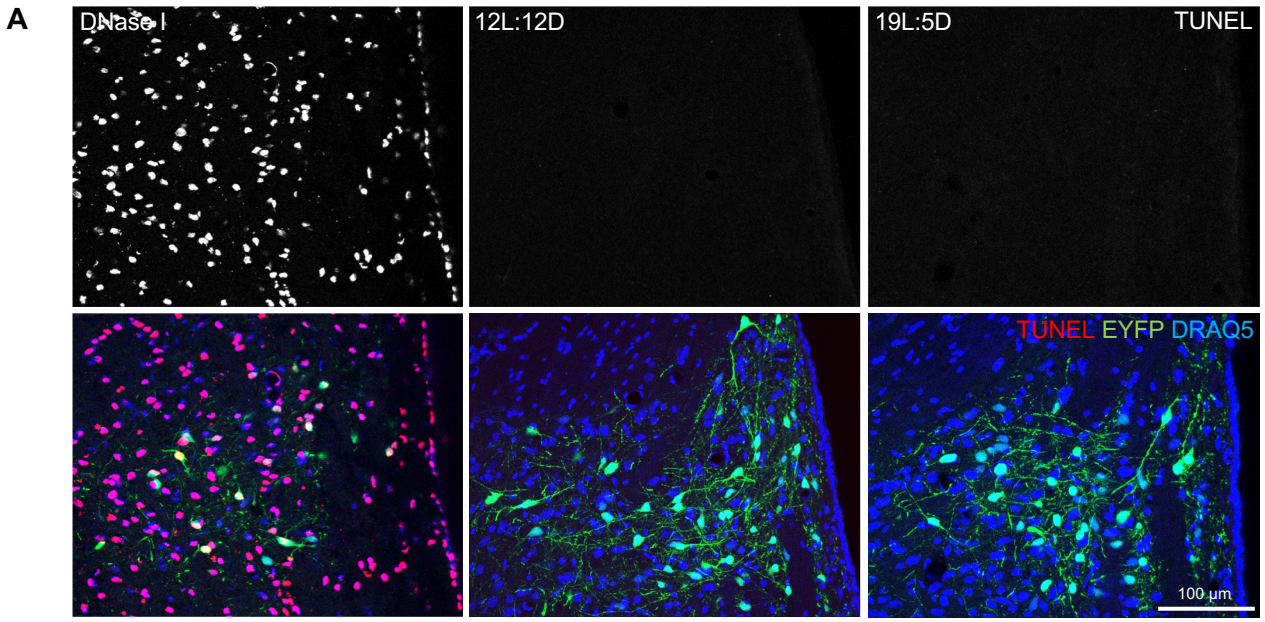
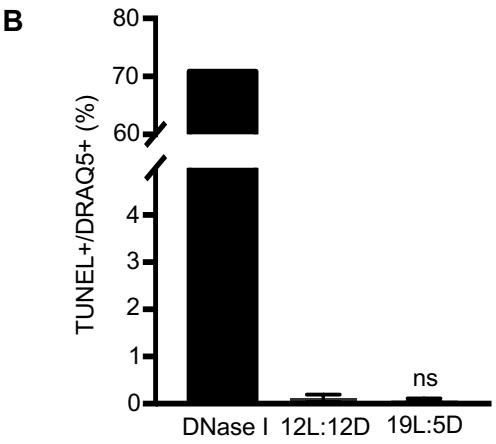
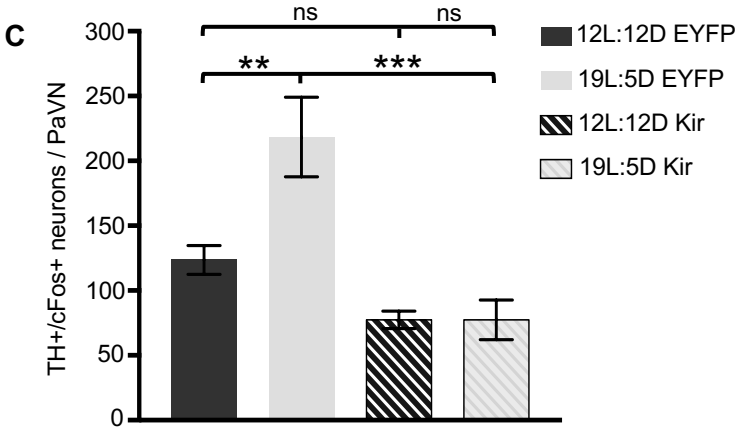
Figure S1





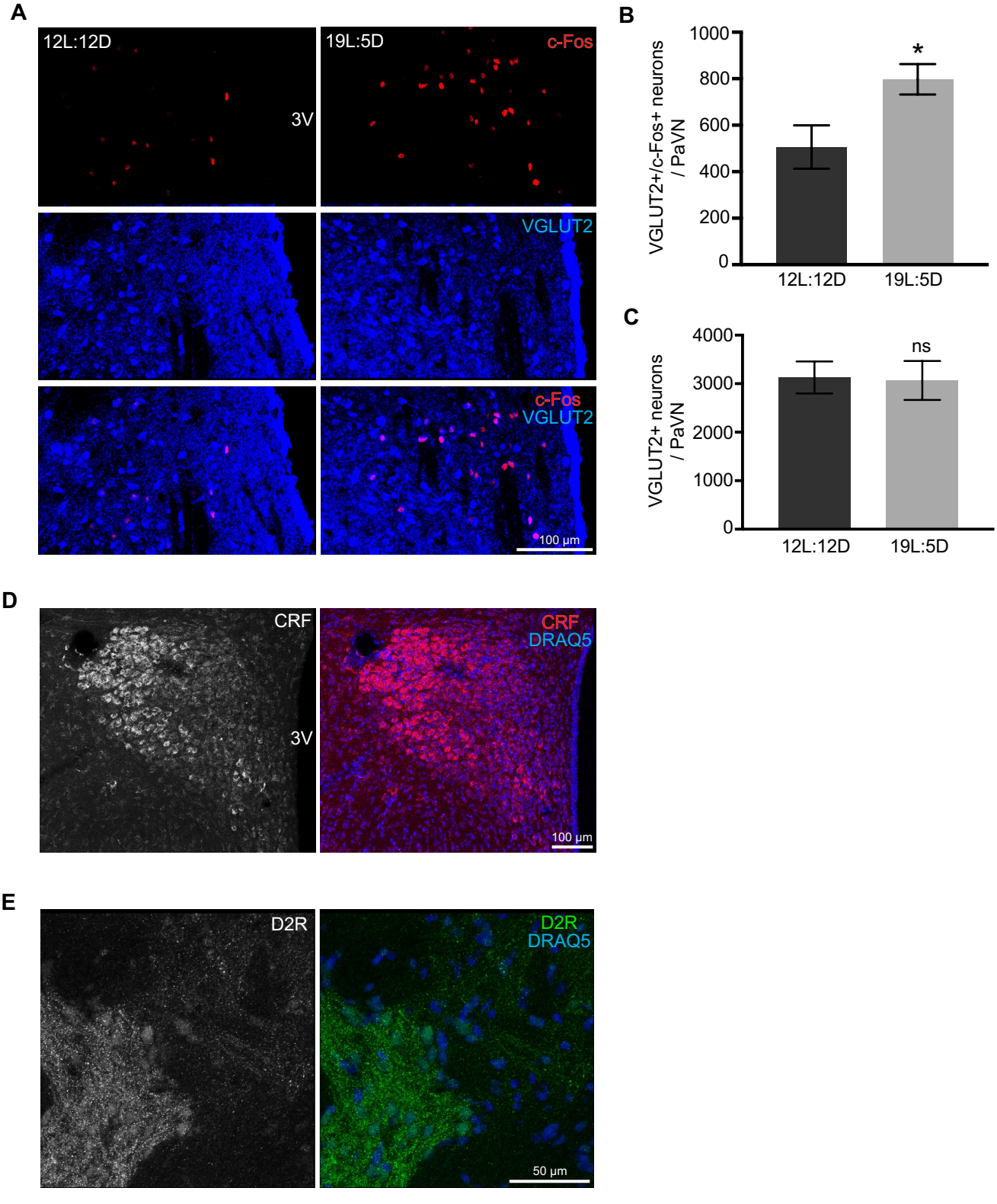


D

Percentage of EYFP+ neurons that are TH+	74.3 ± 2.2 %
Percentage of EYFP+ neurons that are TH-	25.7 ± 2.2 %

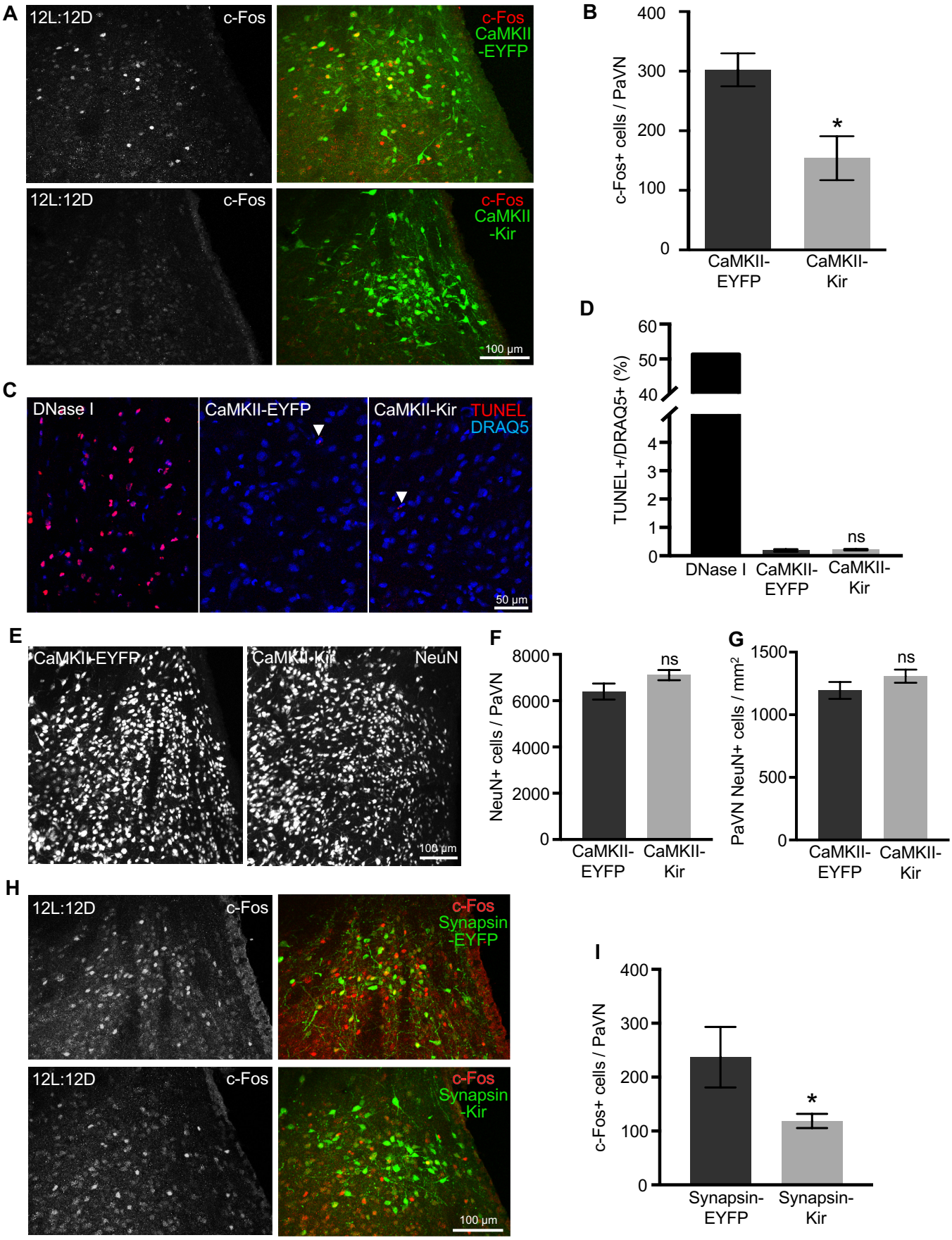
Supplementary Figure 1 Characterization of apoptosis and virus expression in the PaVN of TH-Cre rats (Related to Figures 1 and 2). (A) Brain sections of the virus-expressing side of the PaVN of TH-Cre rats were examined after 2-week 19L:5D or 12L:12D photoperiod exposure. TUNEL assay was used to detect apoptotic cells. Top: TUNEL+ signal. Bottom: Merged view of TUNEL, EYFP, and DRAQ5 DNA counterstain. Left: Positive control (DNase I treated); Middle: 12L:12D; Right: 19L:5D. (B) Quantification of the TUNEL assay showing the percentage of TUNEL+ cells of all DRAQ5+ cells per animal. 12L:12D, n=4 animals; 19L:5D, n=5 animals. 590-1073 cells were analyzed per animal. Welch's t test ($p=0.6633$). Data are mean ± SEM. ns, not significant. (C) The number of PaVN TH+/c-Fos+ cells in the PaVN of TH-Cre rats expressing AAV-DIO-hKir2.1 or AAV-DIO-EYFP virus after 4-day photoperiod exposure was examined by immunofluorescent staining and quantified (n=5 animals per condition). One-way ANOVA followed by Bonferroni post hoc analysis corrected for multiple comparison (12L:12D EYFP vs. 19L:5D EYFP, $p=0.0085$; 19L:5D EYFP vs. 19L:5D, Kir, $p=0.0002$; 12L:12D EYFP vs. 12L:12D Kir, $p=0.3753$; 12L:12D EYFP vs. 19L:5D Kir, $p=0.3753$). Data are mean ± SEM. **, $p<0.01$; ***, $p<0.001$; ns, not significant. (D) Percentage of Cre-dependent virus-expressing neurons (EYFP+) that express endogenous TH protein in TH-Cre rats 4 weeks after PaVN virus injection. n=4 animals per condition. 234-781 neurons were analyzed per animal. Data are mean ± SEM.

Figure S2


A
12L:12D
19L:5D
c-Fos
3V
VGLUT2
c-Fos
VGLUT2
100 µm
B
VGLUT2+/c-Fos+ neurons / PaVN
1000
800
600
400
200
0
*
12L:12D
19L:5D
C
VGLUT2+ neurons / PaVN
4000
3000
2000
1000
0
ns
12L:12D
19L:5D
D
CRF
3V
CRF
DRAQ5
100 µm
E
D2R
D2R
DRAQ5
50 µm

Supplementary Figure 2 Number and activity of PaVN glutamatergic neurons after long-day photoperiod exposure, and corroboration of antibodies against CRF and D2R in the PaVN (Related to Figure 3). (A) Immunofluorescent co-staining of VGLUT2 and c-Fos in the PaVN after 4-day 12L:12D or 19L:5D exposure. Top: c-Fos; Middle: VGLUT2; Bottom: merged view. (B) Quantification of the number of PaVN VGLUT2+/c-Fos+ neurons per animal after 4-day 12L:12D or 19L:5D exposure. 12L:12D, n=4 animals; 19L:5D, n=5 animals. Welch's t test ($p=0.0457$). Data are mean ± SEM. *, $p<0.05$. (C) Quantification of the number of PaVN VGLUT2+ neurons per animal after 2-week 12L:12D or 19L:5D exposure. 12L:12D, n=5 animals; 19L:5D, n=6 animals. Welch's t test ($p=0.9096$). Data are mean ± SEM. ns, not significant. (D) Confocal image of WT rat PaVN CRF neurons stained with guinea-pig anti-CRF antibody (T-5007; Peninsula, San Carlos, CA, 1:5000). Left: CRF; Right: merged view with CRF and DRAQ5. As expected, CRF neurons are most densely stained in the medial parvocellular division of PaVN and are sparsely distributed along the PaVN rostrocaudal axis (1). (E) Confocal image of D2R expression in the rat striatum stained with rabbit anti-D2 receptor antibody (AB5084P, Millipore, 1:500). D2R+ and D2R- patches in the striatum validate the affinity of the antibody (2). Left: D2R; Right: Merged view with D2R and DRAQ5.

Figure S3


A
12L:12D
c-Fos
c-Fos
CaMKII
-EYFP
12L:12D
c-Fos
c-Fos
CaMKII
-Kir
100 µm
B
c-Fos+ cells / PaVN
400
300
200
100
0
*
CaMKII-
EYFP
CaMKII-
Kir
C
DNase I
CaMKII-EYFP
CaMKII-Kir
TUNEL
DRAQ5
50 µm
D
TUNEL+/DRAQ5+ (%)
60
50
40
4
3
2
1
0
ns
DNase I
CaMKII-
EYFP
CaMKII-
Kir
E
CaMKII-EYFP
CaMKII-Kir
NeuN
100 µm
F
NeuN+ cells / PaVN
8000
6000
4000
2000
0
ns
CaMKII-
EYFP
CaMKII-
Kir
G
PaVN NeuN+ cells / mm²
1500
1000
500
0
ns
CaMKII-
EYFP
CaMKII-
Kir
H
12L:12D
c-Fos
c-Fos
Synapsin
-EYFP
12L:12D
c-Fos
c-Fos
Synapsin
-Kir
100 µm
I
c-Fos+ cells / PaVN
400
300
200
100
0
*
Synapsin-
EYFP
Synapsin-
Kir

Supplementary Figure 3 Neuronal activity and cell survival after suppressing activity of PaVN glutamatergic neurons or all neurons (Related to Figure 4). (A) Co-expression of c-Fos and viruses in the PaVN by immunofluorescence after suppressing activity of PaVN glutamatergic neurons. Left: c-Fos; Right: merged view of c-Fos and virus; Top: CaMKII-EYFP; Bottom: CaMKII-Kir. (B) Quantification of the number of PaVN c-Fos+ neurons per animal in CaMKII-EYFP versus CaMKII-Kir groups. CaMKII-EYFP, n=5 animals; CaMKII-Kir, n=6 animals. Welch's t test ($p=0.0109$). Data are mean ± SEM. *, $p<0.05$. (C) TUNEL assay was used to detect cell death after suppressing activity of PaVN glutamatergic neurons. TUNEL+ signal is shown in red and DRAQ5 DNA staining is shown in blue. White arrowheads indicate TUNEL+ cells. Left: Positive control (DNase I treated); Middle: CaMKII-EYFP; Right: CaMKII-Kir. (D) Quantification of the TUNEL assay showing the percentage of TUNEL+ cells of all DRAQ5+ cells per animal. CaMKII-EYFP, n=5 animals; CaMKII-Kir, n=4 animals. 698-1112 cells were analyzed per animal. Welch's t test ($p=0.5134$). Data are mean ± SEM. ns, not significant. (E) NeuN immunostaining was performed in the PaVN of CaMKII-EYFP (left) and CaMKII-Kir (right) groups. (F) Quantification of the number of PaVN NeuN+ cells per animal. CaMKII-EYFP, n=4 animals; CaMKII-Kir, n=3 animals. Welch's t test ($p=0.1445$). Data are mean ± SEM. ns, not significant. (G) Quantification of the number of PaVN NeuN+ cells per mm^2 per animal. CaMKII-EYFP, n=4 animals; CaMKII-Kir, n=3 animals. 5478-7533 cells were analyzed per animal. Welch's t test ($p=0.2401$). Data are mean ± SEM. ns, not significant. (H) Co-expression of c-Fos and viruses in the PaVN by immunofluorescence after suppressing activity of all PaVN neurons. Left: c-Fos; Right: merged view of c-Fos and virus; Top: Synapsin-EYFP; Bottom: Synapsin-Kir. (I) Quantification of the number of PaVN c-Fos+ cells per animal in Synapsin-EYFP versus Synapsin-Kir groups. Synapsin-EYFP, n=5 animals; Synapsin-Kir, n=10 animals. Unpaired t test ($p=0.0159$). Data are mean ± SEM. *, $p<0.05$.

References

1. Simmons DM, Swanson LW (2009) Comparison of the spatial distribution of seven types of neuroendocrine neurons in the rat paraventricular nucleus: Toward a global 3D model. *J Comp Neurol* 516:423–441.

2. Hurd YL, Suzuki M, Sedvall GC (2001) D1 and D2 dopamine receptor mRNA expression in whole hemisphere sections of the human brain. *J Chem Neuroanat* 22:127–137.