

Figure S1. Process of merging immunofluorescent and DAB images from a single site

Representative images obtained in the same brain region across different channels using an epifluorescence/brightfield microscope. Using the epifluorescence mode, clusters of dual labeled RE neurons (yellow) were first identified in dorsal ( $A_{i-}A_{ii}$ ) and ventral ( $B_{i-}B_{ii}$ ). Images of retrogradely labeled RE to mPFC cells were captured in the red channel, while RE to HC cells were captured in the green channel. Immediately after, using the brightfield mode, DAB RE CR<sup>+</sup> or RE CB<sup>+</sup> cells were captured. The red and green channel were merged, allowing the visualization of RE dual mPFC-HC projecting cells (yellow). Finally, the fluorescent merged image was transposed with its corresponding brightfield capture using Adobe Photoshop for the purpose of obtaining the final merged images shown in *Fig.* 6. Manual cell counts were done in each independent channel. Scale Bar = 50µm.

Abbreviations: CB, calbindin; CR, calretinin; DAB, 3,3'-Diaminobenzidine; mPFC, medial prefrontal cortex; RE, nucleus reuniens; HC, hippocampus

Table S1. Mean soma size area of  $CR^+$ ,  $CB^+$  and  $CR^+/CB^+$  neurons in paraventricular (PVT) and nucleus reuniens (RE)

Axis	Regional	Calcium	PVT	PVT		RE	
	Subdivision	Binding Protein	Mean (µm²)	SD	Mean (µm²)	SD	
Rostral	Dorsolateral	CR+	142.0	50.0	155.6	37.5	
		CB+	136.9	37.5	192.0	58.7	
		Dual	132.5	44.0	169.0	32.7	
	Ventrolateral	CR+	165.1	29.8	111.4	25.2	
		CB+	148.8	35.1	133.0	27.1	
		Dual	126.9	45.9	122.3	39.1	
	Medial	CR+	134.2	27.8	128.6	35.7	
		CB+	106.3	42.6	115.7	57.5	
		Dual	132.6	37.3	133.0	57.4	
Mid	Dorsolateral	CR+	132.4	27.0	164.7	48.8	
		CB+	126.3	27.2	161.7	49.7	
		Dual	125.7	31.3	161.1	60.3	
	Ventrolateral	CR+	163.0	36.3	112.1	29.7	
		CB+	172.0	33.9	124.8	30.0	
		Dual	142.6	28.4	116.5	34.1	
	Medial	CR+	143.6	31.8	116.7	27.7	
		CB+	138.1	37.9	122.4	31.1	
		Dual	123.0	44.2	132.4	40.5	
Caudal	Dorsolateral	CR+	145.7	38.1	151.6	26.1	
		CB+	114.5	50.4	131.6	42.0	
		Dual	126.8	39.1	136.3	37.7	
	Ventrolateral	CR+	134.6	38.2	135.4	31.8	
		CB+	152.1	59.6	139.2	30.4	
		Dual	138.8	77.7	140.5	35.7	
	Medial	CR+	138.5	35.3	116.3	31.6	
		CB+	128.4	42.0	101.3	41.5	
		Dual	100.9	28.7	87.7	43.0	

SD denotes standard deviation

## Table S2. Mean cell area density of DAB CR<sup>+</sup> and CB<sup>+</sup> cells in all reuniens (RE) internal subregions across the rostro-caudal axis of the thalamus

Axis	RE subregion	Calretinin (CR⁺)		Calbindin (CB⁺)	
		Mean	SD	Mean	SD
Rostral	RE anterior (REa)	7.8	3.0	9.2	1.6
Rostromedial	RE median (REm)	11.1	7.9	9.0	4.7
	RE lateral (REI)	6.7	3.6	22.0	18.4
	RE dorsal REd)	5.3	1.9	9.4	5.0
	RE ventral (REv)	7.8	1.2	16.3	9.9
	RE other (REother)	6.3	1.8	10.4	3.6
Medial	RE caudal median (REcm)	10.9	6.0	11.8	3.9
	RE caudal dorsal (REcd)	7.5	1.4	12.9	3.7
	RE caudal posterior (REcp)	6.2	1.1	8.6	1.9
	Perireuniens (PRe)	5.5	1.1	10.5	6.0
Caudomedial	RE caudal median (REcm)	7.0	4.3	14.2	5.8
	RE caudal dorsal (REcd)	12.3	6.0	22.7	18.2
	RE caudal posterior (REcp)	16.0	17.7	14.3	13.8
	Perireuniens (PRe)	4.8	1.7	14.8	13.7
Caudal	RE caudal posterior (REcp)	8.8	5.2	14.4	10.9
	RE caudal dorsal (REcd)	12.5	7.4	22.2	14.6
	Perireuniens (PRe)	10.0	3.3	20.9	8.9

\* means are in cells/.01mm<sup>2</sup>

SD denotes standard deviation