Supplementary Material

Simultaneous Detection Of Both GDNF And GFRα1 Expression Patterns In The Mouse Central Nervous System

Clara Ortega-de San Luis¹ and Alberto Pascual¹,*

* Correspondence: Alberto Pascual; apascual-ibis@us.es

1 Supplementary Figures and Tables

1.1 Supplementary Figures

Supplementary Figure 1. GDNF expressing areas in Gdnf-Egfp adult mouse. Immunostaining anti-EGFP (green) in coronal brain sections (50 µm thickness) from 1 month old Gdnf-Egfp mouse. Scale bar represents 5 mm. Distance from bregma (B) is indicated (mm). Abbreviations are listed in Supp. Table 3.
Supplementary Figure 2. Perinatal expression of GFRα1 and GDNF. Coronal brain sections from Gfrα1^{−/−}; Gdnf^{−/−} mice brain showing GFRα1 (anti-EGFP, green) and GDNF expression (X-gal positive, blue areas drawn over left hemispheres). Scale bar represents 2.5 mm. Abbreviations are listed in Supp. Table 4. Distance from the most anterior part of the brain (mm) is shown.
**Supplementary Figure 3.** GDNF expressing areas in *Gdnf-Egfp* perinatal mouse. Immunostaining anti-EGFP (green) in coronal brain sections (50 µm thickness) from P0 *Gdnf-Egfp* mouse. Scale bar represents 1.2 mm. Distance from the most anterior part of the brain is indicated (mm). Abbreviations are listed in Supp. Table 4.

### 1.2 Supplementary Tables

**Supplementary Table 1. Mouse Lines Used in This Study**

| Mouse line | Mutations | Objective | Figure correspondence |
|------------|-----------|-----------|-----------------------|
| *Gdnf*^{−/−} ; *Gfra1*^{−/−} | GDNF+ cells express LacZ marker; GFRα1+ cells express EGFP marker. | Description of expression patterns. | 1A (left and center), 1B, 2, 3, 4A (center), 4C (even panels), 4D (2nd, 4th and 6th panels), 4E (2nd panel), 5A (2nd, 3rd, 4th and 7th panels), 5C (5th panel), 5D (2nd panel), 6A-E, 6F (middle panel), 9, 10, 11A (left), 11B (middle panel), 12, 13, 14, 15F and S2. 1A (right), 4A (3rd panel), 4B (2nd panel), 4C (odd panels), 4D (3rd, 6th panels), 4E (3rd and 4th panels), 5A (5th, 6th and 8th panels), 5B (2nd panel), 5C (2nd, 3rd, 4th and 6th panels), 5D (3rd and 4th panels), 6F (3rd panel), 11A (right), 11B (upper-right panel, bottom panel), 15A-E, S1 and S3. |
| *Gdnf*-Egfp | GDNF+ cells express EGFP marker. | Description of expression pattern. | 1A (right), 4A (3rd panel), 4B (2nd panel), 4C (odd panels), 4D (3rd, 6th panels), 4E (3rd and 4th panels), 5A (5th, 6th and 8th panels), 5B (2nd panel), 5C (2nd, 3rd, 4th and 6th panels), 5D (3rd and 4th panels), 6F (3rd panel), 11A (right), 11B (upper-right panel, bottom panel), 15A-E, S1 and S3. |
| Th-ires-Cre; *Gfra1*^{Flox/+} | Germline cells express CRE recombinase | Generation of Gfra1^{−/−} allele. | 1A (left). |
| *Gfra1*^{−/−} | *Gfra1* deficient mouse; GFRα1+ cells express EGFP marker. | Analysis of GFRα1 function during development. | 7. |
| *Gdnf*^{−/−} ; *Gfra1*^{−/−} | *Gdnf* deficient mouse; GFRα1+ cells express EGFP marker. | Analysis of GDNF function during development. | 8. |
| *Emx1*^{Cre/+}; *Gfra1*^{Flox/+} | EMX1+ cells recombine *Gfra1* and express EGFP marker. | Analysis of GFRα1+ cells in dorsal corticospinal tract. | 1A (left), 15G (left panel). |
| *Emx1*^{Cre/+}; R26R-YFP | EMX1+ cells express YFP marker. | Control of recombination in EMX1+ cells. | 15G (right panel). |
## Supplementary Table 2. Primary Antibody Used in This Study

| Antibody                        | Immunizing antigen                      | Host species         | Manufacturer / catalog No. | RRID#     | Dilution |
|---------------------------------|-----------------------------------------|----------------------|---------------------------|-----------|----------|
| Green fluorescent protein (GFP) | Recombinant GFP protein                 | Rabbit polyclonal    | Invitrogen A11122         | AB_2307355| 1:1000   |
| Bromodeoxyuridine (BrdU)        | Non described                           | Rat monoclonal       | Abcam; ab6326             | AB_305426 | 1:200    |
| Neuronal Nuclei (NeuN)          | Purified cell nuclei from mouse brain   | Mouse monoclonal (Clone A60) | Millipore MAB377         | AB_11210778| 1:500    |
## Supplementary Table 3. GFRα1 and GDNF expression in adult (P30) mouse brain

The study is based on at least three brains analyzed per structure. 1 Semi-quantitative analysis of the expression of GFRα1 and GDNF. –: None GFRα1/GDNF positive cell found; +: 1-20 GFRα1 positive / 1-5 GDNF positive; ++: 20-50 GFRα1 positive / 5-20 GDNF positive; and +++: more than 50 GFRα1 positive / more than 20 GDNF positive cells/analyzed structure. Cells are classified as Neurons when expression of NeuN was detected. 2 Semi-quantitative analysis of the expression of GFRα1 and GDNF in projections identified by morphology. High levels, ++; low, +; and absence of signal, –. 3 Discrepancies between LacZ and EGFP reporter mice are shown separated by a slash. 4 References where expression of GFRα1 and GDNF has been reported, 1: Trupp et al. 1997; 2: Hidalgo-Figueroa et al. 2012; 3: Pascual et al. 2008. GFRα1 and GDNF references are separated by a slash. 5 Concordance with Allen Brain Atlas GFRα1 in situ hybridization data. +: detected area; –: non detected area. n.a. not applicable.

| Structure | Abbreviation | Figures | GFRα1 | GDNF (X-gal/EGFP) | References 4 | Allen Brain Atlas 5 |
|-----------|--------------|---------|-------|-------------------|----------------|-------------------|
| Cerebral cortex | | | | | | |
| Somatosensory areas | SS | 1B | – | – | – | n.a. |
| Secondary motor area | MO | 1B | Neurons (+) | – | – | n.a. |
| Auditory area | AUD | 1B | Neurons (+) | – | – | n.a. |
| Visual areas | VIS | 1B | Neurons (+) | – | – | n.a. |
| Retrosplenial area | RSP | 1B, 2I | Neurons (+) | – | – | n.a. |
| Posterior parietal association areas | PTLp | 1B | Neurons (+) | – | – | n.a. |
| Olfactory areas | | | | | | |
| Main olfactory bulb, outer plexiform layer | MOBopl | 1B, 2A | Cells (+) | – | – | n.a. |
| Accessory olfactory bulb | AOB | 1B, 2A, 4B | ++ | Cells (−/+ | – | n.a. |
| Nucleus of the lateral olfactory tract | NLOT | 1B, 5A, S1 | – | – | n.a./+ | n.a. |
| Hippocampal formation | | | | | | |
| Field CA1 / CA2 | CA1/CA2 | 1B, 2G | Neurons (+) | – | – | n.a. |
| Field CA3 | CA3 | 1B, 2G | Neurons (+) | – | – | n.a. |
| – Stratum oriens | | | | | | |
| – Pyramidal layer | | | | | | |
| Dentate gyrus | DG | 1B, 2I | Neurons (++) | – | – | n.a. |
| Entorhinal area, layer V | ENT | 1B, 3A | Neurons (+) | – | – | n.a. |
| Subiculum | SUB | 1B, 3C, D, 9B | Neurons (++) | – | – | n.a. |
| Cortical-subplate area | | | | | | |
| Claustrum | CLA | 1B, 2C, 11B, S1 | Neurons (++) | – | – | n.a./+ |
| Endopiriform nucleus | EP | 1B, 2C, S1 | Neurons (++) | – | – | n.a./ |
| Amygdala | | | | | | |
| Central amygdalar nucleus | CEA | 1B, 11B | Neurons (+) | – | – | n.a. |
| Medial amygdalar nucleus | MEA | 1B, 3A, 11B | Neurons (+) | – | – | n.a. |
| Basolateral amygdalar nucleus | BLA | 1B, 11B, S1 | Neurons (+) | – | – | n.a./+ |
| Striatum | | | | | | |
| Caudoputamen | CP | 1B, 4C, 9A, 12A, S1 | – | ++ | Neurons (+) | n.a./1, 2, 3 |
| Accumbens | ACB | 1B, 2D, 4C, 12A, S1 | Neurons (++) | ++ | Neurons (++) | /1, 2, 3 |
| Fundus of Striatum | FS | 1B, 2E, 4E, 12C, S1 | Neurons (++) | – | Neurons (++) | – |
| Olfactory tubercle | OT | 1B, 2C, 2D, 4A, 12A, S1 | Neurons (++) | – | Neurons (++) | – |
| Lateral septal nucleus | LS | 1B, 2D, 4E, 4D, 12A, S1 | Neurons (++) | – | Neurons (++) | – |
| Pallidum | | | | | | |
| Substantia innominata | SI | 1B, 2D, 4A, 12A, S1 | Neurons (+) | – | Neurons (+) | – |

References where expression of GFRα1 and GDNF has been reported, 1: Trupp et al. 1997; 2: Hidalgo-Figueroa et al. 2012; 3: Pascual et al. 2008. GFRα1 and GDNF references are separated by a slash. Concordance with Allen Brain Atlas GFRα1 in situ hybridization data. +: detected area; –: non detected area. n.a. not applicable.
| Supplementary Material |
|------------------------|
| **Medial septal complex** | MSC | 1B, 4D, 12B, S1 | – | + | Neurons (+) | – | 1/2 | n.a. |
| **Bed nuclei of the stria terminalis; anterior division** | BST | 1B, 2E, 4E, S1 | Neurons (+++) | – | – | n.a./+ | /n.a. | + |
| **Thalamus** | | | | | | | | |
| Lateral posterior nucleus thalamus | LP | 1B, 3A | Neurons (+++) | – | – | – | /n.a. | + |
| Anteroventral nucleus thalamus | AV | 1B, 5A, S1 | – | – | Neurons (+++/+) | – | n.a./1, 3 | n.a. |
| Anteromedial nucleus thalamus | AM | 1B, 5A, S1 | – | – | Neurons (+++/+) | – | n.a./1, 3 | n.a. |
| **Nucleus of reunions** | RE | 1B, 5A, S1 | – | – | Neurons (+++) | – | n.a./ | n.a. |
| **Reticular nucleus thalamus** | RT | 1B, 2G | Neurons – | – | – | 1/n.a. | + |
| Geniculate complex | GEN | 1B, 3A, 5C, S1 | Neurons (+) | – | – | Neurons (–) | + |
| **Medial habenula** | MH | 1B, 2G, 10 | Neurons (++) | – | – | – | 1/n.a. | + |
| **Hypothalamus** | | | | | | | | |
| Anteroventral periventricular nucleus | AVPV | 1B, 2E | Neurons (+++) | – | – | – | /n.a. | + |
| Dorso-medial nucleus | DMH | 1B, 2G | Neurons (+++) | – | – | – | /n.a. | + |
| Periventricular nucleus | PV | 1B, 3A, 5C | Neurons (+++) | – | – | n.a./+ | /n.a. | – |
| Parasubthalamic nucleus | PSTN | 1S, 5C | – | – | Neurons (–) | – | n.a./ | n.a. |
| **Mammillary nucleus** | MOB | 1B, 9B, 10B | – | ++ | – | – | n.a./n.a. | + |
| **Posterior hypothalamic nucleus** | PH | 1B, 3A | Neurons (+++) | – | – | – | /n.a. | + |
| **Tuberal nucleus** | TU | 1B, 2H | Neurons (+) | – | – | – | /n.a. | + |
| **Zona incerta** | ZI | 1B, 2F | Neurons (+++) | – | – | – | 1/n.a. | + |
| **Midbrain** | | | | | | | | |
| Superior colliculus | SC | 1B, 3B, 5D, S1 | Neurons (+++) | – | – | Neurons (–) | /+ |
| Oculomotor nucleus | III | 1B, 3E | Neurons (+++) | – | – | – | /n.a. | + |
| **Midbrain reticular nucleus** | MRN | 1B, 3D, 14A | Neurons (+++) | – | – | Neurons (+++) | (+) |
| Ventral tegmental area | VTA | 1B, 3C | Neurons (+++) | – | – | – | 1/n.a. | + |
| Periaqueductal gray | PAG | 1B, 3D, 5D, S1 | Neurons (+) | – | – | Neurons (+/+) | + |
| **Substantia nigra** | | | | | | | | |
| – Compact part | SNC | 1B, 3C | Neurons (+++) | – | – | – | /n.a. | + |
| – Reticular part | SNr | 1B, 3C | Neurons (+++) | – | – | – | /n.a. | + |
| Anterior pretectal nucleus | APN | 1B, 3A, C | Neurons (+) | – | – | – | /n.a. | + |
| **Nucleus of the Optic tract** | NOT | 1B, 3C | Neurons (+) | – | – | – | /n.a. | + |
| Pedunculopontine nucleus | PPN | 1B, 3F, AC, S1 | Neurons (+) | – | – | Neurons (+) | + |
| **Interpeduncular nucleus** | IPN | 1B, 10 | – | +++ | – | – | 1/n.a. | n.a. |
| **Dorsal nucleus raphae** | DR | 1B, 3F | Neurons (+++) | – | – | – | 1/n.a. | + |
| **Hindbrain** | | | | | | | | |
| Principal sensory nucleus of the trigeminal | PSV | 1B, 3F | Neurons (+++) | – | – | – | /n.a. | + |
| **Parabrahicial nucleus** | PB | 1B, 4G | Neurons (+) | – | – | – | /n.a. | + |
| **Superior olivary complex** | SOC | 1B, 3G | Neurons (+++) | – | – | – | /n.a. | + |
| V Motor nucleus of trigeminal | V | 1B, 3H | Neurons (+++) | – | – | – | /n.a. | + |
| **Superior central nucleus raphe** | CS | 1B, 3F | Neurons (+++) | – | – | – | /n.a. | + |
| Locus ceruleus | LC | Neurons (+) | – | Neurons (+) | – | 1/2 | + |
| **Cerebellum** | | | | | | | | |
| Cerebellar cortex, granular layer | CBXgr | 11A | – | +++ | Neurons (–) | – | n.a./1 | + |
| **Fiber tracts** | | | | | | | | |
| Olfactory nerve layer of main olfactory bulb | onl | 1B, 2A, 4A, S1 | – | ++ | – | n.a./+ | n.a./n.a. | n.a. |
| **Optic tract** | opt | 1B | – | + | – | – | n.a./n.a. | n.a. |
| Corpus callosum, anterior forceps | fa | 1B, 2C | – | + | – | – | n.a./n.a. | n.a. |
| **Internal capsule** | int | 1B | – | + | – | – | n.a./n.a. | n.a. |
| **Fimbria** | fi | 1B, 2G, 5B, S1 | Neurons (+++) | – | – | n.a./+ | n.a./n.a. | n.a. |
| **Columns of the fornix** | fx | 1B, 2E | Neurons (+++) | – | – | – | n.a./n.a. | n.a. |
| **Stria terminalis** | st | 1B | – | + | – | – | n.a./n.a. | n.a. |
| **Mammillothalamic tract** | mtt | 1B, 2G, 9B, 10B | – | + | – | – | n.a./n.a. | n.a. |
| **Fasciculus retroflexus** | fr | 1B, 10 | – | +++ | – | – | n.a./n.a. | n.a. |
| Others                  |         |                  | Cells (+++) | – | Cells (+) | – | + |
|------------------------|---------|------------------|-------------|---|-----------|---|---|
| Subependymal zone      | SEZ     | 1B, 2A, 4A, S1   | Cells (+++) | – | Cells (+) | – | + |
| Subventricular zone    | SVZ     | 1B, 2D, 4D, 12A, | Cells (+++) | – | Cells (+) | – | + |
|                        |         | S1               |             |   |           |   |   |
| Subcommissural organ   | SCO     | 1B, 3A, 5C, S1   | Cells (+++) | – | Cells (+++) | – | + |
|                        |         |                  |             |   |           |   |   |/1, 2


Supplementary Table 4. GFRα1 and GDNF expression in neonatal (P0) mouse brain

The study is based on at least three brains analyzed per structure. 1 Semi-quantitative analysis of the expression of GFRα1 and GDNF: None GFRα1/GDNF positive cell found; +: 1-20 GFRα1 positive / 1-5 GDNF positive; ++: 20-50 GFRα1 positive / 5-20 GDNF positive; and +++: more than 50 GFRα1 positive / more than 20 GDNF positive cells/analyzed structure. 2 Semi-quantitative analysis of the expression of GFRα1 and GDNF in projections identified by morphology. High levels, ++; low, +; and absence of signal, −. 3 Discrepancies between LacZ and EGFP reporter mice are shown separated by a slash. 4 References where expression of GFRα1 and GDNF has been reported. 1: Golden et al. 1999; 2: Hidalgo-Figueroa et al. 2012. 5 Concordance with Allen Brain Atlas GFRα1 in situ hybridization data. +: detected area; −: non detected area. n.a. not applicable. n.i.: structure not identified in the Allen Brain Atlas.

| Structure                                   | Abbreviation | Figures | GFRα1 Cells | GFRα1 Projections | GDNF (X-gal/EGFP) Cells | GDNF (X-gal/EGFP) Projections | References 5 | Allen Brain Atlas 5 (GFRα1) |
|--------------------------------------------|--------------|---------|--------------|------------------|------------------------|-------------------------------|----------------|-----------------------------|
| Cerebral cortex                            |              |         |              |                  |                        |                               |                |                             |
| Cingulate cortex                           | Cg           | S2      | ++           | –                | –                      | –                             | 1/ n.a.        |                             |
| Motor cortex, secondary                    | MOs          | S2      | ++           | –                | –                      | –                             | / n.a.         | +                           |
| Olfactory areas                            |              |         |              |                  |                        |                               |                |                             |
| Main olfactory bulb, granular cell layer   | MOBgr        | S2      | +            | –                | –                      | –                             | / n.a.         | +                           |
| Outer plexiform layer                      | MOBpl        | S2, S3  | +            | –                | –/ +                  | –                             | n.a./+         | n.a./ n.a.                  |
| Accessory olfactory bulb                   | AOB          | S2, S3  | –            | ++               | –                      | n.a./+                        | n.a./ n.a.     |                             |
| Hippocampal region                         |              |         |              |                  |                        |                               |                |                             |
| Dentate Gyrus                              | DG           | S2      | +++          | –                | –                      | –                             | / n.a.         | +                           |
| Subiculum                                  | SUB          | S2      | +++          | –                | –                      | –                             | / n.a.         | +                           |
| Cortical subplate                          |              |         |              |                  |                        |                               |                |                             |
| Claustrum                                  | CLA          | S2      | ++           | –                | –                      | –                             | 1/ n.a.        | +                           |
| Endopiriform nucleus                       | EP           | S2      | ++           | –                | –                      | –                             | 1/ n.a.        | +                           |
| Amygdala                                   | AMG          | S2      | ++           | –                | –                      | –                             | 1/1            | +                           |
| Striatum                                   | S2           |         |              |                  |                        |                               |                |                             |
| Caudoputamen                                | CP           | S2, S3  | –            | ++               | –/ +                  | –                             | 1/2, n.a.      |                             |
| Accumbens                                  | ACB          | S2, S3  | ++           | –                | +                      | –                             | 1/2            | +                           |
| Olfactory tubercle                         | OT           | S2, S3  | +++          | –                | ++                    | –                             | 1/ n.a.        |                             |
| Lateral septal nucleus                     | LS           | S2      | +++          | –                | –                      | –                             | / n.a.         | +                           |
| Pallidum                                   | S2           |         |              |                  |                        |                               | 1/2            |                             |
| Substantia innominata                      | SI           | S2, S3  | ++           | –                | +                      | –                             | 1/ n.a.        |                             |
| Medial septal complex                      | MSC          | S2, S3  | +            | ++               | –                      | –                             | 1/2, n.i.      |                             |
| Thalamus                                   |              |         |              |                  |                        |                               |                |                             |
| Lateral posterior nucleus of the thalamus  | LP           | S2      | ++           | +                | –                      | –                             | n.a.           |                             |
| Reticular nucleus thalamus                 | RT           | S2      | +++          | –                | –                      | –                             | / n.a.         | +                           |
| Ventral geniculate nucleus                 | GENv         | S2      | +            | –                | –                      | –                             | / n.a.         | +                           |
| Medial habenula                            | MH           | S2      | +++          | –                | –                      | –                             | / n.a.         | +                           |
| Lateral habenula                           | LH           | S2      | +            | –                | –                      | –                             | / n.a.         | n.i.                        |
| Hypothalamus                               | S2           |         |              |                  |                        |                               | 1/2            |                             |
| Medial mammillary nucleus                  | MM           | S2      | –            | +                | –                      | –                             | n.a./n.a.      | n.a.                        |
| Posterior hypothalamic nucleus             | PH           | S2      | ++           | –                | –                      | –                             | / n.a.         | +                           |
| Arcuate hypothalamic nucleus               | ARH          | 6C, S2  | ++           | –                | –                      | –                             | / n.a.         | +                           |
| Dorsomedial hypothalamic nucleus dorsal part| DMH         | S2      | +            | –                | –                      | –                             | / n.a.         | +                           |
| Preoptic area                              | PO           | 6A, S2  | ++           | –                | –                      | –                             | / n.a.         | +                           |
| Anterior hypothalamic area                 | AH           | 6F, S2, S3 | – | – | ++ | – | n.a./ n.a. | + | |
| Lateral hypothalamic area                  | LHA          | 6B, S2 | ++ | – | –/+ | – | n.a. | + | |
| Zona incerta                              | ZI           | S2, S3 | ++ | – | –/+ | – | n.a. | + | |
| Midbrain                                  |              |         |              |                  |                        |                               |                |                             |
| Superior Colliculus                        | SC           | S2, S3  | ++           | +                | –/ +                  | –                             | +              |                             |
| Oculomotor nucleus                         | III          | 13A, S2 | ++           | –                | ++/+                  | –                             | +              |                             |
| Structure                          | S3          | S2          | Notes       |
|-----------------------------------|-------------|-------------|-------------|
| Cuneiform nucleus                 | CUN 6E, 13B| S2          | ++ – +/- – +|
| Midbrain reticular nucleus        | MRN S2     | ++ – +/- – /2 | +           |
| Ventricle tegmental area           | VTA S2     | ++ – – – /n.a. | +           |
| Periaqueductal gray               | PAG S2, S3 | ++ + – – – | +           |
| Substantia nigra                  | S2         | ++ – – – 1/n.a. | +           |
| – Compact part                    | SNC S2     | ++ – – – /n.a. | +           |
| – Reticular part                  | SNR S2     | ++ – – – /n.a. | +           |
| Anterior pretectal nucleus        | APN S2     | + – – – 1/n.a. | +           |
| Nucleus of the Optic tract        | NOT S2     | + – – – /n.a. | +           |
| Pedunculopontine nucleus          | PPT S2     | + – +/- – | +           |
| Interpedunicular nucleus          | IPN 10C, S2| ++ +/ – – | n.a./2 +    |
| Dorsal raphe nuclei               | DR S2      | ++ – – – | /n.a. +     |
| Hindbrain                         | NLL 6D, S2 | + – – – | /n.a. n.i. |
| Medial paralemniscal nucleus      | PLN 6D, S2 | + – +/- – | n.i. n.i.   |
| Principal sensory nucleus of the  | PSV S2     | ++ – – – | /n.a. +     |
| trigeminal                        |            |            |             |
| Parabrachial nucleus              | PB S2      | + – – – | /n.a. +     |
| Olivary Complex                   | SOC S2     | + – – – | /n.a. +     |
| V motor nucleus of trigeminal     | V 14B, S2  | +++ – +/- – | 2 +         |
| Locus ceruleus                    | LC 13C, S2 | ++ – ++ – | 2 +         |
| Medulla                           | VII 6E, S2 | ++ – – – | /n.a. +     |
| Fiber tracts                      | onl S2     | – ++ + | n.a./ n.a. |
| Olfactory nerve layer             | int S2     | – – – n.a./ | n.a/n.a. n.a. |
| Internal capsule                  | fr S2      | – +++ – | n.a/n/a n.a. |
| Fasciculus retroflexus            | fi S2      | – ++ – | n.a/n/a n.a. |
| Others                            | Subependymal zone | SEZ S2 | ++ – – – | /n.a. – |
| Subcommisural organ               | SCO S2, S3 | +++ ++ – | n.i.        |