iScience, Volume *23*

Supplemental Information

A Glial-Neuronal Circuit in the Median Eminence Regulates Thyrotropin-Releasing Hormone-Release via the Endocannabinoid System

Erzsébet Farkas, Edina Varga, Balázs Kovács, Anett Szilvásy-Szabó, Antonieta Cote-Vélez, Zoltán Péterfi, Magdalini Matziari, Mónika Tóth, Dóra Zelena, Zsolt Mezriczky, Andrea Kádár, Dóra Kővári, Masahiko Watanabe, Masanobu Kano, Ken Mackie, Balázs Rózsa, Yvette Ruska, Blanka Tóth, Zoltán Máté, Ferenc Erdélyi, Gábor Szabó, Balázs Gereben, Ronald M. Lechan, Jean-Louis Charli, Patricia Joseph-Bravo, and Csaba Fekete

Supplementary Figures

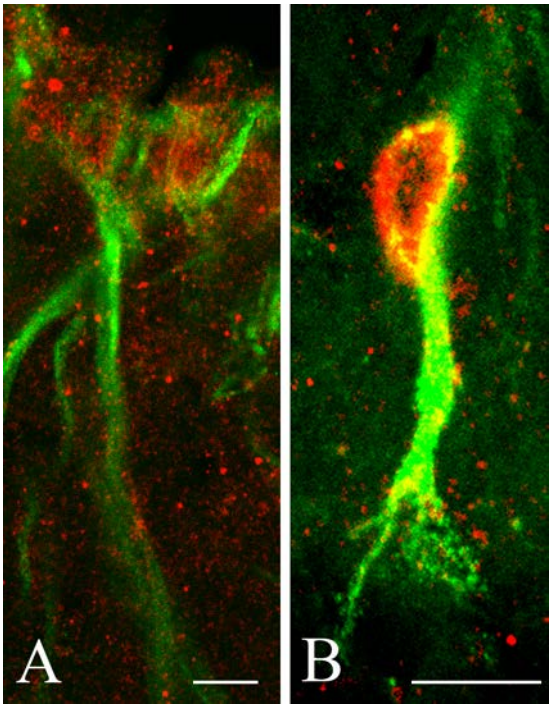

Figure S1. Expression of DAGLα in tanycytes of the rat median eminence. **Related to Figure 1.** DAGLα-immunoreactivity (red) is present in the cell bodies and basal process of vimentin-immunoreactive (green) β2- (A) and γ-tanycytes (B). γ-tanycytes are located in the external zone of the median eminence (Wittmann et al., 2017).

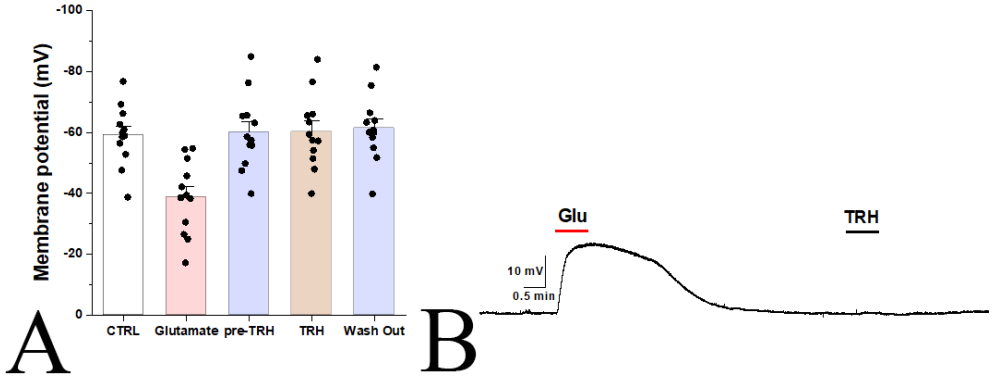

Figure S2. Effect of TRH on the membrae potential of tanycytes (N=12). **Related to Figure 3.** Bar graphs (A) show the effects of glutamate (500µM) and TRH (1µM) on the membrane potential of β2-tanycytes. While glutamate (500µM) caused a marked depolarization of tanycytes, TRH administration had no effect. A representative trace (B) illustrates the effects of glutamate and TRH on a β2-tanycyte.

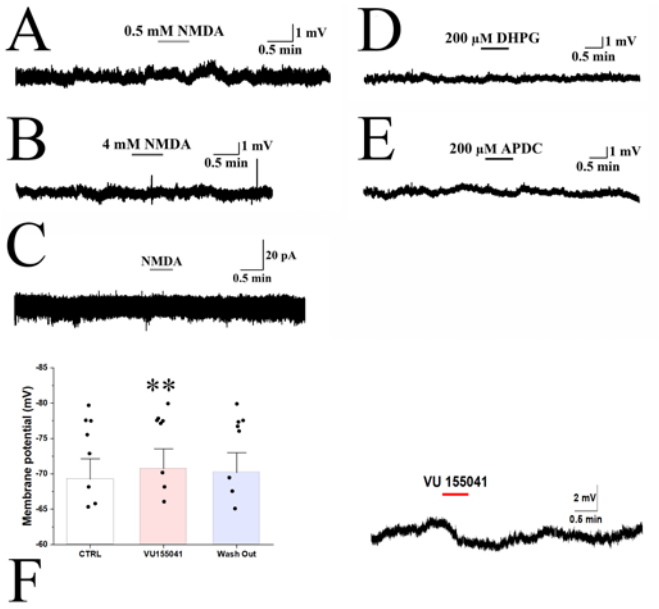

Figure S3. Effect of NMDA and metabotropic glutamate receptor agonists on the membrane potential of the β2-tanycytes. **Related to Figure 3.** Representative traces demonstrate that neither (A) 0.5 mM nor (B) 4 mM NMDA had effect on the membrane potential of β2-tanycytes. To exclude the possibility that the lack of NMDA effect is due to the magnesium block of the receptor at the resting membrane potential, the effect of NMDA was tested on β2-tanycytes held at -40 mV. (C) NMDA (0.5 mM) had also no effect on the β2-tanycytes under this condition. Neither Type I (D) nor Type II (E) metabotropic glutamate receptor agonists had an effect on the membrane potential of tanycytes. In contrast, the GRM4 agonist VU155041 (1mM) caused a small, but significant hyperpolarization of β2-tanycytes (n=10). Data are shown as mean ± SEM, for statistical comparison repeated measure ANOVA was used followed by Bonferroni post hoc test. ** = significantly different from control $P < 0.01$.

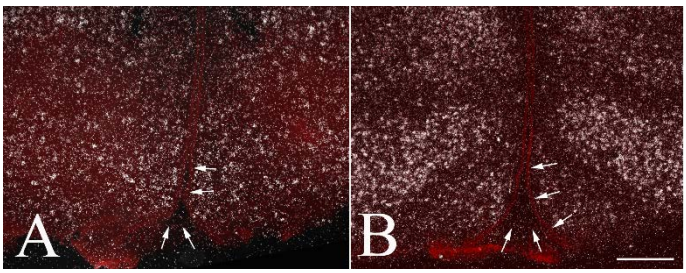

Figure S4. Distribution of *GRM1* and *GRM5* mRNA in the hypothalamus. **Related to Table 1**. Images of in situ hybridization for (A) *Grm1* and (B) GRM5 demonstrate the absence of hybridization signal (silver grains) for the tanycytes (arrows) lining the floor and lateral wall of the third ventricle. cresyl-violet counterstaining (red) labels the cell nuclei. Scale bar = 200µm

Table S1

Assay ID	Gene Symbol(s)	Gene Name(s)
18S-Hs99999901_s1	18S	18s rRNA
Mm02619580_g1	Actb	actin, beta
Mm03302249_g1	Gapdh	glyceraldehyde-3-phosphate dehydrogenase
Mm03024075_m1	Hprt	hypoxanthine guanine phosphoribosyl transferase
Mm00813830_m1	Dagla	diacylglycerol lipase, alpha
Mm00515664_m1	Dio2	deiodinase, iodothyronine, type II
Mm00436590_m1	Slc1a1	solute carrier family 1, member 1
Mm01275814_m1	Slc1a2	solute carrier family 1, member 2
Mm00600697_m1	Slc1a3	solute carrier family 1, member 3
Mm01173279_m1	Slc1a6	solute carrier family 1, member 6
Mm00525562_m1	Slc1a7	solute carrier family 1, member 7
Mm00433753_m1	Gria1	glutamate receptor, ionotropic, AMPA1 (alpha 1)
Mm00442822_m1	Gria2	glutamate receptor, ionotropic, AMPA2 (alpha 2)
Mm00497506_m1	Gria3	glutamate receptor, ionotropic, AMPA3 (alpha 3)
Mm00444754_m1	Gria4	glutamate receptor, ionotropic, AMPA4 (alpha 4)
Mm00446882_m1	Grik1	glutamate receptor, ionotropic, kainate 1
Mm00599860_m1	Grik2	glutamate receptor, ionotropic, kainate 2 (beta 2)
Mm01179716_m1	Grik3	glutamate receptor, ionotropic, kainate 3
Mm00615472_m1	Grik4	glutamate receptor, ionotropic, kainate 4
Mm00433774_m1	Grik5	glutamate receptor, ionotropic, kainate 5 (gamma 2)
Mm00433790_m1	Grin1	glutamate receptor, ionotropic, NMDA1 (zeta 1)
Mm00433802_m1	Grin2a	glutamate receptor, ionotropic, NMDA2A (epsilon 1)
Mm00433820_m1	Grin2b	glutamate receptor, ionotropic, NMDA2B (epsilon 2)
Mm00439180_m1	Grin2c	glutamate receptor, ionotropic, NMDA2C (epsilon 3)
Mm00433822_m1	Grin2d	glutamate receptor, ionotropic, NMDA2D (epsilon 4)
Mm01341723_m1	Grin3a	glutamate receptor, ionotropic, NMDA3A
Mm00504568_m1	Grin3b	glutamate receptor, ionotropic, NMDA3B
Mm01187089_m1	Grm1	glutamate receptor, metabotropic 1
Mm01235831_m1	Grm2	glutamate receptor, metabotropic 2
Mm00725298_m1	Grm3	glutamate receptor, metabotropic 3

Mm01306128_m1	Grm4	glutamate receptor, metabotropic 4
Mm00690332_m1	Grm5	glutamate receptor, metabotropic 5
Mm00841148_m1	Grm6	glutamate receptor, metabotropic 6
Mm01189424_m1	Grm7	glutamate receptor, metabotropic 7
Mm00433840_m1	Grm8	glutamate receptor, metabotropic 8
Mm00443262_m1	TRHR1	thyrotropin-releasing hormone receptor 1
Mm01241598_m1	TRHR2	thyrotropin-releasing hormone receptor 2

Table S1. The table summarizes the assay ID, the symbol and the name of the genes included in the Custom TaqMan Gene Expression Array Cards that was used to investigate the presence and absence of glutamate transporters and glutamate receptor subunits in β-tanycytes. **Related to Table 1.**

The 18s ribosomal RNA, the actin beta (*Actb*), the glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) and the hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) served as housekeeping genes. Diacylglycerol lipase alpha (*Dagla*) and *Dio2* served as positive controls.

Supplementary results

Presence of DAGLα in the tanycytes of the rat median eminence

Similarly to that observed in mice, DAGLα-immunoreactivity was observed in the cell bodies of tanycytes lining the ventricular wall of the median eminence (Fig. S1A) and the basal processes of these cells. The cell bodys of these tanycytes had, however, stronger DAGLα-immunoreactivity than the cell bodies of tanycytes in mice. In addition, the γ-tanycytes, that are located in the external zone of the median eminence (Wittmann et al., 2017), were strongly labeled with DAGLα-immunoreactivity (Fig. S1B)

Effects of NMDA and metabotropic glutamate receptor agonists on the β2 tanycytes of mice

As magnesium can block the NMDA receptors when the membrane potential is low, like the membrane potential of tanycytes, the effect of NMDA was also tested in voltage-clamp mode at -40 mV holding potential, where the NMDA receptors relieved from the magnesium block (Mayer et al., 1984). However, NMDA had no effect on the β2-tanycytes even under this condition (control: 6.14±7.58 pA, n=3 vs. 0.5 mM NMDA: 13.43±15.66 pA, n=3, P=1.00; Fig. S3) suggesting that the tanycytes do not have functional NMDA receptors.

Similarly, treatment of tanycytes with agonists of Type I (DHPG, used in mM: 0.1, 0.2, 1, 2 and 2.5; n=6), Type II (APDC, used in mM: 0.2, 2 and 2.5; n=5) metabotropic glutamate receptors had no effect on the membrane potential of β2-tanycytes (Fig. S3).

Grm1 and Grm5 mRNA levels are under the detection limit in tanycytes

While high level of Grm1 and Grm5 mRNA are detectable in the neuropil around the third ventricle, silver grains denoting the in situ hybridization signal were not observed above the cell bodies of tanycytes (Fig. S4).

Transparent Methods

Animals

Adult, male CD1 mice weighing 28-32 g were used for the morphological studies. Male C57Bl/6J mice between P40 and P60 days of age were used for *in vitro* patch clamp electrophysiology studies. Adult, male TRH-IRES-Cre mice between P55 and P70 days of age were used for the optogenetic studies. Rax/CreERT2//Gt(ROSA)26Sor_CAG/LSL_ZsGreen1 were used in studies examining the presence of DAGLα in the β-tanycytes in mice. DAGLα mice were used to demonstrate the DAGLα-immunoreactivity in the median eminence. Mice were housed under standard conditions (lights on between 06.00 and 18.00 h, temperature 22±1 °C, chow and water *ad libitum*). All experimental protocols were reviewed and approved by the Animal Welfare Committees at the Institute of Experimental Medicine of the Hungarian Academy of Sciences.

Male Wistar rats P80 days old, raised as an outbred colony at the Institute of Biotechnology, UNAM, and 250g male Sprague-Dawley rats from AnimaLab Hungary Kft. (Budapest, Hungary) were used for median eminence explant experiments and study the presence of DAGLα-immunoreactivity in the tanycytes of rats. They were kept under controlled temperature (20-22 °C), lights on from 7:00 to 19:00 h, with water and food (Harlan 2018S) *ad libitum*.

All experimental protocols were reviewed and approved by the Animal Welfare Committee at the Institute of Experimental Medicine of the Hungarian Academy of Sciences or by the Bioethics Committee of the Institute of Biotechnology, UNAM.

Generation of the TRH-IRES-Cre mouse line

An IRES Cre-recombinase cassette was inserted into the mouse TRH locus using CRISPR/Cas9 on an FVB.129P2-Pde6b^{+} Tyr$^{c\text{-}ch/}$AntJ (FVB/Ant) background. This was performed by the

modification of the targeting construct that was recently used to create the TRH-IRES-tdTomato mouse using 1473 bp long 5' and the 1375 bp long 3' TRH arms (Varga et al., 2019). The targeting construct was cut with by EcoRI and NotI to remove the TdTomato cassette. A Nuclear Localization Signal (NLS) containing Cre-recombinase encoding cassette was generated with Vent PCR on a Turbo-Cre plasmid (kindly porvided by Dr Ley (Revell et al., 2005)). (sense oligo: ggaattcactATGCCCAAGAAGAAGAGGAAGGTGTCCAATT; antisense oligo: ataagaatgc ggccgc CTAATCGCCATCTTCCAGCAGGCGCA) followed by the insertion of the NLS-Cre cassette between EcoRI and NotI of the targeting construct. The construct was confirmed by sequencing.

Pronuclear microinjection was carried out on fertilized eggs of FVB/Ant mice, using of a single guide RNA with the target sequence of GGAGTAAGGTTAGAGTC and Cas9 mRNA (Trilink).

Founders were identified with PCR checking the insertion with outer and inner PCR oligos (outer antisense: CTTCCATGAGAGGAGTATTTATCA; NLS-Cre sense1: GCTGGAAGATGGCGATTAG; NLS-Cre sense2: GAAGCAACTCATCGATTGATT).

A founder with a single copy of the targeting cassette was selected for breeding.

Heterozygote F1 animals were crossbred with littermate animals of identical genotype. Mice were bred and maintained as homozygous colonies.

Tissue fixation and preparation for morphological studies

For morphological studies the animals were anaesthetized with a mixture of ketamine and xylazine (ketamine 50 mg/kg, xylazine 10 mg/kg body weight, i.p.) and perfused transcardially with fixative solution. The details of tissue fixation and tissue preparation are described below.

For *in situ* hybridization (N=4), mice were perfused transcardially with 10 ml 0.01 M phosphate-buffered saline pH 7.4 (PBS), followed by 50 ml 4% paraformaldehyde in 0.1 M

phosphate buffer pH 7.4 (PB) (all solutions were RNAse-free). The brains were rapidly removed and were cryoprotected in 20% sucrose in 0.01 M PBS overnight at 4 ^{o}C. The brains were then snap frozen with powdered dry ice and stored at -80 ^{o}C until used.

For light microscopy, the anaesthetized mice (N=9) and rats (N=3) were perfused transcardially with 10 ml 0.01 M PBS, followed by 50 ml 4% paraformaldehyde in 0.1M PB or by 150 ml 4% paraformaldehyde in 0.1M PB, respectively. The brains were rapidly removed and were cryoprotected in 30% sucrose in 0.01 M PBS overnight at room temperature (RT). The brains were then snap frozen with powdered dry ice and stored at -80 ^{o}C until used.

For electron microscopy (N=3), the anaesthetized animals were perfused transcardially with 10 ml 0.01 M PBS, followed by 50 ml 4% acrolein in 2% paraformaldehyde in 0.1 M PB.

For light microscopic immunocytochemistry or for immunofluorescence, serial, 25 µm thick coronal sections were cut through the median eminence (ME) using freezing microtome (Leica Microsystems, Wetzlar, Germany). Series of sections, obtained at 100 µm intervals, were collected into antifreeze solution (30% ethylene glycol; 25% glycerol; 0.05 M PB) and stored at -20 °C until their use for immunohistochemistry. The sections were then pretreated first with 0.5% Triton X-100 and 0.5% H_2O_2 in 0.01 M PBS for 15 min. Nonspecific antibody binding was blocked with treatment in 2% normal horse serum (NHS) in PBS for 20 min at room temperature (RT).

For ultrastructural studies, serial 25 µm thick coronal sections were cut on a Leica VT 1000S vibratome (Leica Microsystems, Wetzlar, Germany) through ME. The sections were treated with 0.5% H_2O_2 in PBS for 15 min, cryoprotected in 15% sucrose in PBS for 15 min at RT and in 30% sucrose in PBS overnight at 4 °C. Then, the sections were quickly frozen over liquid nitrogen and thawed. This cycle was repeated three times to improve antibody penetration.

Single-labeling immunocytochemistry for DAGLα

One-in-four series of sections from each brain and sections from 2 DAGLα mice were incubated in guinea-pig anti DAGLα serum (Keimpema et al., 2013) at 1:500 dilutions in 2% NHS overnight at RT. Then, sections were incubated in a biotinylated donkey anti guinea-pig IgG (1:500, Jackson) in 2% NHS for 2 h. After washing steps in PBS and treatment with avidin-biotin-peroxidase complex (ABC 1:1000, Vector lab, Vectastain kit), the DAGLα-immunoreactivity was detected in Ni-DAB developer (0.05% DAB/0.15% Ni-ammonium-sulfate/0.005% H_2O_2 in 0.05 M Tris buffer, pH 7.6). Then, the sections were mounted onto glass slides and coverslipped with DPX mounting medium (Sigma). Immunoreaction signal was completely absent from the ME of DAGLα mice.

Detection of DAGLα-immunoreactivity in the tanycytes of the ME in mice and rats

To detect the tanycytes in the median eminence of mice Rax/CreERT2 mice (Gift from Dr Blackshaw (Pak et al., 2014)) were crossed with Gt(ROSA)26Sor_CAG/LSL_ZsGreen1 mice (Jackson Laboratoratory, Bar Harbor, ME). The ZsGreen1 expression of tanycytes was induced in the resulted double transgenic Rax/CreERT2//Gt(ROSA)26Sor_CAG/LSL_ZsGreen1 mice by tamoxifen treatment. Tamoxifen (175 mg/kgBW) dissolved in corn oil was administered daily for 4 days by oral gavage. The day after the last tamoxifen treatment, the mice were anaesthetized, perfused transcardially with 4% PFA and the brains were sectioned with freezing microtome as described above. After pretreatment, one-in-four series of sections from each brain were incubated in rabbit anti-DAGLα antibody (Katona et al., 2006) in 2µg/ml concentration overnight at RT. Then the sections were incubated in Alexa 555-conjugated donkey anti rabbit IgG (1:500, Invitrogen) for 2h, mounted onto glass slides and coverslipped with Vectashield mounting medium (Vector).

To detect the tanycytes in the median eminence of rats, one-in-four series of sections from each brain were incubated in a mixture of rabbit anti-DAGLα antibody (Katona et al., 2006) in 2µg/ml concentration and goat anti vimentin antibody (Santa Cruz Biotech) at 1:3000 dilution overnight at RT. Then the sections were incubated in a mixture of Alexa 555-conjugated donkey anti rabbit IgG (1:500, Invitrogen) and Alexa 488-conjugated donkey anti sheep IgG (1:500, Invitrogen) for 2h, mounted onto glass slides and coverslipped with Vectashield mounting medium (Vector). Nikon A1R confocal microscope was used for imaging.

Triple-labeling immunofluorescence for CB1, DAGLα and TRH

One-in-four series of sections from each brain were incubated in goat anti-CB1 antibody at 1:1000 dilution (Makara et al., 2007), guinea-pig anti-DAGLα antibody (Malenczyk et al., 2015) at 1:500 and rat anti-mouse proTRH 178-200 serum (Peterfi et al., 2018) at 1:250 in 2% NHS overnight at RT. Then, the sections were incubated in biotinylated donkey anti guinea-pig IgG (1:500) for 1 h, at RT followed by incubation in a mixture of Streptavidin conjugated cy5 (1:250, Jackson), Alexa 555 conjugated donkey anti-sheep IgG (1:500, Invitrogen) and Alexa 488 conjugated donkey anti-rat IgG (1:250, Invitrogen) for 2 h, mounted onto glass slides and coverslipped with Vectashield mounting medium (Vector). Images were taken using Zeiss LSM 780 confocal microscope (Zeiss Company, Jena, Germany) using line by line sequential scanning with laser excitation lines 410-483 nm for Dylight 405, 490-553 nm for Alexa Fluor 488 and 566-697 nm for Alexa Fluor 555; beamsplitter/emission filters, MSB 405 nm for Alexa Fluor 405, MSB488/561 nm for Alexa Fluor 488 and Alexa Fluor 555. For 40x and 60x oil lenses, pinhole sizes were optimized resulting in 0.9 µm thin optical slices, respectively. The series of optical sections were recorded with 0.7 µm Z steps. Images were analyzed with Zen 2012 (Zeiss Company, Jena, Germany) and with Adobe Photoshop (Adobe System Inc., California, US.).

Single-labeling immuno-electron microscopy using silver-intensified Ni-DAB chromogen

One-in-four series of pretreated sections from each brain were incubated in guinea-pig anti DAGLα serum at 1:500 dilution in 2% NHS for 4 days at 4 °C. After rinsing in PBS, the sections were incubated in biotinylated donkey anti-guinea-pig IgG (1:500, Jackson) diluted in 2% NHS for 2 h at RT, followed by treatment in ABC (1:1000, Vector lab). The DAGLα-immunoreactivity was NiDAB developed. The resultant reaction product was silver-gold-intensified using the Gallyas method (Liposits et al., 1982). The sections were osmicated for 1h at RT, and then treated with 2% uranyl acetate in 70% ethanol for 30 min. Following dehydration in an ascending series of ethanol and acetonitrile (Sigma Aldrich), the sections were flat embedded in Durcupan ACM epoxy resin (Sigma Aldrich) on liquid release agent (Electron Microscopy Sciences) coated slides and polymerized at 56 °C for 2 days. After polymerization, 60–70 nm thick ultrathin sections were cut with Leica ultracut UCT ultramicrotome (Leica Microsystems, Wetzlar, Germany). The ultrathin sections were mounted onto Formvar-coated, single slot grids, contrasted with 2% lead citrate (Sigma Aldrich, Hungary) and examined with a JEOL-100 C transmission electron microscope.

Specificity of the antibodies

The specificity of the goat anti-CB1 antibody was proved in CB1 KO animals (Hajos et al., 2000), and the specificity of the rat anti-mouse proTRH 178-200 serum was demonstrated by double labeling immunofluorescence showing that the rat anti-mouse proTRH 178-200 antiserum and the sheep TRH antiserum had identical distribution (Peterfi et al., 2018). The specificity of the guinea-pig anti-DAGLα serum was established by the complete absence of the immunoreaction signal in the median eminence of mice when sections of DAGLα knock-out mice (Tanimura et al., 2010) were used.

Double labeling in situ hybridization for TRH and CB1 mRNAs

Coronal, 20 µm thick sections were cut with a cryostat (Leica) through the antero-posterior extent of the PVN. The sections were collected in an RNAse free cryoprotective solution (30% ethylene glycol; 25% glycerol; 0.05 mM PB) and stored at -20 °C until use.

Serial sections were washed in 2× SSC, acetylated with 0.25% acetic anhydride (Sigma-Aldrich) in 0.9% triethanolamine (Sigma-Aldrich) for 20 min. To facilitate the penetration of labeled RNA probes, the sections were delipidated by treatment in 50%, 70% and again 50% acetone (Sigma-Aldrich). After additional washes in 2×SSC for 5 min, the sections were hybridized with the mixture of digoxigenin-labeled cRNA probe for proTRH and [35S]UTP-labeled cRNA probe for CB1.

The hybridization was performed for 16 h at 56 °C in 200 µl polypropylene tubes in a hybridization buffer (50% formamide, 2× SSC, 10% dextran sulfate, 0.5% SDS, and 250 µg/ml denatured salmon sperm DNA) that contained the digoxigenin-labeled proTRH probe diluted 1:50 and 50000 cpm/µl of [35S]-labeled CB1 cRNA probe. The sections were washed in 1× SSC for 15 min and then treated with 25 µg/ml RNase for 1 hour at 37 °C. After additional washes in 0.1× SSC (four times for 15 min) at 65 °C, sections were washed in PBS, treated with a mixture of 0.5% Triton X-100 and 0.5% H_2O_2 for 15 min, and then treated with 2% bovine serum albumin (BSA) in PBS for 20 min to reduce the nonspecific antibody binding. The sections were incubated with sheep anti-digoxigenin-peroxidase Fab fragments (1:100; Boehringer Mannheim Corp., Indianapolis, IN) in 1% BSA in PBS overnight at 4 °C. The sections were then rinsed in PBS and incubated in 0.1% biotinylated tyramide and 0.01% H_2O_2 in PBS for 10 min to intensify the hybridization signal. After additional washes, the sections were incubated in streptavidin conjugated Alexa 555 (1:250; Vector Laboratories, Burlingame, CA) and mounted on gelatin coated glass slides. The slides were dipped into Kodak NTB

autoradiography emulsion (Eastman Kodak, Rochester, NY), and the autoradiograms were developed after 3 weeks of exposure at 4 °C. The specificity of hybridization was confirmed using sense probes, which resulted in the absence of specific hybridization in the paraventricular nucleus (PVN).

Both sides of the PVN from three sections of each brain containing the mid-level of PVN were imaged with a Zeiss AxioImager M1 microscope equipped with MRC5 digital camera (Carl Zeiss) using a 20X objective. Both darkfield and epifluorescent images of the same field were taken. The images were overlaid in Adobe Photoshop CS5 software (Adobe Systems Inc.). The ratio of red fluorescent TRH neurons that had accumulation of silver grains were counted.

Determination of the role of the endocannabinoid system in the regulation of TRH release in the ME

To understand the effects of endocannabinoids on the TRH release of median eminence explants, a total of 70 WISTAR rats were used in two independent experiments. Rats were individually transported from their nearby house-room to the procedural room and decapitated, by an experienced technician, with a sharp guillotine; between each killing the area was thoroughly cleaned. Each brain was excised from skull, taking care first to cut the optic nerve to preserve ME intact. Under a stereo microscope, the ME together with part of the arcuate nucleus was dissected. Explants were incubated as described (Joseph-Bravo et al., 1979) with the following modifications: two explants were placed together in a microcentrifuge tube containing 300 µl of Hank's solution saturated with 95% O_2/5% CO_2, kept at 4 ^oC until end of dissections (max. 40 min); medium was then replaced with 200 µl fresh artificial CSF medium (ACSF) saturated with 95% O_2/5% CO_2 containing in mM: 125 NaCl, 2.5 KCl, 25 glucose, 25 $NHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, pH 7.4 and 200 nM of a phosphinic analogue of TRH (GlpΨ[P(O)(OH)]HisProNH2; P-TRH), a TRH-DE inhibitor (Matziari et al., 2008).

Explants were pre-incubated at 37 ºC for 10 min. After preincubation, medium was replaced with 200 µl of ACSF-P-TRH and the drugs (Sigma-Aldrich) under study: CB1 antagonist, AM125 at 1 µM (Kola et al., 2008); CB1 agonist, WIN55,212-2 at 1 µM (Farkas et al., 2010); DAGLα inhibitor, tetrahydrolipstatine (THL) at 10 µM (Farkas et al., 2010); either alone or combined as stated in Fig. 2; control medium was ACSF-P-TRH. Drugs were initially dissolved in DMSO (final concentration: 0.0025%). Explants were incubated for 10 min at 37 ºC; then, 170 µl of medium were recovered into 800 µl of cold methanol. Another 200 µl of ACSF-P-TRH and respective drug was added and the explants incubated 10 more min at 37 ºC; afterwards a 170 µl aliquot of medium was recovered into 800 µl of cold methanol. Tubes containing medium/methanol were kept overnight at -20 °C, centrifuged at 12100 g for 30 min at 4 ºC and supernatant evaporated. Residues were resuspended in 90% methanol, kept overnight at -20 °C, centrifuged, supernatant evaporated and TRH quantified by radioimmunoassay (RIA) using the R2 antibody (Joseph-Bravo et al., 1979). The amount of TRH detected in the two subsequent incubates was added, and the average quantity detected in control tubes considered 100%; each value was calculated as % of control. The explant was recovered and extracted as reported to determine the residual intracellular level of TRH (Joseph-Bravo et al., 1979).

Determination of the role of endogenous glutamate in the regulation of 2-AG content of the ME

To elucidate the effect of glutamate on the 2-AG content of the median eminence, 14 Sprague-Dawley rats were used. ME explants were prepared as described above. One explant was placed in each microcentrifuge tube containing 300 µl of Hank's solution saturated with 95% O_2/5% CO_2, kept at on ice until end of dissections (max. 40 min); medium was then replaced with 200

µl fresh ACSF saturated with 95% O_2/5% CO_2. Explants were pre-incubated at 37 ^oC for 10 min. After preincubation, medium was replaced with 200 µl of ACSF+1%DMSO or 200 µl of ACSF+1% DMSO containing the mixture 500 µM DNQX and 1mM TBOA to block the effects of glutamate on the tanycytes. After 10 min incubation, 170µl solution was removed and replaced with 200µl similar solution. All solutions were saturated with 95% O_2/5% CO_2.

After additional 10 min incubation, all solutions were removed and the ME samples were frozen on powdered dry ice.

To measure endocannabinoid levels, we used a PerkinElmer Life and Analytical Sciences HPLC Series 200 system, which was coupled to an Applied Biosystems/Sciex 4000 QTRAP triple quadrupole/linear ion trap tandem mass spectrometer operated in positive electrospray ionization mode. The electronspray ionization ion source parameters were set as follows: curtain gas, 10; ion spray voltage, 5000 V; temperature, 500°C; collisionally activated dissociation gas, medium; gas 1, 50; gas 2, 40. Chromatographic separation was achieved with a Phenomenex Kinetex C18 column (50mmx3.00 mm) using methanol and 10 mM ammonium formate as elution solvents at a flow rate of 500 l/min. The injection volume was 50 µl. The initial eluent condition was 80% methanol/20% buffer that was increased to 85% organic phase during 3 min and then further elevated to 95% during 2 min and was kept at this condition for 2 min. Afterward, the column was equilibrated to the initial condition. Analytes were detected in multiple reaction monitoring (MRM) mode at the following ion transitions and parameter settings: (1) 2-AG, MRM transition [mass/charge ratio (m/z), 379.4 - 287.2, 379.4 - 91.1], declustering potential (81 V), collision energy (23 V, 81 V), cell exit potential (10 V, 8 V); (2) 2-arachidonoylglycerol-d5, MRM transition (m/z, 384.4 - 287.2, 384.4 - 91.1), declustering potential (81 V), collision energy (23 V, 81 V), cell exit potential (10 V, 8 V). The peak areas were determined with Analyst 1.4.2. software. The quantity of the analytes was calculated by comparing their peak areas with those of the deuterated internal standards, and it was

normalized to the sample weight. All endocannabinoid standards were purchased from Cayman Chemical. HPLC gradient-grade methanol was supplied by Merck. Ammonium formate was from Sigma. Water was purified with a MilliQ Direct 8 system (Millipore).

Sample preparation for chromatography started with tissue incubation on ice for 5 min in 100 µl of methanol containing the deuterated internal standards 2-arachidonoylglycerol- d5 (100 ng/ml). After ultrasonic homogenization, samples were centrifuged on an Eppendorf miniSpin microtube centrifuge at 13,400 rpm for 15 min. The supernatants were diluted to initial HPLC eluent composition with 10 mM ammonium formate solution.

In situ hybridization detection of Grm1 and Grm5 mRNAs

Serial, 12 µm thick coronal sections of fresh frozen brains of adult male CD1 mice were cut with a cryostat (Leica Microsystems GmbH, Wetzlar, Germany) through the antero-posterior extent of the median eminence. The sections were mounted on Superfrost Plus slides (Fisher, Hampton, NH), and dried at 42 ^{o}C overnight, as described (Hrabovszky and Petersen, 2002). On the day of hybridization, the sections were fixed with 4% paraformaldehyde in PBS (pH 7.4) for 1 h, washed in 2-fold concentration of standard sodium citrate (2× SSC), acetylated with 0.25% acetic anhydride in 0.9% triethanolamine for 20 min, and then treated in graded solutions of ethanol (70, 80, 96, and 100%), chloroform, and a descending series of ethanol (100 and 96%) for 5 min each, and hybridized with *Grm1* or *Grm5* single-stranded ^{35}S-UTP labeled cRNA probes. The hybridizations were performed under plastic coverslips in a buffer containing 50% formamide, 2-fold concentration of SSC (2× SSC), 10% dextran sulfate, 0.5% sodium dodecyl sulfate, 250 µg/ml denatured salmon sperm DNA, and $5x10^4$ cpm/µl radiolabeled probe for 16 h at 56 ^{o}C. The slides were washed in 1× SSC for 15 min and then treated with RNase (25 µg/ml) for 1 h at 37 ^{o}C, followed by additional washes in 0.1× SSC (2

× 30 min) at 65 ºC. After dehydration in graded dilutions of ethanol, the slides were dipped into Kodak NTB autoradiography emulsion (Eastman Kodak, Rochester, NY), and the autoradiograms were developed after 6-wk exposure at 4 ºC. The specificity of hybridization was confirmed using sense probes that resulted in the complete absence of hybridization signal in the brain. Sections were immersed in 0.0005% cresyl violet acetate (Sigma-Aldrich) for 2 minutes to obtain fluorescent labeling of cell nuclei, dehydrated in ascending ethanol series and xylenes, and coverslipped with DPX mountant (Alvarez-Buylla et al., 1990).

Examination of the glutamate receptor and transporter expression in β-tanycytes

Adult, male, CD1 mice were transcardially perfused with ice-cold 10% RNAlater solution (Ambion) diluted in DEPC treated 0.1M PBS. Brains were quickly removed and placed into -35-40 °C cold 2-methylbutane (Sigma-Aldrich), cooled down with a mixture of 100% ethanol and dry ice. Brains were kept in -80 °C freezer until sectioning.

12 µm coronal brain sections were cut at -18 °C using a cryostat (Leica, Germany). Sections were mounted on PEN-membrane slides (Zeiss, Germany), thawed and counterstained with 0.6% cresyl violet dissolved in 70% ethanol. Then, the sections were dehydrated in a series of ascending ethanol and stored at -80 °C until further processing.

The sections were thawed under vacuum and the cell bodies of β-tanycytes lining the floor of the third ventricle were microdissected using Zeiss Microbeam Laser Capture Microdissection (LCM) system and PALM software. The β-tanycyte samples were pressure-catapulted with a single laser pulse into 0.5 ml adhesive cap tubes (Carl Zeiss Microimaging) using a ×20 objective lens.

RNA isolation was performed from the isolated β-tanycytes using Arcturus PicoPure RNA Isolation Kit (Applied Biosystems). To remove potential genomic DNA contamination, DNase treatment was carried out on the RNA purification column using RNase-Free DNase Set (Qiagen). The quality and the concentration of the isolated RNA samples were measured with

Bioanalyzer using Agilent RNA 6000 Pico Kit and 2100 Expert software (Agilent Technologies).

The RNA samples were transcribed using ViLO Superscript III cDNA Reverse Transcription Kit (Invitrogen) according to the manufacturer's protocol. The cDNA concentration was measured with Qubit® Fluorometer by using Qubit® ssDNA Assay Kit (Invitrogen). The cDNA product served as a template for preamplification by using Preamp Master Mix Kit (Applied Biosystems) and the double stranded DNA concentration was measured with Qubit® Fluorometer using the Qubit® dsDNA BR Kit (Invitrogen).

384-well Custom TaqMan® Gene Expression Array Cards (Applied Biosystems) were used to identify the expression of glutamate receptor subunits and glutamate transporters in the β-tanycytes. The microfluidic card was preloaded by the manufacturer with selected gene expression assays for our target receptors, transporters and housekeeping genes listed in Supplementary Table 1. ViiA 7 real-time PCR platform with Array Card Block and comparative CT method (Life Technologies) was used for thermal cycles of the qPCR.

The 18s ribosomal RNA, the actin beta (*Actb*), the glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) and the hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) served as housekeeping genes, namely, the CT values of each genes of our interest were normalized to the CT value of the geometric mean of these housekeeping genes. *Dio2* is known to be expressed by tanycytes (Fekete et al., 2000, Tu et al., 1997), and our current immunocytochemical data showed that diacylglycerol lipase alpha (*Dagla*) is also expressed in tanycytes, thus, these genes served as positive controls. As our in situ hybridization studies demonstrated that the metabotropic glutamate receptor 5 (*Grm5*) and the metabotropic glutamate receptor 1 *(Grm1)* are not expressed in tanycytes (Supplementary Fig. 4), the expression levels of these genes were used as a threshold value. Thus, genes that had CT value

equal or lower than that of *Dagla* were considered to be expressed in β-tanycytes, while genes above the threshold of the CT values of *Grm5* were considered to be absent in β-tanycytes.

Slice preparation for electrophysiological recordings

The mice were deeply anaesthetized with isoflurane and decapitated. The brains were rapidly removed and immersed in ice-cold slicing solution containing, in mM: 87 NaCl, 2.5 KCl, 0.5 $CaCl_2$, 7 $MgCl_2$, 25 $NaHCO_3$, 25 D-glucose, 1.25 NaH_2PO_4, 75 sucrose saturated with 95% O_2/5% CO_2. Coronal 250 µm slices were cut using a VT1200S vibratome (Leica), then the slices were transferred into a holding chamber filled with artificial cerebrospinal fluid (aCSF; 36 °C) containing in mM: 126 NaCl, 2.5 KCl, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 10 glucose; pH 7.4; 280-300 mOsm/L. The slices were kept in holding solution for at least 1.5 hours and gradually cooled down to room temperature.

Chemicals used for electrophysiology

The chemicals for the intracellular and extracellular solutions, carbenoxolone disodium salt (carbenoxolone) and L-Glutamic acid (glutamate) were purchased from Sigma-Aldrich. Tetrodotoxin (TTX), (2S,3S,4S)-Carboxy-4-(1-methylethenyl)-3-pyrrolidineacetic acid (kainate), (RS)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-Methyl-D-aspartic acid (NMDA), 6,7-Dinitroquinoxaline-2,3-dione disodium salt (DNQX) (S)-3,5-Dihydroxyphenylglycine (DHPG), (2R,4R)-4-Aminopyrrolidine-2,4-dicarboxylate (APDC), L-(+)-2-Amino-4-phosphonobutyric acid (L-AP4) and DL-threo-β-Benzyloxyaspartic acid (TBOA), Vu 155041 were purchased from the Tocris Bioscience. TBOA was dissolved in dimethylsulfoxide (DMSO) and then diluted in aCSF. The final concentration of DMSO used in the experiments was <0.01%. TRH was purchased from Bachem (Switzerland).

Data acquisition and analysis for electrophysiology

The slices were transferred to a submersion type of recording chamber containing aCSF at 32-33 °C and were perfused with aCSF at a rate of approximately 1 mL/min. As tanycytes express connexin 43 gap junctions and hemichannels (Szilvasy-Szabo et al., 2017), gap junction inhibitor carbenoxolone (100 μM) was added to the aCSF to block the intercellular communication *via* gap junctions. To prevent the potential indirect effects of glutamate treatment, the voltage-dependent sodium channel inhibitor, TTX (600 nM) was also continuously administered into the aCSF. β2-tanycytes were identified based on their typical morphology and the location of their cell body in the floor of the third ventricle under infrared differential interference contrast illumination using FN1 Microscope (Nikon) equipped with 40x water-immersion objective with additional zoom (up to 2x) and Zyla CCD camera (ANDOR). The patch pipettes (6-8 MΩ) were pulled from borosilicate capillaries (OD=1.5 mm thin wall, Garner Co.) with a P-1000 horizontal puller (Sutter Instrument Co.) and filled with intracellular recording solution containing, in mM: 110 K-gluconate, 4 NaCl, 20 HEPES, 0.1 EGTA, 10 phosphocreatine di(tris) salt, 2 ATP, 0.3 GTP; pH 7.25; 280-300 mOsm/L. Recordings were performed with Multiclamp 700B patch clamp amplifier, Digidata-1440A data acquisition system and pCLAMP 10.4 software (Molecular Devices). The headstage of the amplifier was fitted to a Luigs&Neumann SM7 micromanipulator system. Whole-cell and outside-out patch-clamp recordings were filtered at 10 kHz using the built-in Bessel filter of the amplifier and digitized at 10 kHz. Slow and fast capacitive components were automatically compensated for. The stability of the patch was checked by repetitively monitoring the access resistance during the experiment. Liquid junction potential was 14.4 mV and not compensated.

Whole-cell patch clamp recording

The membrane potential (MP) was measured immediately after break-in and establishing whole-cell recording in current-clamp mode (I=0). In each series of experiments, a control period was recorded for 2-3 min that was followed by a drug treatment phase for 3-4 min. During the treatment, the perfusion was opened. The final volume of the drug was 300 μL and the treatment lasted until this volume passed through the recording chamber. The concentration of the drug used in the recording chamber is described in the Results section. The effect of the drugs was examined on the MP of β2-tanycytes. The washout of the drugs restored the MP of β2-tanycytes in all cases.

Outside-out patch clamp recording

To exclude the indirect effect of substances that may be released from neighboring non-neuronal cells, the effect of glutamate was examined using outside-out patch clamp preparations. After establishing stable, whole-cell patch clamp connection, β2-tanycytes were isolated from the tissue by pulling them into the third ventricle. The MP was held at -78 mV and the inward current was measured during glutamate treatment (500 μM).

Examination of the influence of optogenetic activation of the hypophysiotropic TRH axon terminals on the membrane potential of tanycytes

The AAV5.EF1a.DIO.hChR2(H134R)-eYFP.WPRE.hGH virus (Addgene 20298P) was bilaterally injected into the PVN of 19 TRH-IRES-Cre mice. The surgeries were performed in a biosafety level 2 (BSL-2) virus injection facility. The mice were anesthetized (intraperitoneal injection of 25mg/kg xylazine and 125mg/kg ketamine in 0.9% NaCl) and their head positioned in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA) with the Bregma and Lambda in the horizontal plane. Through burr holes in the skull, a glass pipette (20-μm outer tip

diameter) connected to a Nanoject II/Nanoliter 2000 microinjector (Drummond Scientific Co. or WPI Inc.) was lowered into the brain at stereotaxic coordinates corresponding to the two sides of the PVN (anteroposterior: -0.7 mm, mediolateral: + or -0.3mm, dorsoventral: -5.0 mm) based on the atlas of Paxinos and Watson (Paxinos and Watson, 1998). The virus containing solution was injected bilaterally into the PVN 20nl with 100nl/min and another 30nl with 57nl/sec speed. Three minutes after the injections, the pipettes were removed slowly, the scalp was sutured and the mice were housed in BSL-2 quarantine for 2 weeks. Then the animals were used within two weeks.

On the day of the experiment, the mice were sacrificed and slices were prepared for electrophysiology as described above. B2-tanycytes were patched and their membrane potential was measured as described above. Carbenoxolone (100 μM) was added to the aCSF to block the intercellular communication *via* gap junctions. To activate the channelrhodopsin-containing TRH axons in the external zone of the ME, a micromanipulator-attached fibre optic cable (200 μm core diameter) delivered light from a laser (473 nm, Roithner Lasertechnik GmbH, Wien, Austria) The fiber optic was placed 1–2 mm away from the target area. Light intensity was calibrated by a Handheld Laser Power Meter (Edmund Optics; Nether Poppleton, York, UK). Maximally, 2.5 mW light was delivered to the tissue. The blue light was delivered in 2ms pulses at a 10 Hz frequency in 0.1s sweeps. The membrane potential changes were measured with Clampfit v10.7 using the average of 10 sweeps. In some of the cells, the effect of photostimulation was also studied in the presence of glutamate receptor (DNQX, 500 μM) and glutamate transporter inhibitors (TBOA, 1mM).

To determine whether the blue laser light can directly influence the tanycytes, control experiment was performed using slices of TRH-IRES-tdTomato mice where the TRH axons contained the tdTomato fluorescent protein.

Calcium imaging of tanycytes

Coronal slices (250 μm thick) containing intact ME were incubated with Fluo-4 AM (20 μM; Invitrogen) dissolved in extracellular solution containing low glucose (aCSF containing 1 mM glucose and 9 mM sucrose), DMSO (0.125% final concentration; Invitrogen), and Pluronic F-127 (0.05% final concentration; Invitrogen). Dye was allowed to diffuse into the tanycytes for at least 60-90 min before starting calcium imaging. Incubation of sections and calcium imaging experiments were carried out at room temperature to decrease intracellular compartmentalization of the dye. Despite the long incubation period, only few β2-tanycytes (3-8 cells) were loaded with dye, while almost all α-tanycytes cells were labelled.

First, we recorded a stabile baseline for 1 min without any treatment. This is considered as control period, then drugs were directly pipetted into the recording chamber and recording was continued for another 2-4 min. Tanycytes that did not show stable FIV during the control (baseline) period were excluded from analyses.

Andor AMH-200-FS6 halide light source was used to illuminate the sections. Fluorescence signal was collected, using a Nikon 40× objective lens, and images were collected at a 0.5 Hz sampling rate with 200 ms exposure time using an Andor Zyla 5.5 camera (Andor Technology, UK) equipped with an optical sectioning device (DSD2, Andor Technology, UK) to generate semi-confocal images. All semi-confocal image data were collected and analyzed using Nikon control and analysis software (NIS-Elements AR 4,40 64 bit versions).

Statistical analyses

Changes of the MP and inward current were analyzed using Clampfit module of the pCLAMP 10.4 software (Molecular Devices) and OriginPro 2015. Statistical tests were performed using one-way analysis of variance (ANOVA) followed by the Bonferroni correction to determine differences among treatment groups in the dose-response and mGLU4 agonist experiment and

ANOVA with repeated measure followed by the Bonferroni correction for all other patch clamp experiments. Treatment values were compared to their own control period. In calcium imaging recordings, nonparametric pairwise Wilcoxon test was carried out.

For TRH release of ME explants, significance was calculated by one-way ANOVA, followed by Tukey *post hoc* test, while the 2-AG content of ME explants was compared by Student's t-test. All data are reported as mean ± standard error of mean (SEM). The p value <0.05 was considered significant in all cases.

Supplementary References

Alvarez-Buylla, A., Ling, C. Y. & Kirn, J. R. (1990). Cresyl violet: a red fluorescent Nissl stain. J Neurosci Methods*,* 33**,** 129-33.

Farkas, I., Kallo, I., Deli, L., Vida, B., Hrabovszky, E., Fekete, C., Moenter, S. M., Watanabe, M. & Liposits, Z. (2010). Retrograde endocannabinoid signaling reduces GABAergic synaptic transmission to gonadotropin-releasing hormone neurons. Endocrinology*,* 151**,** 5818-29.

Fekete, C., Mihaly, E., Herscovici, S., Salas, J., Tu, H., Larsen, P. R. & Lechan, R. M. (2000). DARPP-32 and CREB are present in type 2 iodothyronine deiodinase-producing tanycytes: implications for the regulation of type 2 deiodinase activity. Brain Res*,* 862**,** 154-61.

Hajos, N., Katona, I., Naiem, S. S., Mackie, K., Ledent, C., Mody, I. & Freund, T. F. (2000). Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. Eur J Neurosci*,* 12**,** 3239-49.

Hrabovszky, E. & Petersen, S. L. (2002). Increased concentrations of radioisotopically-labeled complementary ribonucleic acid probe, dextran sulfate, and dithiothreitol in the hybridization buffer can improve results of in situ hybridization histochemistry. J Histochem Cytochem*,* 50**,** 1389-400.

Joseph-Bravo, P., Charli, J. L., Palacios, J. M. & Kordon, C. (1979). Effect of neurotransmitters on the in vitro release of immunoreactive thyrotropin-releasing hormone from rat mediobasal hypothalamus. Endocrinology*,* 104**,** 801-6.

Katona, I., Urban, G. M., Wallace, M., Ledent, C., Jung, K. M., Piomelli, D., Mackie, K. & Freund, T. F. (2006). Molecular composition of the endocannabinoid system at glutamatergic synapses. J Neurosci*,* 26**,** 5628-37.

Keimpema, E., Alpár, A., Howell, F., Malenczyk, K., Hobbs, C., Hurd, Y. L., Watanabe, M., Sakimura, K., Kano, M., Doherty, P., et al. (2013). Diacylglycerol lipase α manipulation reveals developmental roles for intercellular endocannabinoid signaling. Scientific Reports*,* 3**,** 2093.

Kola, B., Farkas, I., Christ-Crain, M., Wittmann, G., Lolli, F., Amin, F., Harvey-White, J., Liposits, Z., Kunos, G., Grossman, A. B., et al. (2008). The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. PLoS One*,* 3**,** e1797.

Liposits, Z., Gorcs, T., Gallyas, F., Kosaras, B. & Setalo, G. (1982). Improvement of the electron microscopic detection of peroxidase activity by means of the silver intensification of the diaminobenzidine reaction in the rat nervous system. Neurosci Lett*,* 31**,** 7-11.

Makara, J. K., Katona, I., Nyiri, G., Nemeth, B., Ledent, C., Watanabe, M., De Vente, J., Freund, T. F. & Hajos, N. (2007). Involvement of nitric oxide in depolarization-induced suppression of inhibition in hippocampal pyramidal cells during activation of cholinergic receptors. J Neurosci*,* 27**,** 10211-22.

Malenczyk, K., Keimpema, E., Piscitelli, F., Calvigioni, D., Bjorklund, P., Mackie, K., Di Marzo, V., Hokfelt, T. G., Dobrzyn, A. & Harkany, T. (2015). Fetal endocannabinoids orchestrate the organization of pancreatic islet microarchitecture. Proc Natl Acad Sci U S A*,* 112**,** E6185-94.

Matziari, M., Bauer, K., Dive, V. & Yiotakis, A. (2008). Synthesis of the phosphinic analogue of thyrotropin releasing hormone. J Org Chem*,* 73**,** 8591-3.

Mayer, M. L., Westbrook, G. L. & Guthrie, P. B. (1984). Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. Nature*,* 309**,** 261-3.

Pak, T., Yoo, S., Miranda-Angulo, A. L., Wang, H. & Blackshaw, S. (2014). Rax-CreERT2 knock-in mice: a tool for selective and conditional gene deletion in progenitor cells and radial glia of the retina and hypothalamus. PLoS One*,* 9**,** e90381.

Paxinos, G. & Watson, C. 1998. *The Rat Brain in Stereotaxic Coordinates,* San Diego, CA, Academic Press.

Peterfi, Z., Farkas, E., Nagyunyomi-Senyi, K., Kadar, A., Otto, S., Horvath, A., Fuzesi, T., Lechan, R. M. & Fekete, C. (2018). Role of TRH/UCN3 neurons of the perifornical area/bed nucleus of stria terminalis region in the regulation of the anorexigenic POMC neurons of the arcuate nucleus in male mice and rats. Brain Struct Funct*,* 223**,** 1329-1341.

Revell, P. A., Grossman, W. J., Thomas, D. A., Cao, X., Behl, R., Ratner, J. A., Lu, Z. H. & Ley, T. J. (2005). Granzyme B and the downstream granzymes C and/or F are important for cytotoxic lymphocyte functions. J Immunol*,* 174**,** 2124-31.

Szilvasy-Szabo, A., Varga, E., Beliczai, Z., Lechan, R. M. & Fekete, C. (2017). Localization of connexin 43 gap junctions and hemichannels in tanycytes of adult mice. Brain Res*,* 1673**,** 64-71.

Tanimura, A., Yamazaki, M., Hashimotodani, Y., Uchigashima, M., Kawata, S., Abe, M., Kita, Y., Hashimoto, K., Shimizu, T., Watanabe, M., et al. (2010). The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. Neuron*,* 65**,** 320-7.

Tu, H. M., Kim, S. W., Salvatore, D., Bartha, T., Legradi, G., Larsen, P. R. & Lechan, R. M. (1997). Regional distribution of type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. Endocrinology*,* 138**,** 3359-68.

Varga, E., Farkas, E., Zseli, G., Kadar, A., Venczel, A., Kovari, D., Nemeth, D., Mate, Z., Erdelyi, F., Horvath, A., et al. (2019). Thyrotropin-Releasing-Hormone-Synthesizing Neurons of the Hypothalamic Paraventricular Nucleus Are Inhibited by Glycinergic Inputs. Thyroid*,* 29**,** 1858-1868.

Wittmann, G., Farkas, E., Szilvasy-Szabo, A., Gereben, B., Fekete, C. & Lechan, R. M. (2017). Variable proopiomelanocortin expression in tanycytes of the adult rat hypothalamus and pituitary stalk. J Comp Neurol*,* 525**,** 411-441.