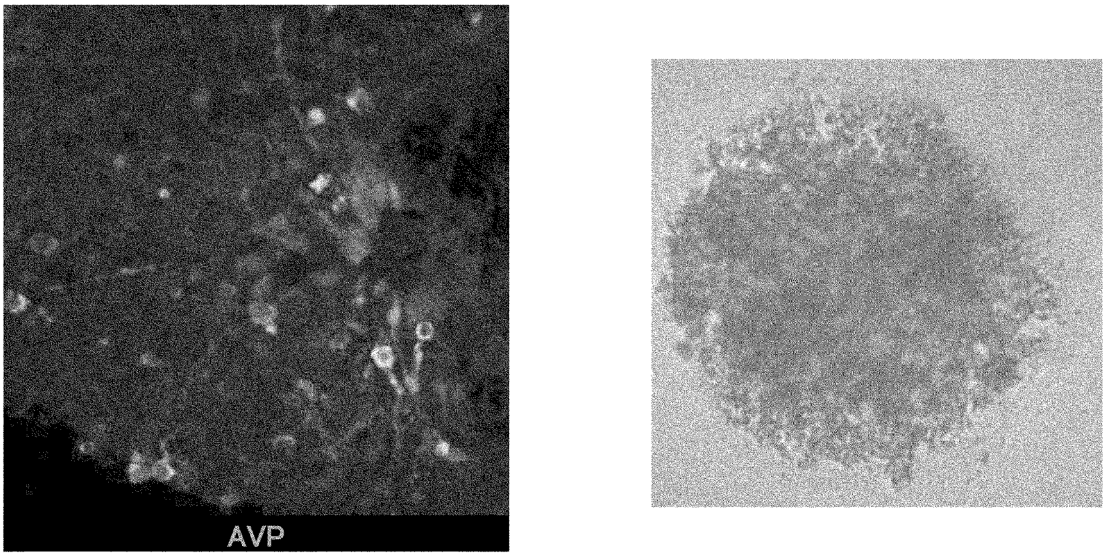


Fig. 1

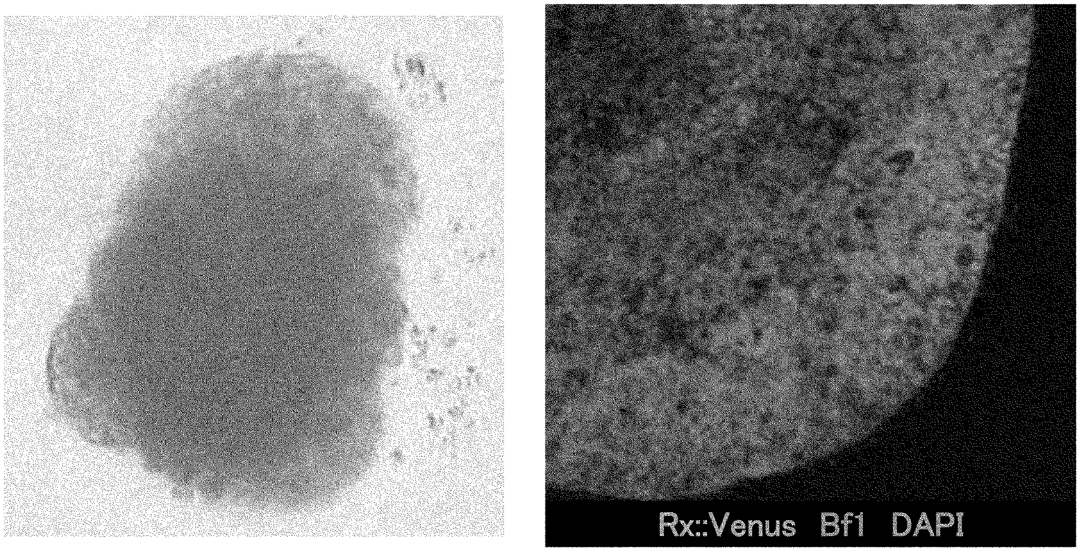
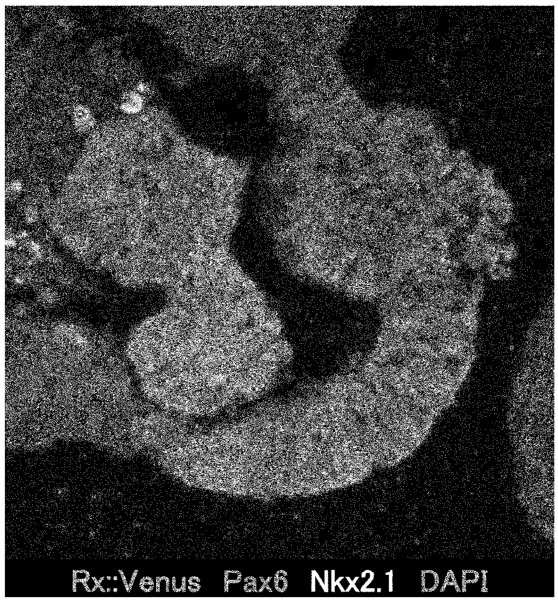


Fig. 2


Rx::Venus Pax6 Nkx2.1 DAPI

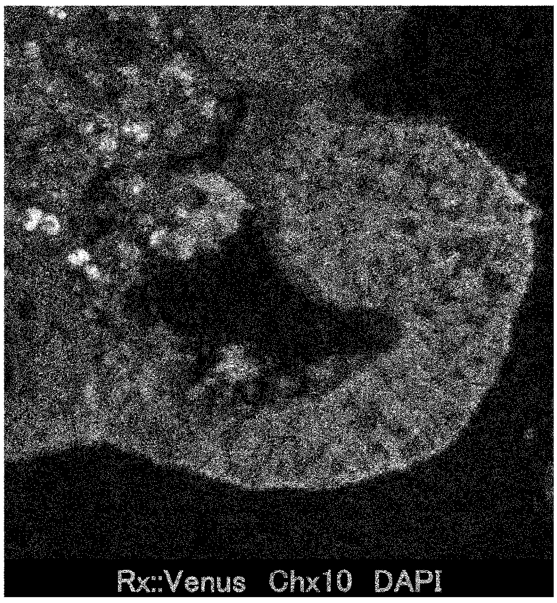
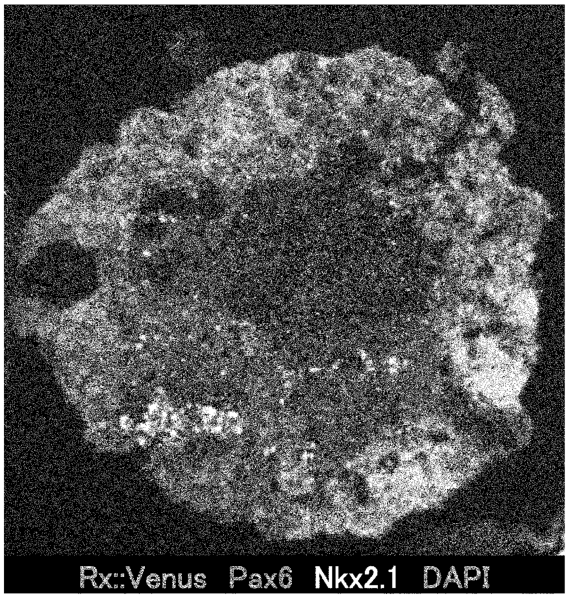

Rx::Venus Chx10 DAPI

Fig. 3


Rx::Venus Pax6 Nkx2.1 DAPI

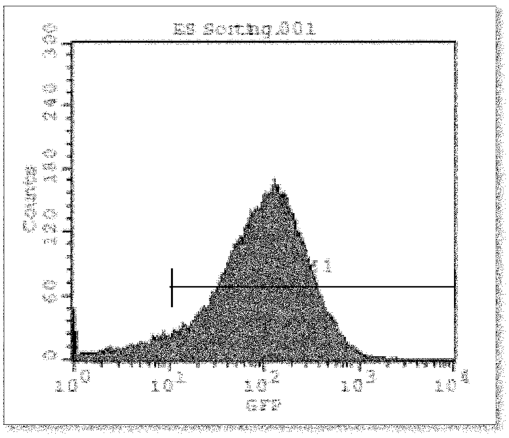

ES Sorting.001
Counts
0
60
120
180
240
300
R1
10^0
10^1
10^2
10^3
10^4
GFP

Fig. 4

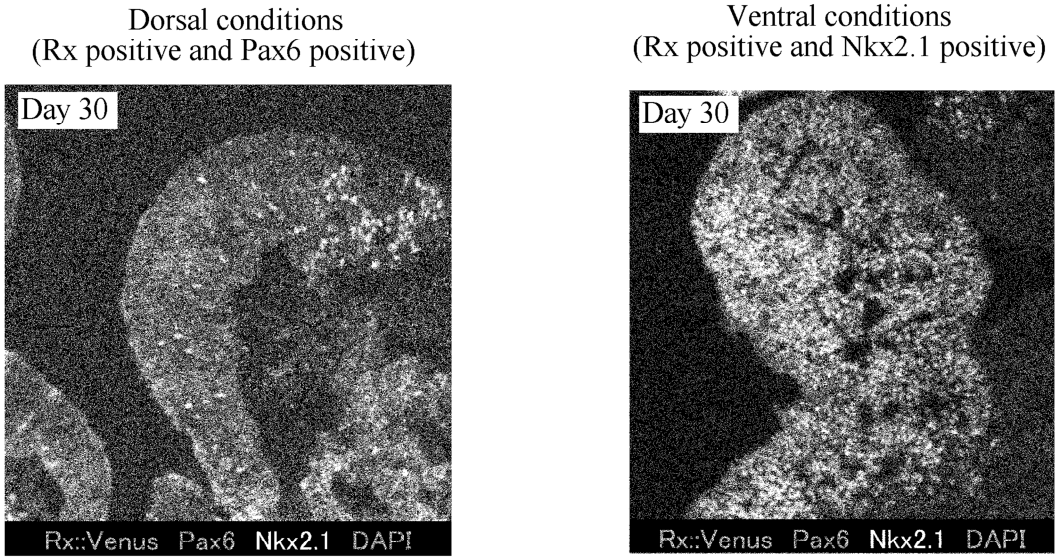

Dorsal conditions
(Rx positive and Pax6 positive)
Day 30
Rx::Venus Pax6 Nkx2.1 DAPI
Ventral conditions
(Rx positive and Nkx2.1 positive)
Day 30
Rx::Venus Pax6 Nkx2.1 DAPI

Fig. 5

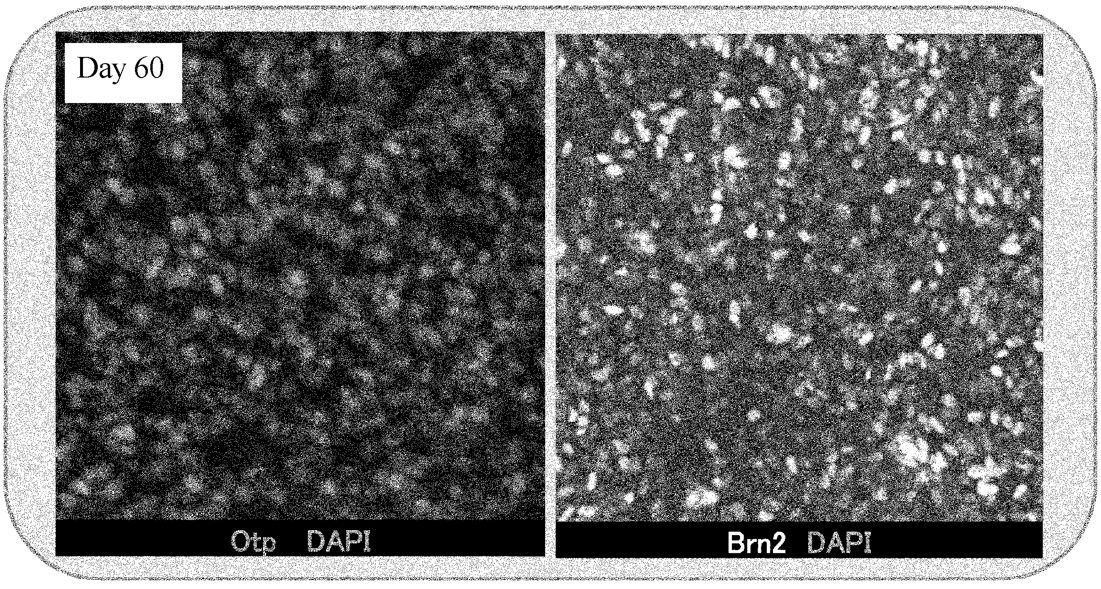

Day 60
Otp DAPI
Brn2 DAPI

Fig. 6

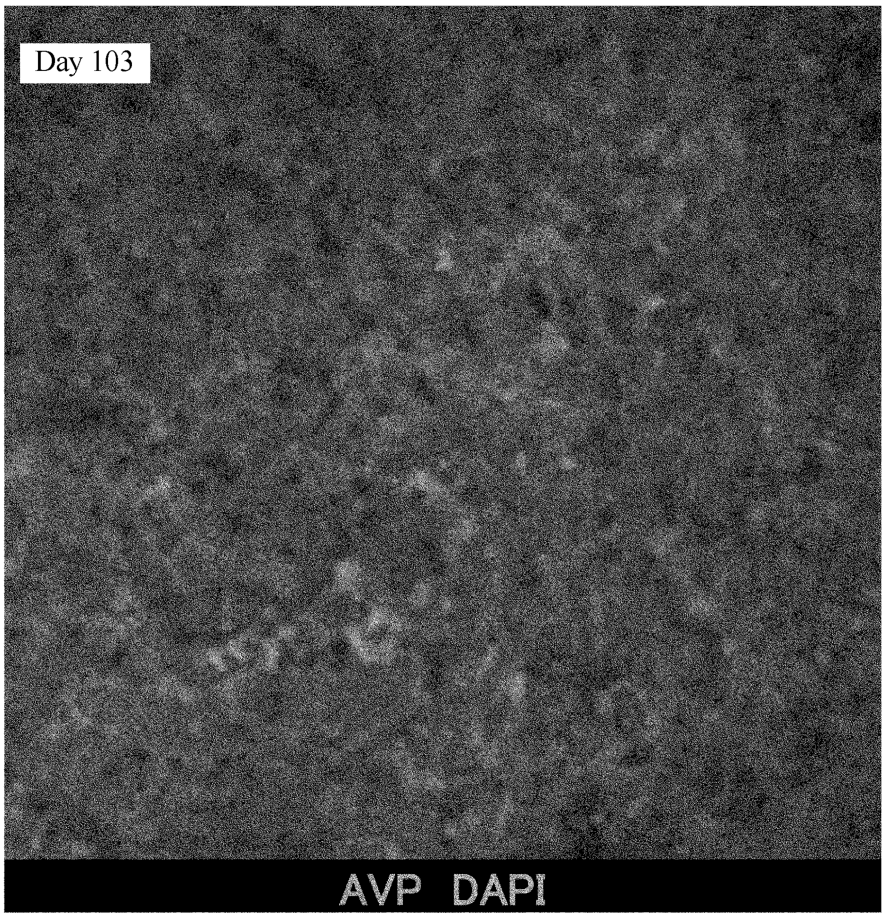


Fig. 7

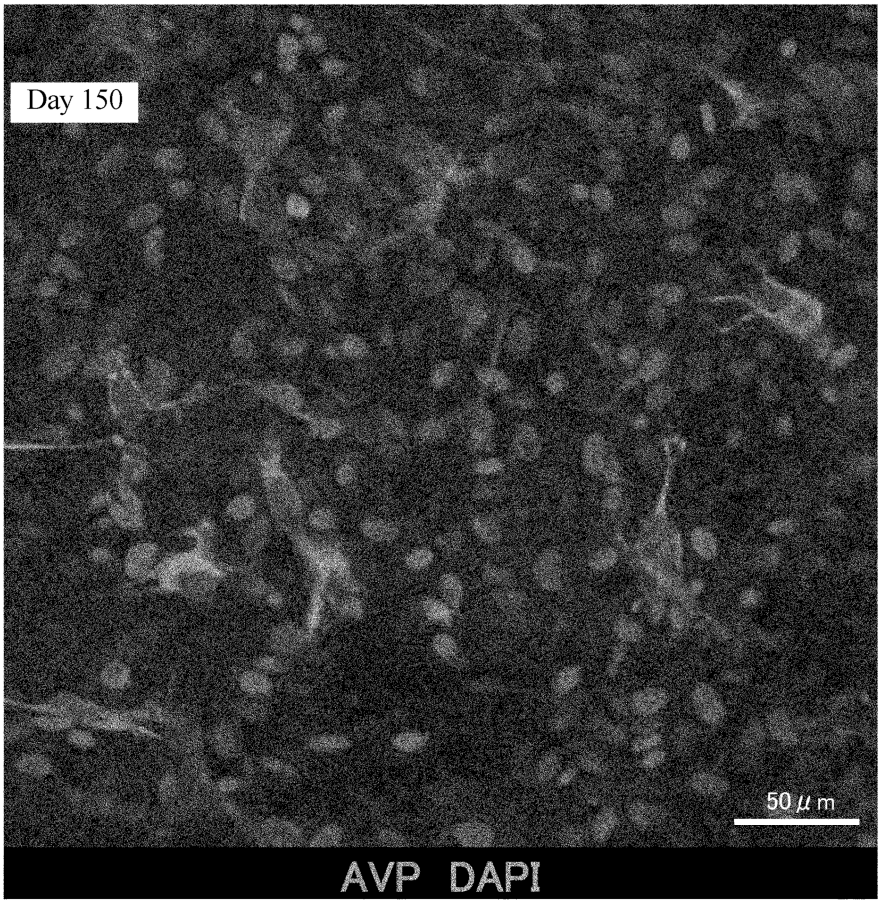


Fig. 8

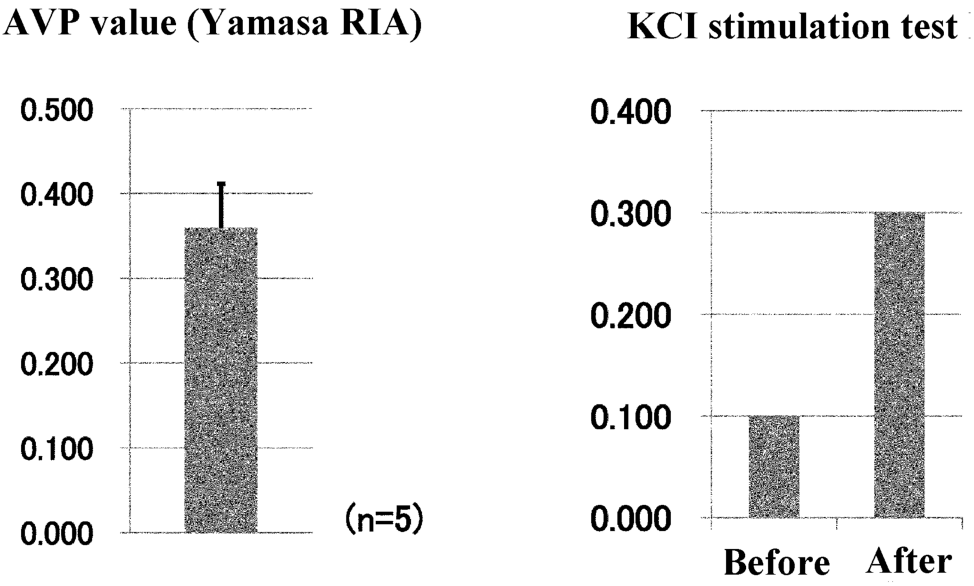

AVP value (Yamasa RIA)
0.500
0.400
0.300
0.200
0.100
0.000
(n=5)
KCI stimulation test
0.400
0.300
0.200
0.100
0.000
Before
After

Fig. 9

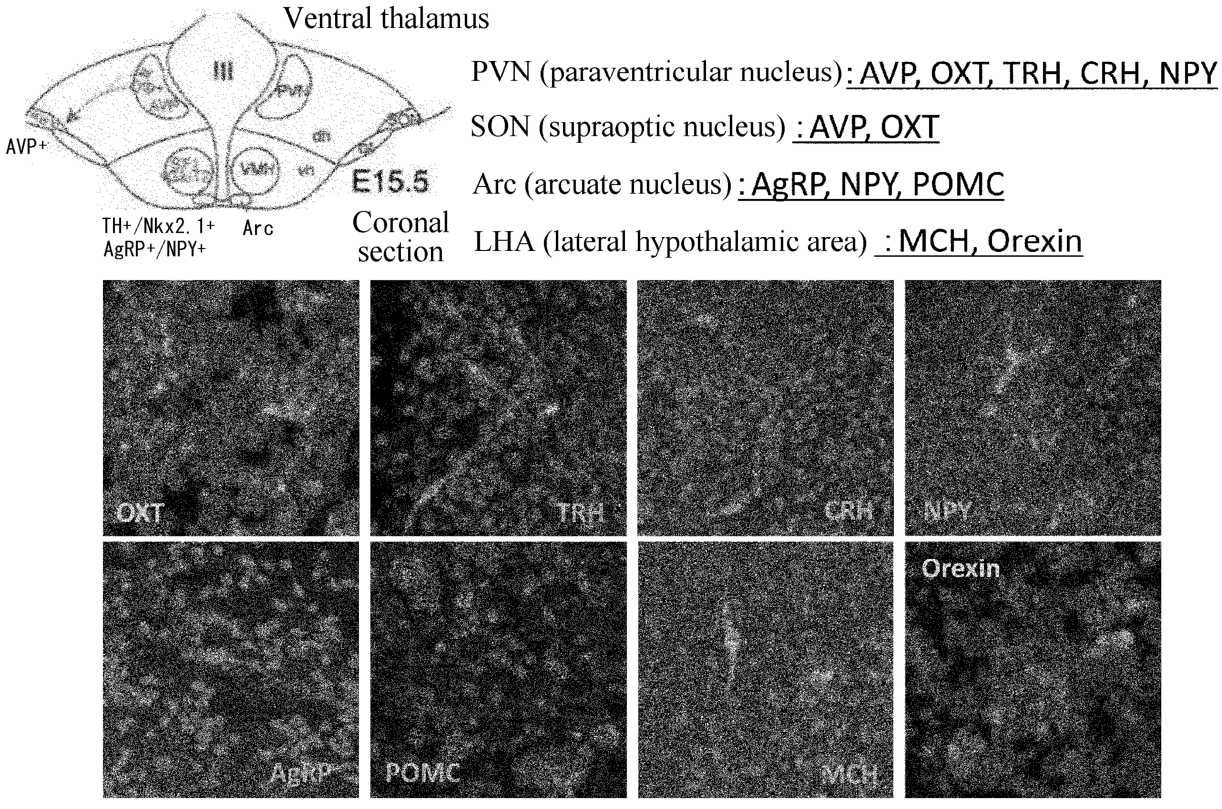

Ventral thalamus
III
PVH
AVP+
E15.5
TH+/Nkx2.1+
AgRP+/NPY+
Arc
Coronal section
PVN (paraventricular nucleus): AVP, OXT, TRH, CRH, NPY
SON (supraoptic nucleus) : AVP, OXT
Arc (arcuate nucleus): AgRP, NPY, POMC
LHA (lateral hypothalamic area) : MCH, Orexin
OXT
TRH
CRH
NPY
AgRP
POMC
MCH
Orexin

Fig. 10

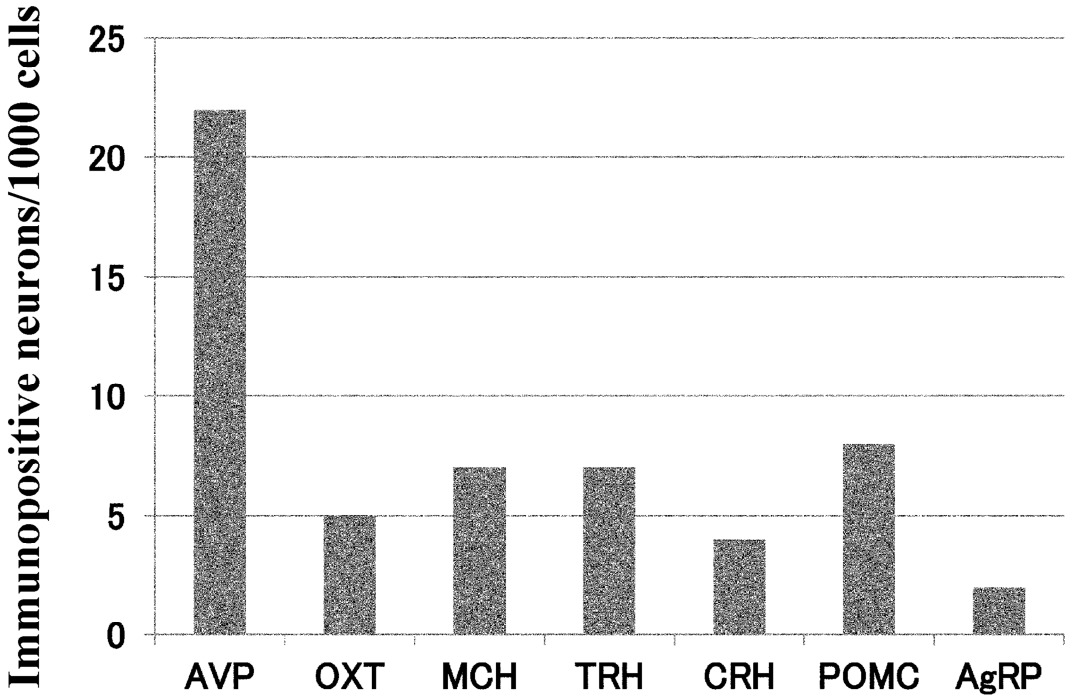

Immunopositive neurons/1000 cells
25
20
15
10
5
0
AVP
OXT
MCH
TRH
CRH
POMC
AgRP

Fig. 11

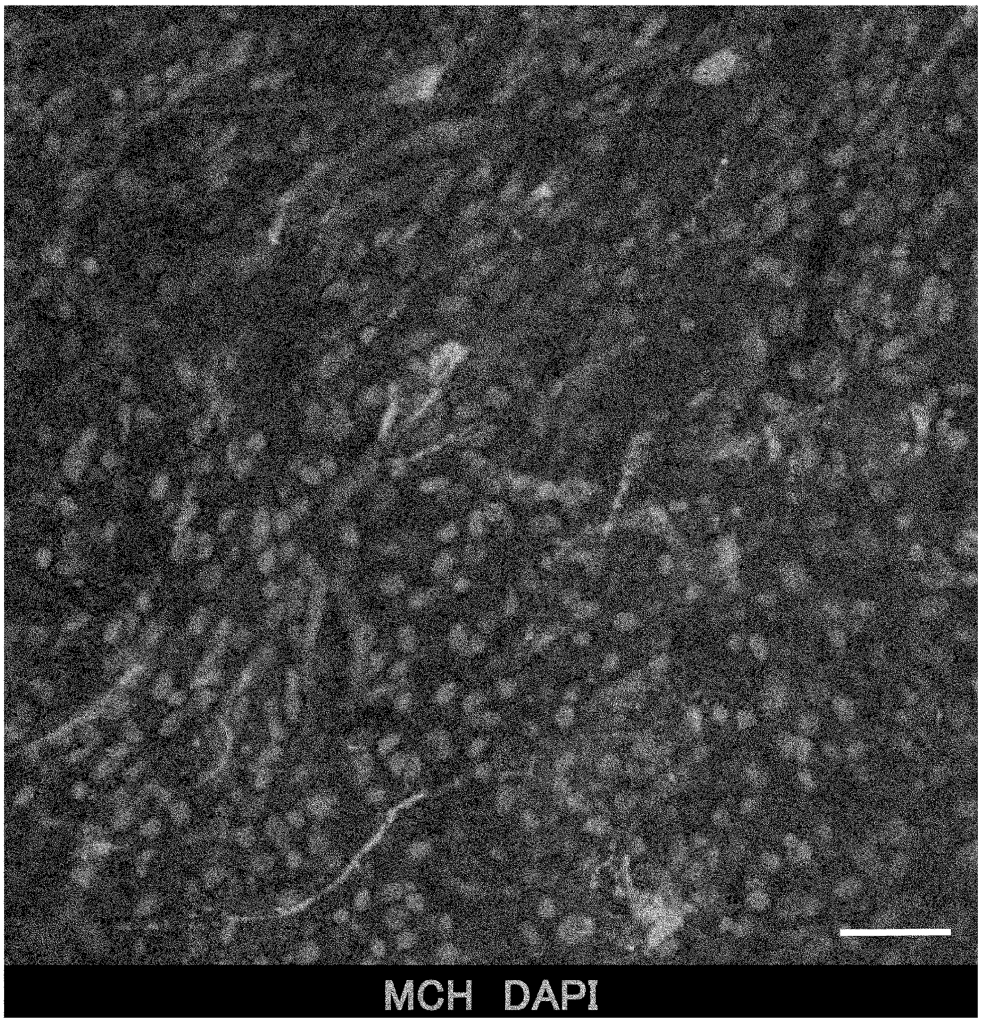


Fig. 12

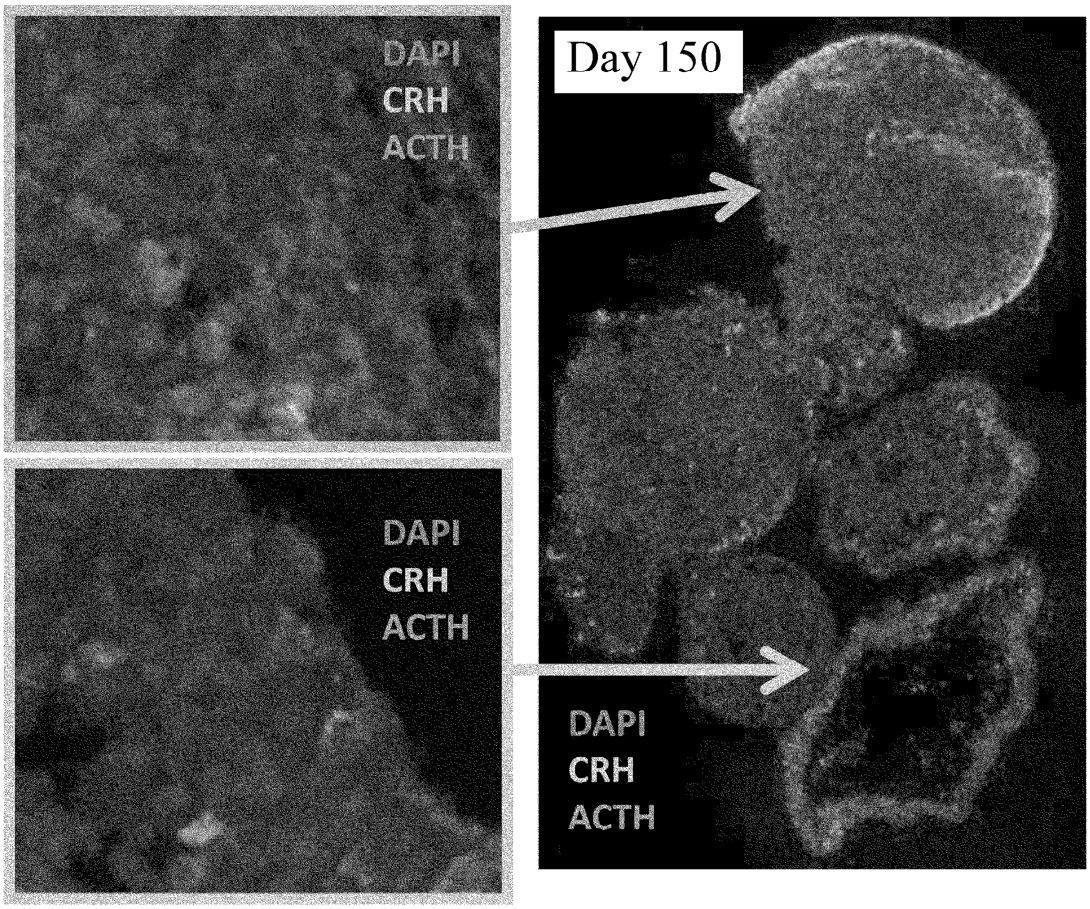
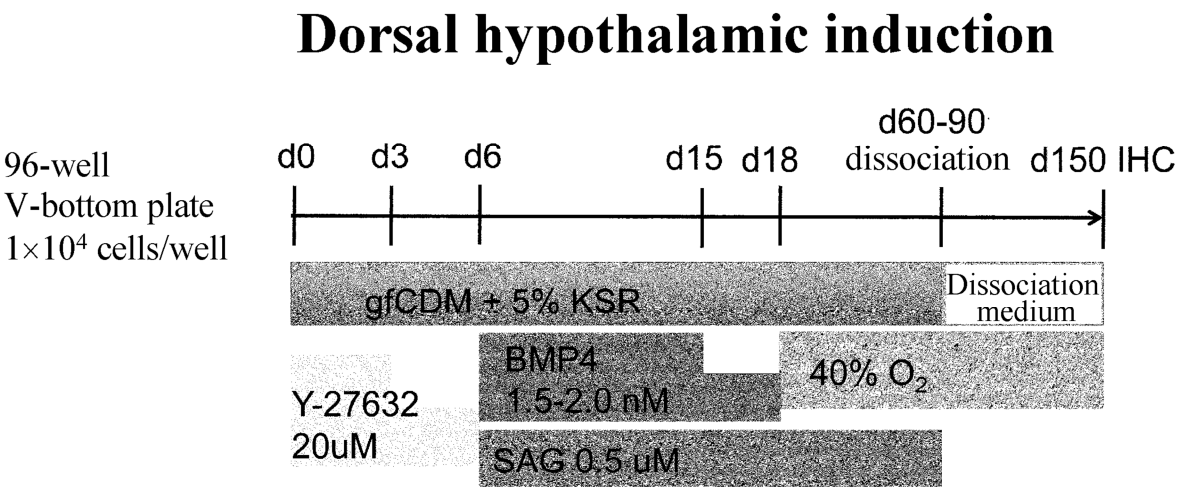


Fig. 13



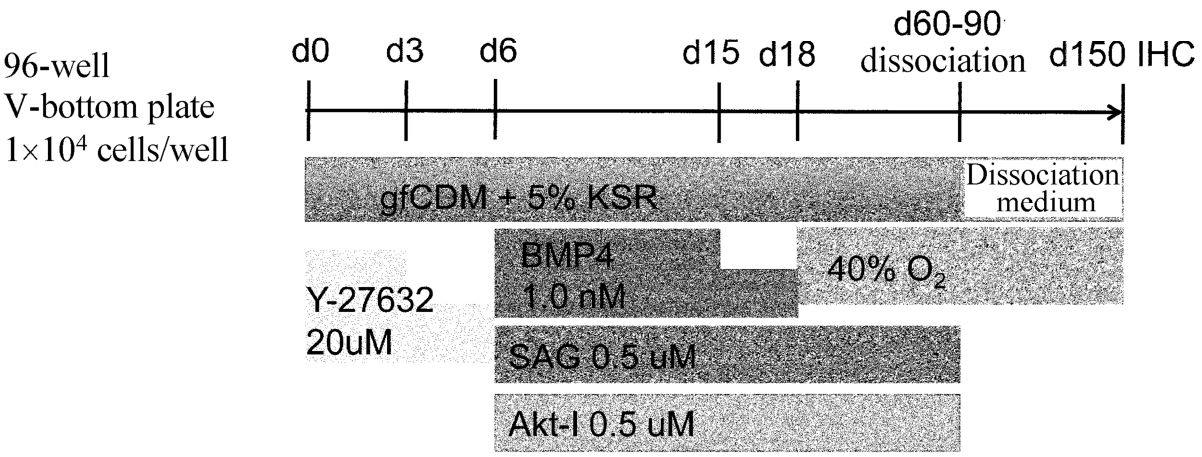
Dissociation medium : **DFNB, CNTF (10 ng/ml), BDNF (50 ng/ml), NT3 (50 ng/ml), FBS** (10% of the total volume in volume ratio)

DFNB : DMEM/F12 (7g/l of glucose, N2 supplement, added B27 supplement)

Coating agent: Matrigel, PDL, laminin

Fig. 14

Ventral hypothalamic induction



Dissociation medium : **DFNB, CNTF (10 ng/ml), BDNF (50 ng/ml), NT3 (50 ng/ml), FBS** (10% of the total volume in volume ratio)

DFNB : DMEM/F12 (7g/l of glucose, N2 supplement, added B27 supplement)

Coating agent: Matrigel, PDL, laminin

Fig. 15

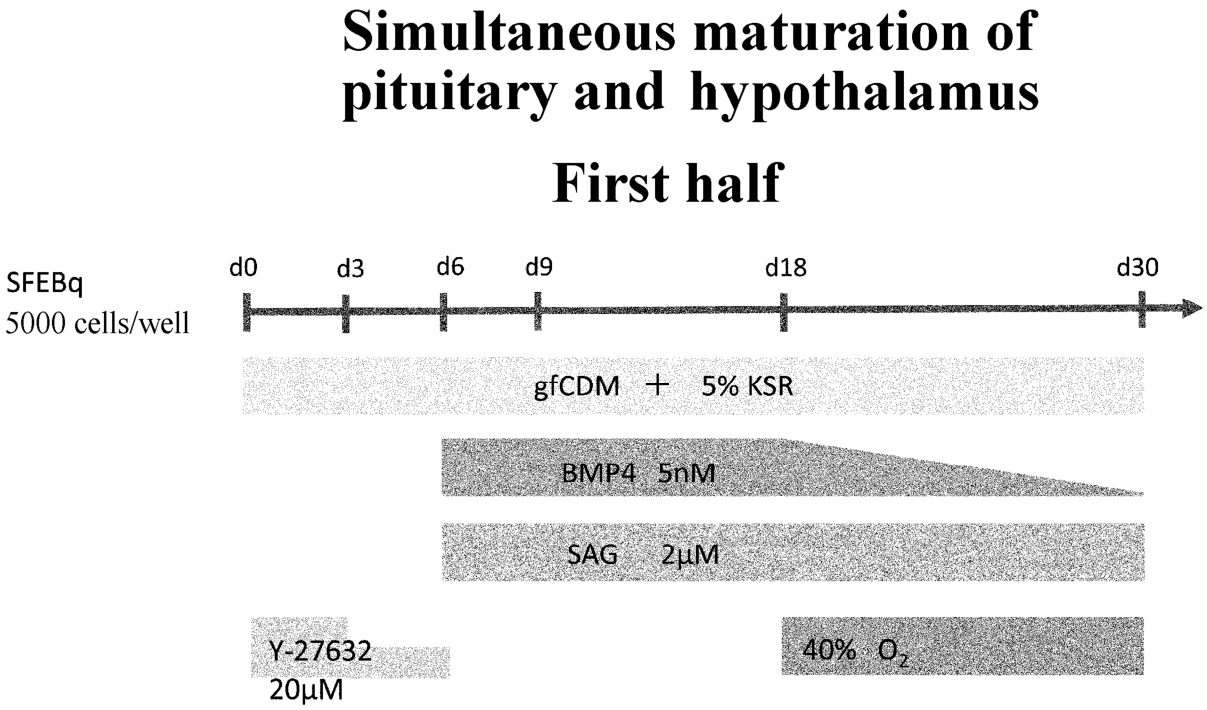

Simultaneous maturation of pituitary and hypothalamus
First half
SFEBq
5000 cells/well
d0
d3
d6
d9
d18
d30
gfCDM + 5% KSR
BMP4 5nM
SAG 2μM
Y-27632
20μM
40% O_2

Fig. 16

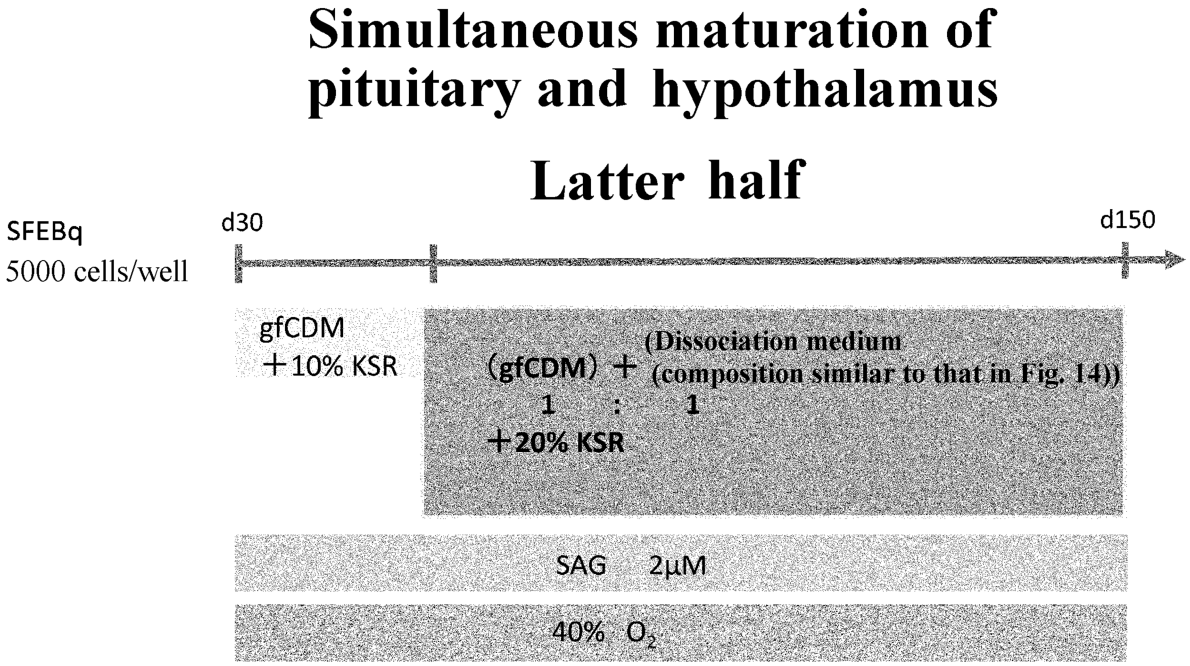

Simultaneous maturation of pituitary and hypothalamus
Latter half
SFEBq
5000 cells/well
d30
d150
gfCDM
＋10% KSR
(gfCDM) ＋ (Dissociation medium (composition similar to that in Fig. 14))
1 : 1
＋20% KSR
SAG 2μM
40% O_2

Fig. 17

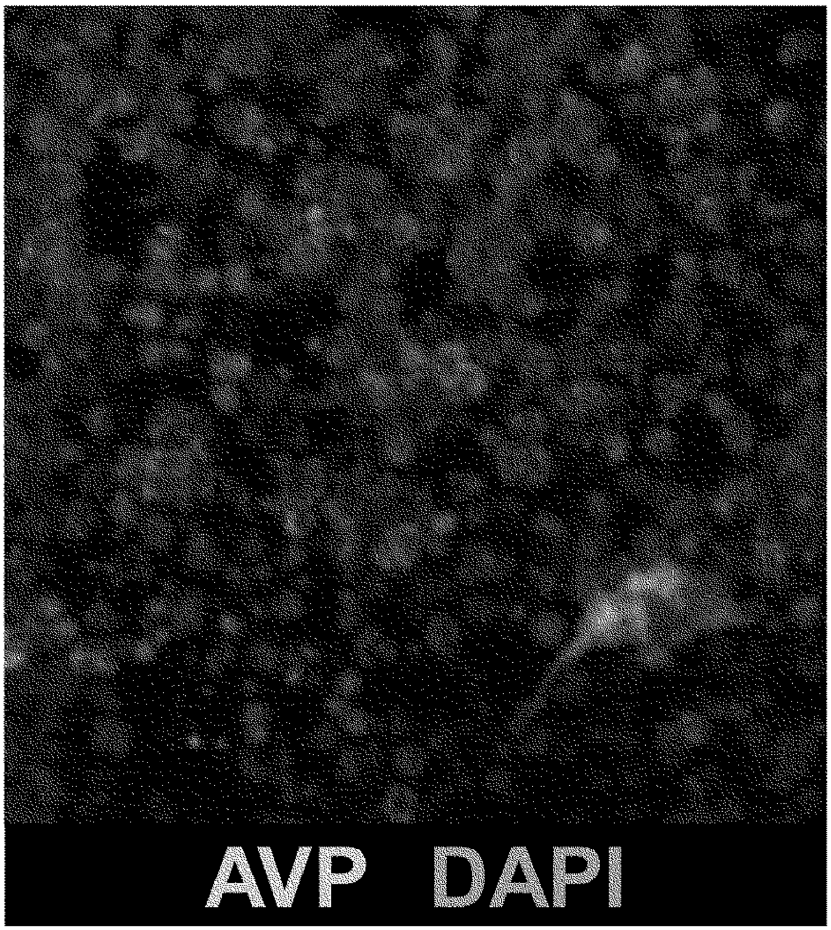


Fig. 18

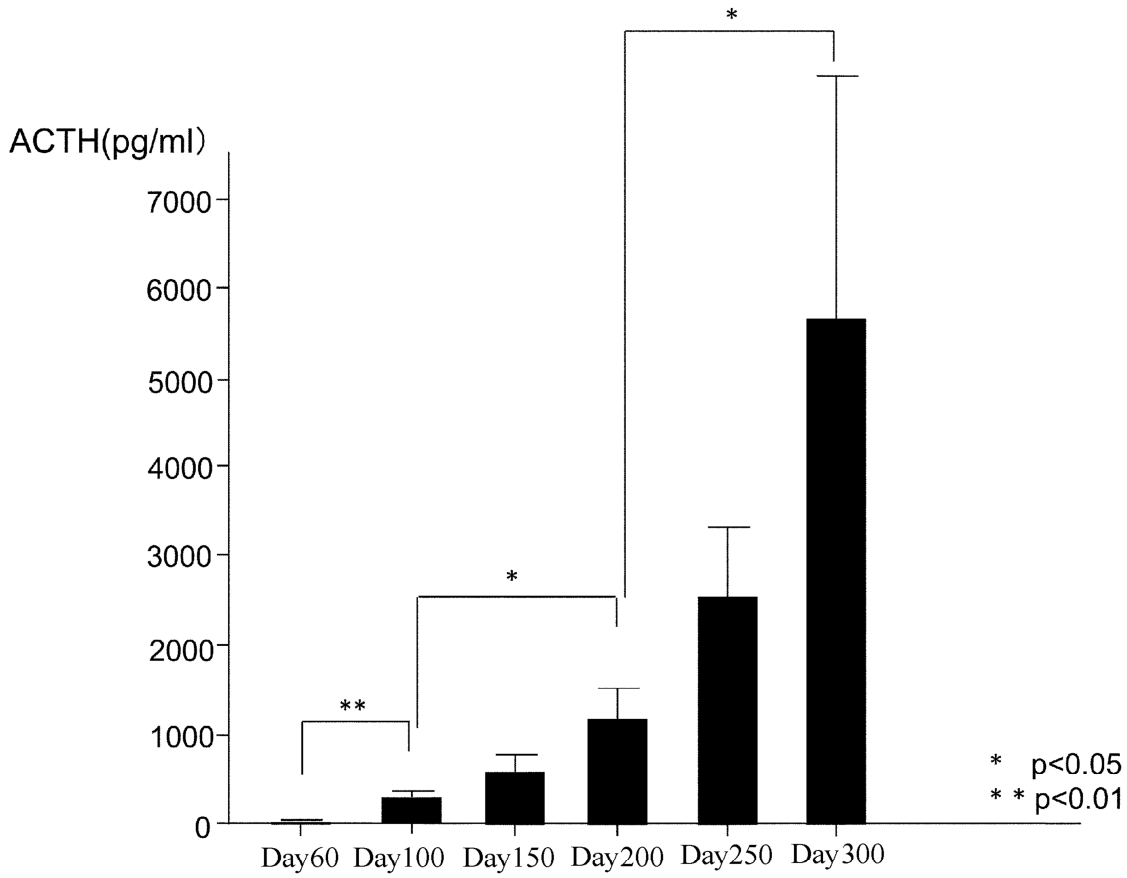
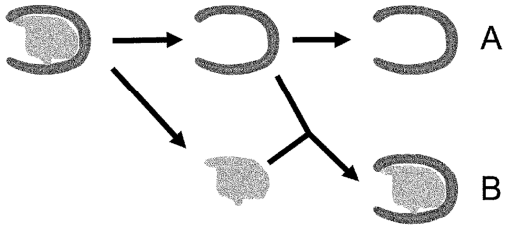

ACTH(pg/ml)
7000
6000
5000
4000
3000
2000
1000
0
Day60
Day100
Day150
Day200
Day250
Day300
*
*
**
*
p<0.05
* *
p<0.01

Fig. 19

Separate anterior pituitary and hypothalamus at Day 70



A: Culture only anterior pituitary
B: Make anterior pituitary and hypothalamus adjacent to each other again and culture

ACTH concentration in culture solution at Day 150

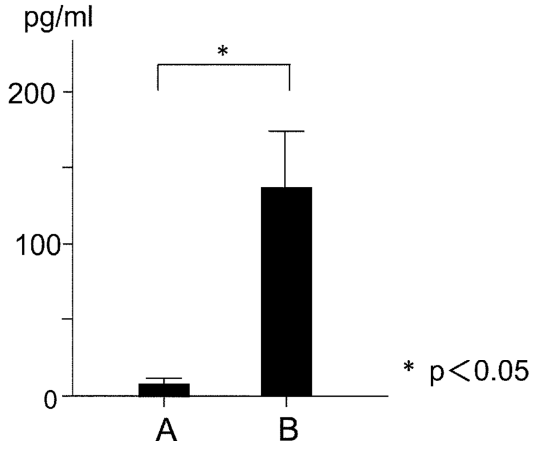
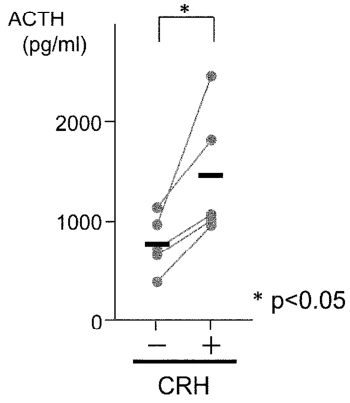
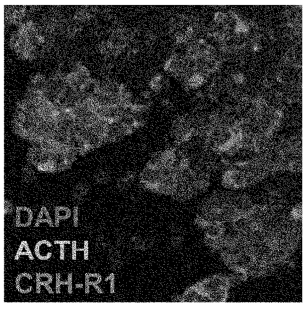


Fig. 20

ACTH stimulation test by CRH

Add 5 μg/ml of CRH





ACTH positive cells are CRH-R1 positive

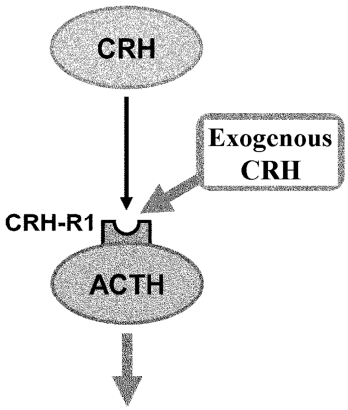


Fig. 21

ACTH suppression test by dexamethasone

Add 500 ng/ml of dexamethasone

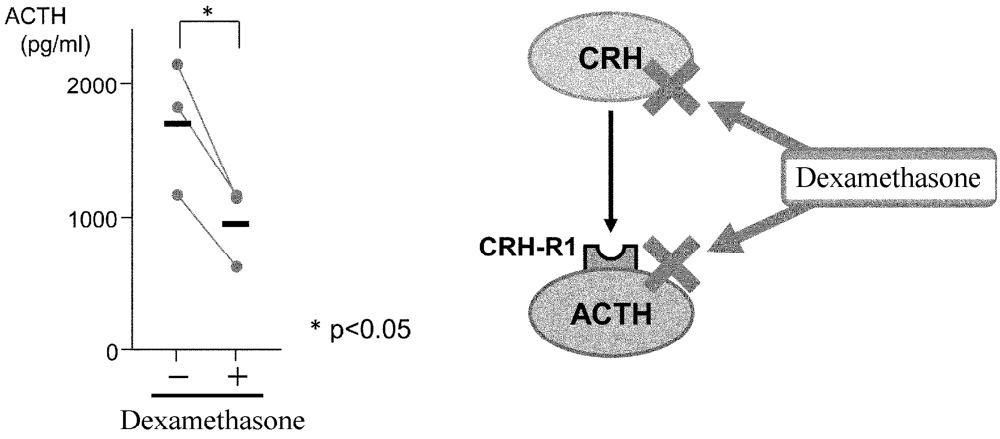


Fig. 22

ACTH suppression test by CRH-R1 inhibitor

CRH-R1 inhibitor: Antalarmin, NBI27194 Add at 10^{-5} M

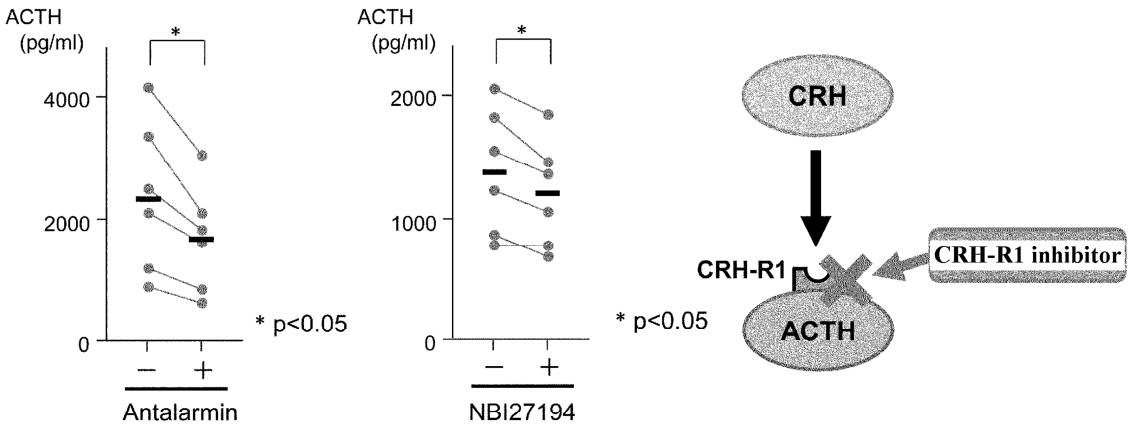


Fig. 23

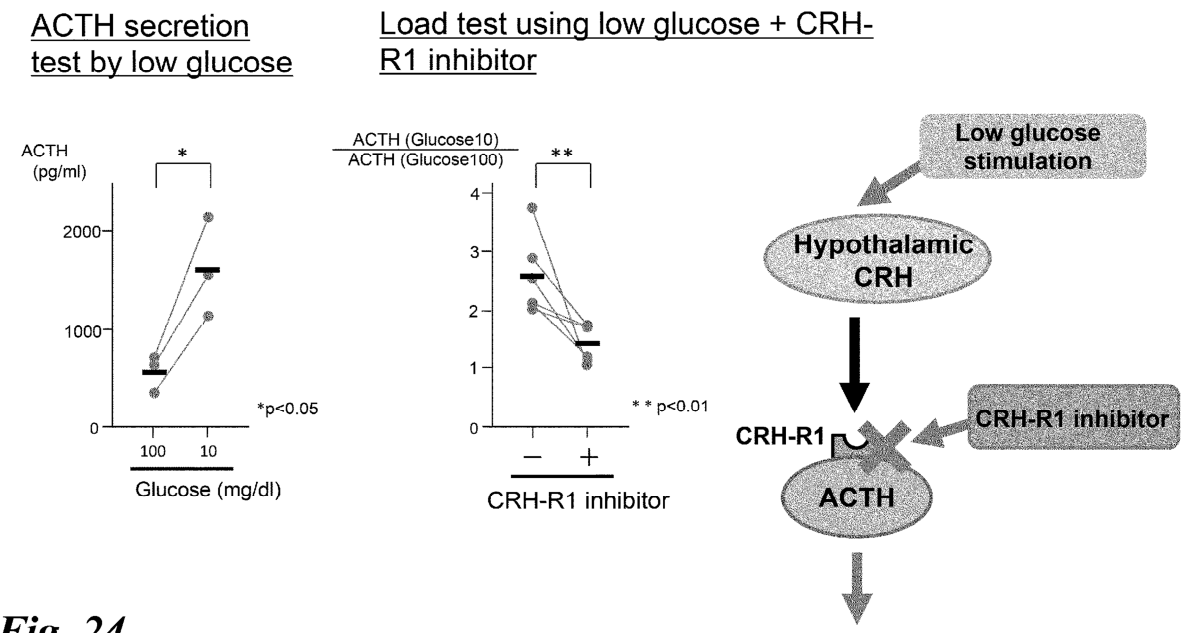

ACTH secretion test by low glucose
Load test using low glucose + CRH-R1 inhibitor
ACTH (pg/ml)
*
2000
1000
0
*p<0.05
100
10
Glucose (mg/dl)
ACTH (Glucose10) / ACTH (Glucose100)
**
4
3
2
1
0
* * p<0.01
–
+
CRH-R1 inhibitor
Low glucose stimulation
Hypothalamic CRH
CRH-R1
CRH-R1 inhibitor
ACTH

Fig. 24

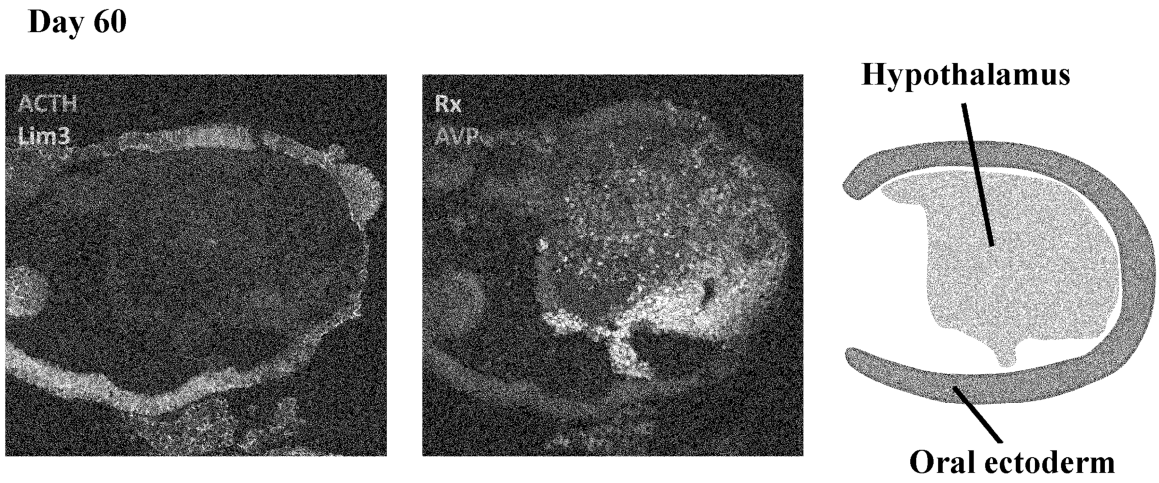

Day 60
ACTH
Lim3
Rx
AVP
Hypothalamus
Oral ectoderm

Fig. 25