonal; Santa Cruz # sc-376938	1:1200	AffiniPure Donkey Anti-Mouse IgG (H+L), HRP-conjugated; Jackson ImmunoResearch Laboratories # 711-035-150	1:10,000
PKA RI $\alpha$	PKA RI $\alpha$ , mouse monoclonal; BD Biosciences # 610610	1:500	AffiniPure Donkey Anti-Mouse IgG (H+L), HRP-conjugated; Jackson ImmunoResearch Laboratories # 711-035-150	1:10,000
PKA C $\alpha$	PKA C $\alpha$ rabbit polyclonal; Santa Cruz #sc-903	1:1000	AffiniPure Donkey Anti-Rabbit IgG (H+L), HRP-conjugated; Jackson ImmunoResearch Laboratories # 711-035-152	1:10,000
PKA C $\alpha$ $\beta$ $\gamma$	PKA C $\alpha$ $\beta$ $\gamma$ (H-95) rabbit polyclonal; Santa Cruz #sc-28892	1:1000	AffiniPure Donkey Anti-Rabbit IgG (H+L), HRP-conjugated; Jackson ImmunoResearch Laboratories # 711-035-152	1:10,000

**immunofluorescence**

c-Fos	c-Fos (4) X rabbit polyclonal; Santa Cruz # sc-52X	floating, 1:300	Donkey anti-Rabbit IgG (H+L), Alexa Fluor 555; Invitrogen # A-31572	1:400
c-Jun	c-Jun (N) X rabbit polyclonal; Santa Cruz # sc-45 X	floating, 1:300	Donkey anti-Rabbit IgG (H+L), Alexa Fluor 555; Invitrogen # A-31572	1:400
DARPP-32	DARPP-32 rabbit monoclonal; Cell Signaling #2302S	mounted, 1:500	Donkey anti-Rabbit IgG (H+L), Alexa Fluor 555; Invitrogen # A-31572	1: 500
GFP	GFP (B-2) mouse monoclonal; Santa Cruz, sc-9996	floating, 1:350; mounted, 1:400	Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 48; Invitrogen # A32766	floating, 1:400; mounted 1:500
MAP2	MAP-2 (AP20) mouse monoclonal; Santa Cruz # sc-32791	mounted, 1:400	Donkey anti-Rabbit IgG (H+L), Alexa Fluor 555; Invitrogen # A-31572	1:400
PKA RII $\alpha$	PKA RII $\alpha$ reg (M-20) rabbit polyclonal; Santa Cruz # sc-909	floating, 1:400; mounted, 1:450	Donkey anti-Rabbit IgG (H+L), Alexa Fluor 647; Invitrogen # A32795	floating, 1:400; mounted, 1:500
PKA C $\alpha$	PKA C $\alpha$ rabbit polyclonal; Santa Cruz #sc-903	floating, 1:400; mounted, 1:450	Donkey anti-Rabbit IgG (H+L), Alexa Fluor 647; Invitrogen # A32795	floating, 1:400; mounted, 1:500
PKA C $\alpha$ $\beta$ $\gamma$	PKA C $\alpha$ $\beta$ $\gamma$ (H-95) rabbit polyclonal; Santa Cruz #sc-28892	floating, 1:400; mounted, 1:450	Donkey anti-Rabbit IgG (H+L), Alexa Fluor 647; Invitrogen # A32795	floating, 1:400; mounted, 1:500

<sup>a</sup> Blocking buffer used was 5% bovine serum albumin in 1X Tris-buffered saline with 0.1% Tween-20 (1X TBST). All other membranes, 5% nonfat milk was used in 1X TBST.

**Supplemental Table 2.** List of probes and dilution used for *in situ* hybridization experiments. The ACD Biotechne RNAscope Fluorescent Multiplex Assay was used after optimization for fresh frozen brain sections.

target	probe name, catalog #	dilution
<b><i>In situ</i> hybridization</b>		
<i>Chat</i>	RNAscope® Probe- Mm-Chat-C2, 408731-C2	1:50
<i>Prkar2a</i>	RNAscope® Probe- Mm-Prkar2a, 472011	1:1
	RNAscope® Probe- Mm-Prkar2a-C3, 472011-C3	1:45
<i>Slc17a7</i>	RNAscope® Probe- Mm-Slc17a7-C2, 416631-C2	1:50
<i>Tac1</i>	RNAscope® Probe- Mm-Tac1-C2, 410351-C2	1:50
<i>Tacr1</i>	RNAscope® Probe- Mm-Tacr1-C3, 428781-C3	1:50
<i>Tac2</i>	RNAscope® Probe- Mm-Tac2-C2, 446391-C2	1:50