Claims

1. A method for producing a cellular structure comprising hypothalamic tissue comprising a hypothalamic neuron, the method comprising the steps of:

(1) culturing an aggregate of human pluripotent stem cells in suspension in a medium containing a bone morphogenetic protein signal transduction pathway activating substance and ~~of~~ a substance acting on the Shh signaling pathway;

(2) further culturing the cell aggregate obtained in the step (1) in a medium containing a substance acting on the Shh signaling pathway, but not containing a bone morphogenetic protein signal transduction pathway activating substance; and

(3) recovering the cell aggregate obtained in the step (2) and subjecting the cells constituting the cell aggregate to dissociation culture,

wherein the bone morphogenetic protein signal transduction pathway activating substance in the step (1) is ~~selected from the group consisting of BMP2,~~ BMP4~~, BMB7 and GDF5~~, and the concentration of BMP4~~thereof~~ in the medium is~~corresponds to~~ 0.1 nM to 5.0 nM ~~of BMP4~~; and

wherein the substance acting on the Shh signaling pathway in the steps (1) and (2) is ~~selected from the group consisting of proteins belonging to the Hedgehog family, Shh receptors, Shh receptor agonists, Purmorphamine,~~ SAG ~~and Hh-Ag 1.5~~, and the concentration of SAG~~thereof~~ in the medium is~~corresponds to~~ 0.1 μM to 2.0 μM ~~of SAG~~,

wherein a medium used for the dissociation culture in step (3) comprises at least one member selected from the group consisting of a ciliary neurotrophic factor (CNTF), a brain-derived neurotrophic factor (BDNF), a neurotrophin 3 (NT-3), a fetal bovine serum, an N2 supplement, a B27 supplement and LM22A-4.

2. The producing method according to claim 1, wherein the medium used for the dissociation culture in step (3) comprises a ciliary neurotrophic factor (CNTF), a brain derived neurotrophic factor (BDNF) or LM22A4, a neurotrophin 3 (NT-3), a fetal bovine serum, an N2 supplement and a B27 supplement.

~~3. The production method according to claim 1 or 2, wherein the bone morphogenetic protein signal transduction pathway activating substance is BMP4.~~

~~4. The production method according to any one of claims 1 to 3, wherein the substance acting on the Shh signaling pathway is SAG.~~

~~5~~3. The producing method according to ~~any one of~~ claim~~s~~ 1 or 2~~to 4~~, wherein the step (2) is carried out under an oxygen partial pressure condition of 30 to 60 %.

~~6~~4. The producing method according to any one of claim~~-~~s 1 to 3~~5~~, wherein the step (3) is carried out under an oxygen partial pressure condition of 30 to 60 %.

~~7~~5. The producing method according to any one of claims 1 to 4~~6~~,

~~wherein the bone morphogenetic protein signal transduction pathway activating substance in the step (1) is BMP4,~~

~~wherein the substance acting on the Shh signaling pathway in the steps (1) and (2) is SAG, and~~

wherein the differentiation into dorsal hypothalamic tissue is induced by the steps (1) and (2).

~~8~~6. The producing method according to claim 5~~7~~, wherein the dorsal hypothalamic tissue comprises one or more neurons selected from the group consisting of vasopressin neurons, oxytocin neurons, thyrotropin releasing hormone neurons, corticotropin releasing hormone neurons and neuropeptide Y neurons.

~~9~~7. The producing method according to any one of claims 1 to 4~~6~~,

wherein the bone morphogenetic protein signal transduction pathway activating substance in the step (1) is BMP4, and the concentration thereof in the medium is 0.1 nM to 3.0 nM,

wherein the substance acting on the Shh signaling pathway in the steps (1) and (2) is SAG, and the concentration thereof in the medium is 0.2 $\mu$M to 1.5 $\mu$M,

wherein the medium further comprises an Akt inhibitor, and

wherein the differentiation into ventral hypothalamus tissue is induced by the steps (1) and (2).

~~10~~8. The producing method according to claim 7~~9~~, wherein the ventral hypothalamic tissue comprises one or more neurons selected from the group consisting of agoutirelated protein neurons, proopiomelanocortin neurons, melanin-concentrating hormone neurons and Orexin neurons.

~~11~~9. The producing method according to any one of claims 1 to 8~~10~~, wherein the suspension culture is carried out in the absence of feeder cells.

~~12~~10. The producing method according to any one of claims 1 to 9~~11~~, wherein the aggregate in the step (1) is formed by culturing dispersed human pluripotent stem cells in suspension.

~~13~~11. The producing method according to claim 10~~12~~, wherein the suspension culture is carried out by SFEBq (Serum-free Floating culture of Embryoid Body-like aggregates with quick reaggregation) method.

~~14~~12. A method for producing a cellular structure comprising hypothalamic tissue comprising a hypothalamic neuron and pituitary tissue, wherein the method comprises the steps of:

(i) culturing an aggregate of human pluripotent stem cells in suspension in a medium containing a bone morphogenetic protein signal transduction pathway activating substance and a substance acting on the Shh signaling pathway,

(ii) further culturing the cell aggregate formed in the step (i) in suspension in a medium containing a bone morphogenetic protein signal transduction pathway activating substance and the substance acting on the Shh signaling pathway, and

(iii) further culturing the cell aggregate obtained in the step (ii) in a medium containing the substance acting on the Shh signaling pathway, but not containing a bone morphogenetic protein signal transduction pathway activating substance; and

(iv) culturing the cell aggregate obtained in the step (iii) in suspension in a medium suitable for simultaneous induction of pituitary and hypothalamus,

wherein the~~said~~ medium suitable for simultaneous induction of pituitary and hypothalamus of the step (iv) is obtained by mixing the medium used in step (iii) in a volume ratio of 1:1 with a medium having a ciliary neurotrophic factor (CNTF), a brain derived neurotrophic factor (BDNF), a neurotrophin 3 (NT-3), a fetal bovine serum, an N2 supplement and a B27 supplement;

wherein the bone morphogenetic protein signal transduction pathway activating substance is selected from the group consisting of BMP2, BMP4, BMB7 and GDF5 and the substance acting on the Shh signaling pathway is selected from the group consisting of Shh protein~~s belonging to the Hedgehog family, Shh receptors, Shh receptor agonists~~, Purmorphamine, SAG and Hh-Ag 1.5.

~~15~~13. The producing method according to claim 12~~14~~, wherein the steps (ii), (iii) and (iv~~ii~~) are carried out under an oxygen partial pressure condition of 30 to 60 %.

~~16. The producing method according to claim 14 or 15,~~

~~wherein the following step (a) is performed between the steps (ii) and (iii), and~~

~~wherein, in the step (iii), the cell aggregate obtained in the step (a) is cultured in suspension:~~

~~(a) further culturing the cell aggregate obtained in the step (ii) in suspension in a medium containing the substance acting on the Shh signaling pathway,~~

~~wherein the substance acting on the Shh signaling pathway is selected from the group consisting of proteins belonging to the Hedgehog family, Shh receptors, Shh receptor agonists, Purmorphamine, SAG and Hh-Ag 1.5.~~

~~17. The producing method according to any one of claims 14 to 16, wherein the step (a) is carried out under an oxygen partial pressure condition of 30 to 60 %.~~

~~18~~14. The producing method according to ~~any one of~~ claim~~s~~ 12 or 13~~14 to 17~~, wherein the suspension culture in each step of (i), (ii), (iii) and (iv) is carried out in the absence of feeder cells.

~~19~~15. The producing method according to any one of claims 12~~14~~ to 14~~18~~, wherein the aggregate in the step (i) is formed by culturing dispersed human pluripotent stem cells in suspension.

~~20~~16. The producing method according to any one of claims 12~~14~~ to 15~~19~~, wherein the bone morphogenetic protein signal transduction pathway activating substance is BMP4.

~~21~~17. The producing method according to any one of claims 12~~14~~ to 16~~20~~, wherein the substance acting on the Shh signaling pathway is SAG.

~~22~~18. A cellular structure comprising hypothalamic tissue comprising a hypothalamic neuron and pituitary tissue, wherein the structure is obtained by the producing method according to any one of claims 12~~14~~ to 17~~21~~, wherein

(a) the hypothalamic tissue is corticotropin-releasing hormone (CRH) positive;

(b) the dorsal hypothalamic tissue is vasopressin neuron (AVP) positive, Rx positive, Pax6 positive and Nkx2.1 negative;

(c) the ventral hypothalamic tissue is positive for melanin concentrating

hormone neurons (MCH) positive, Rx positive, Pax6 negative and Nkx2.1 positive; and (d) the pituitary tissue is Adrenocorticotropic hormone (ACTH) positive.