



S1P1 receptor expression during the embryonic development of the nervous system

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Introduction

BACKGROUND:

- Sphingosine 1 phosphate receptor (S1P1), also known as the endothelial differentiation gene 1 (EDG1), belongs to a large family of G-protein-coupled receptors.
- This receptor, which binds the bioactive signaling molecule Shingosine 1 phospate (S1P), is involved in many cellular processes, including cell differentiation, migration, calcium homeostasis, inflammation and immunity [2].
- S1P1 is also thought to play a number of vital roles in the developing central nervous system (CNS), including astrocyte proliferation, cell survival, neurite outgrowth and neurogenesis [3].
- However, despite the importance of S1P1 in CNS formation, the expression patterns of S1P1 in the CNS are not clearly defined.

AIMS:

- Show the pattern of S1P1 expression in the developing forebrain, midbrain, hindbrain and spinal cord through the development of the CNS.
- Identify the phenotype of cells expressing S1P1.

Materials and Methods

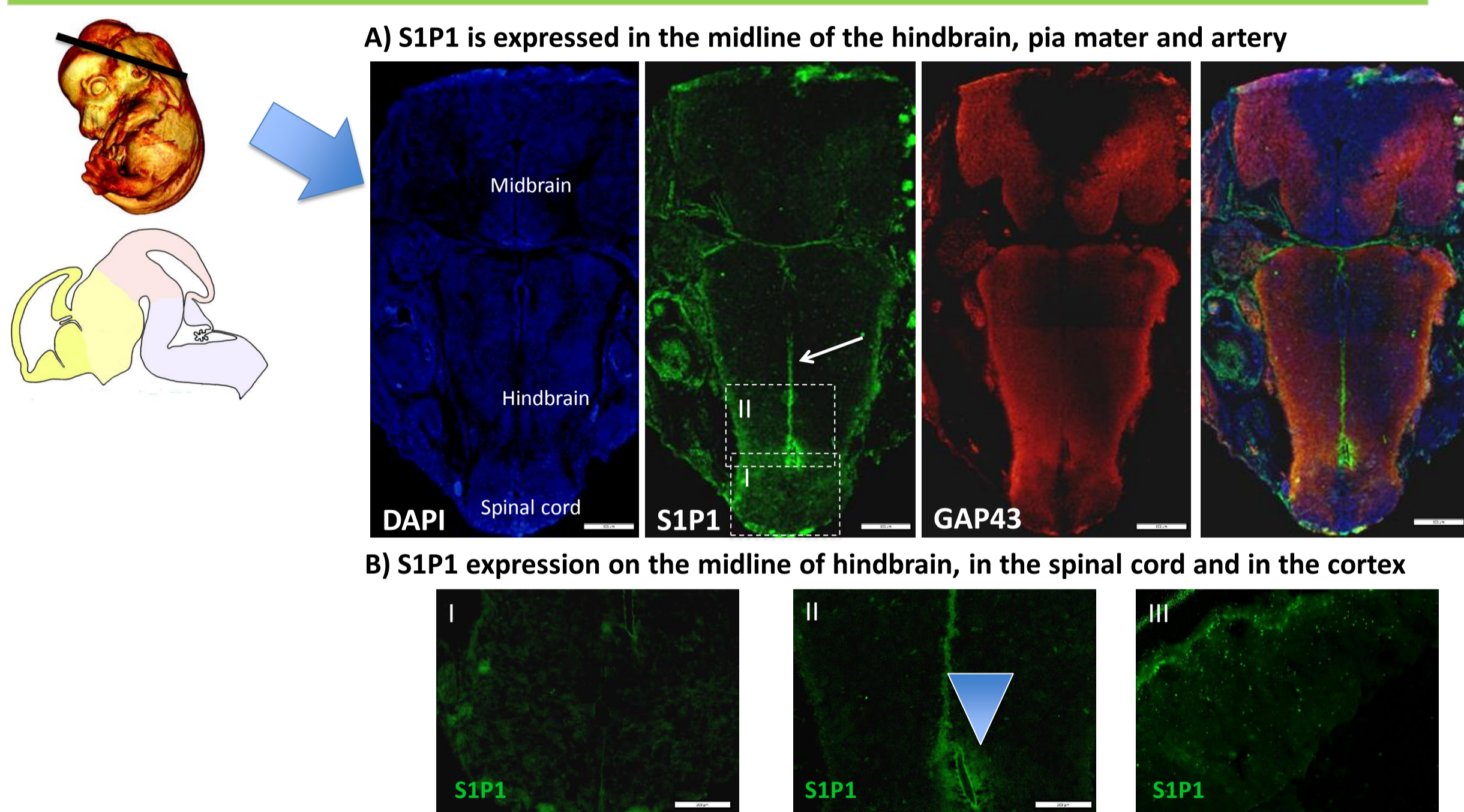
- Immunohistochemistry: 20 μm cryosections of mouse (C57BL6) embryos ages E13.5, E15.5, E17.5 and P2 were cut from the brain and thoracic regions of the spinal cord (n = 3).
- Images were acquired on an Olympus IX81 fluorescent microscope using Cell Sens Imaging software and analysed with ImageJ software.
- The primary antibodies used are shown in table 1. Nuclei were stained with DAPI (1:1000)

Antibodies	Epitope
BIII tubulin (1:500 Abcam)	Mature neurons
EDG1 (1:200 Santa Cruz)	S1P1 receptor
FORSE-1 (1:100 DSHB)	neuroprogenitors
GAP43 (1:200 Abcam)	Axon growth cones
Iba1 (1:100)	Microglia
Nestin (1:100 DSHB)	Radial glial cells
PSA-NCAM (1:100)	neuroprogenitors

- Haematoxyline-eosine staining allowed the identification of nuclei and cytoplasm.

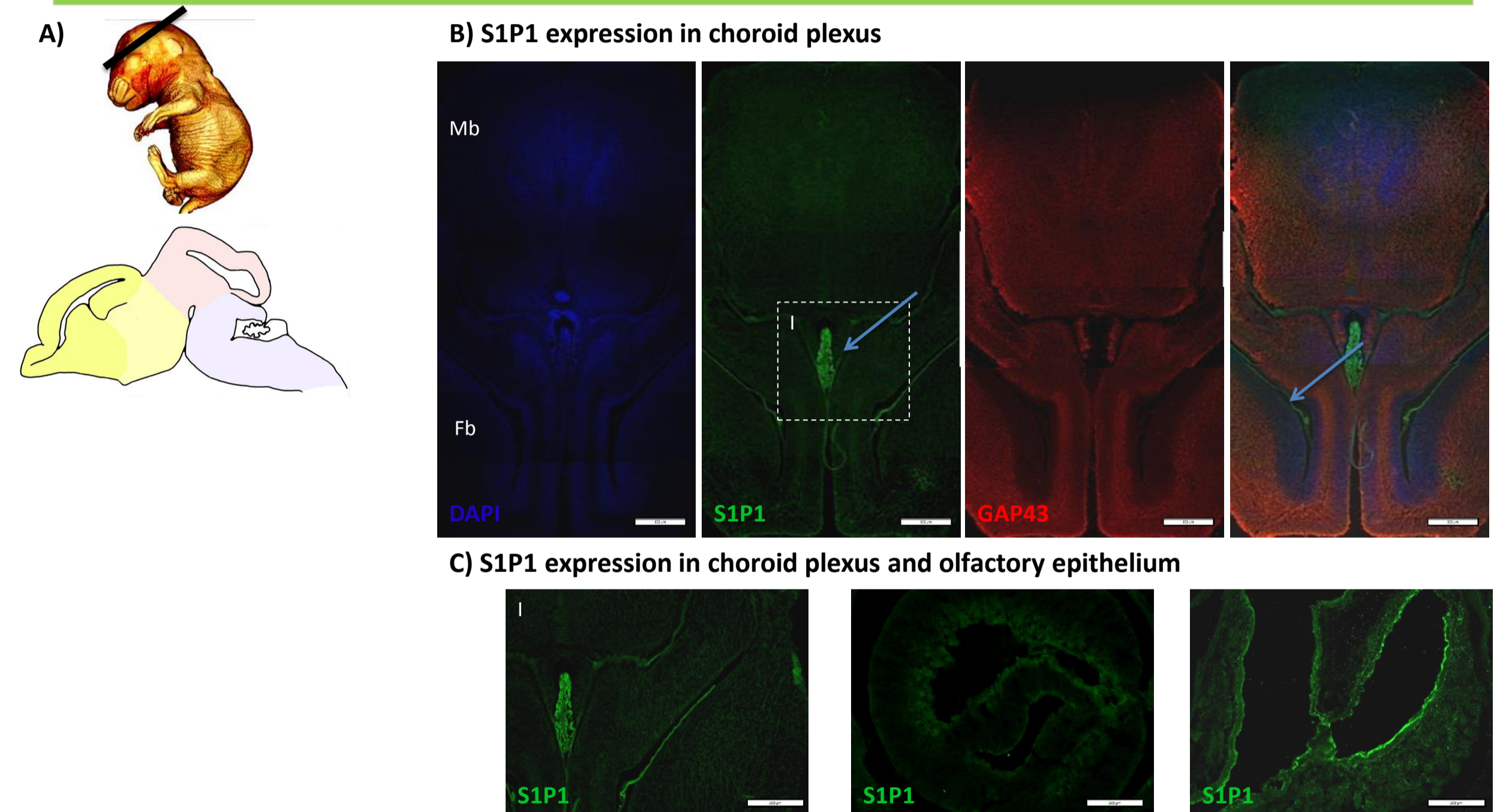
Results & Discussion

S1P1 expression in the early developing CNS: E13.5



A) The staining of S1P1 on the midline of the hindbrain (arrow) might show a role of S1P1 in closure of the neural tube. S1p1 is not expressed by growing axons. S1P1 is also present surrounding an artery in the spinal cord (arrowhead). Scalbar : 200μm

S1P1 expression in the late stages of CNS development: E17.5



S1P1 is expressed in choroid plexus and olfactory epithelium. Unlike some studies showed S1P1 expressed in retina, we didn't find a convinced expression of this receptor. Scalbar : 200μm

S1P1 expression in the mid stages of CNS development: E15.5

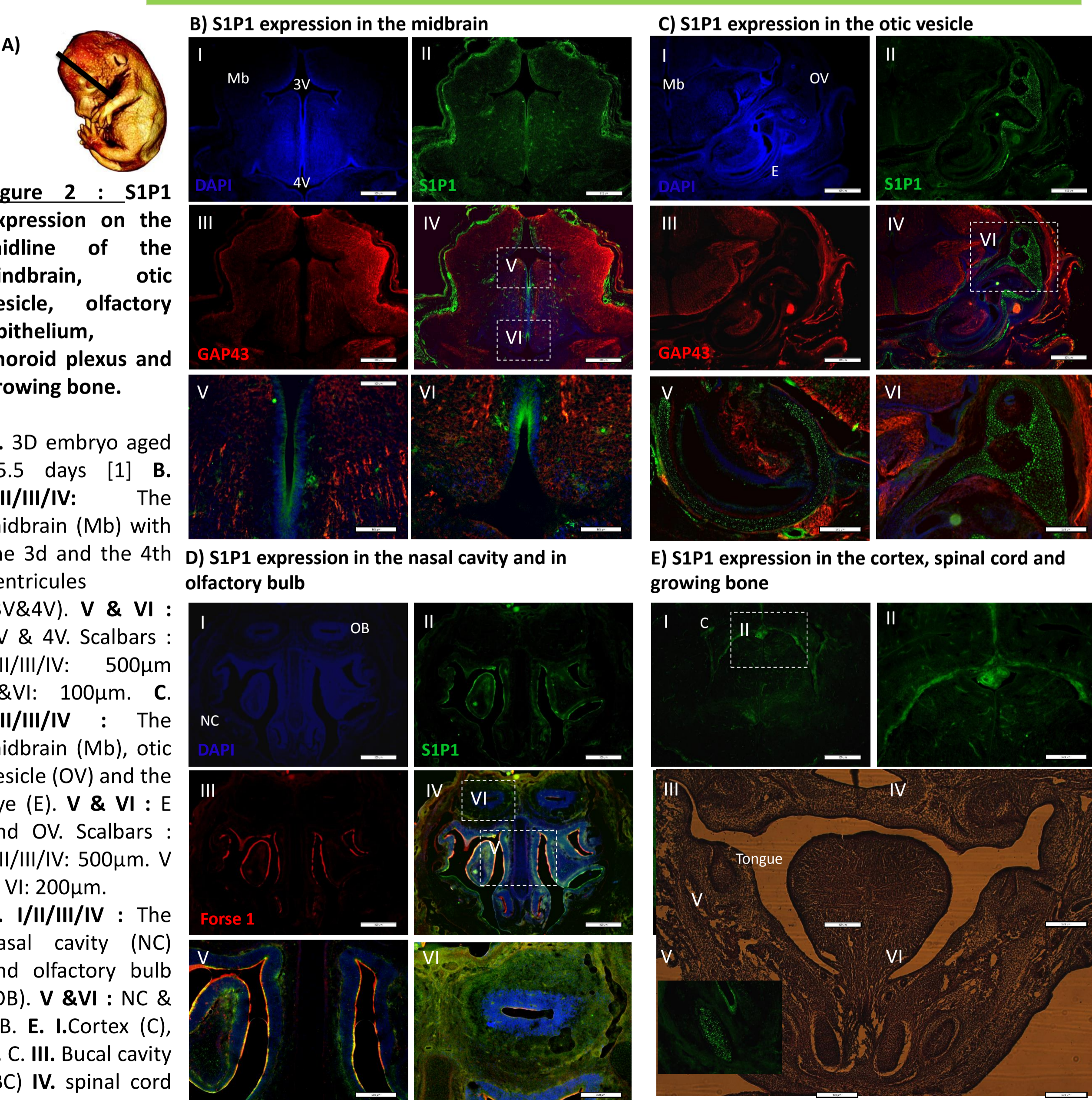
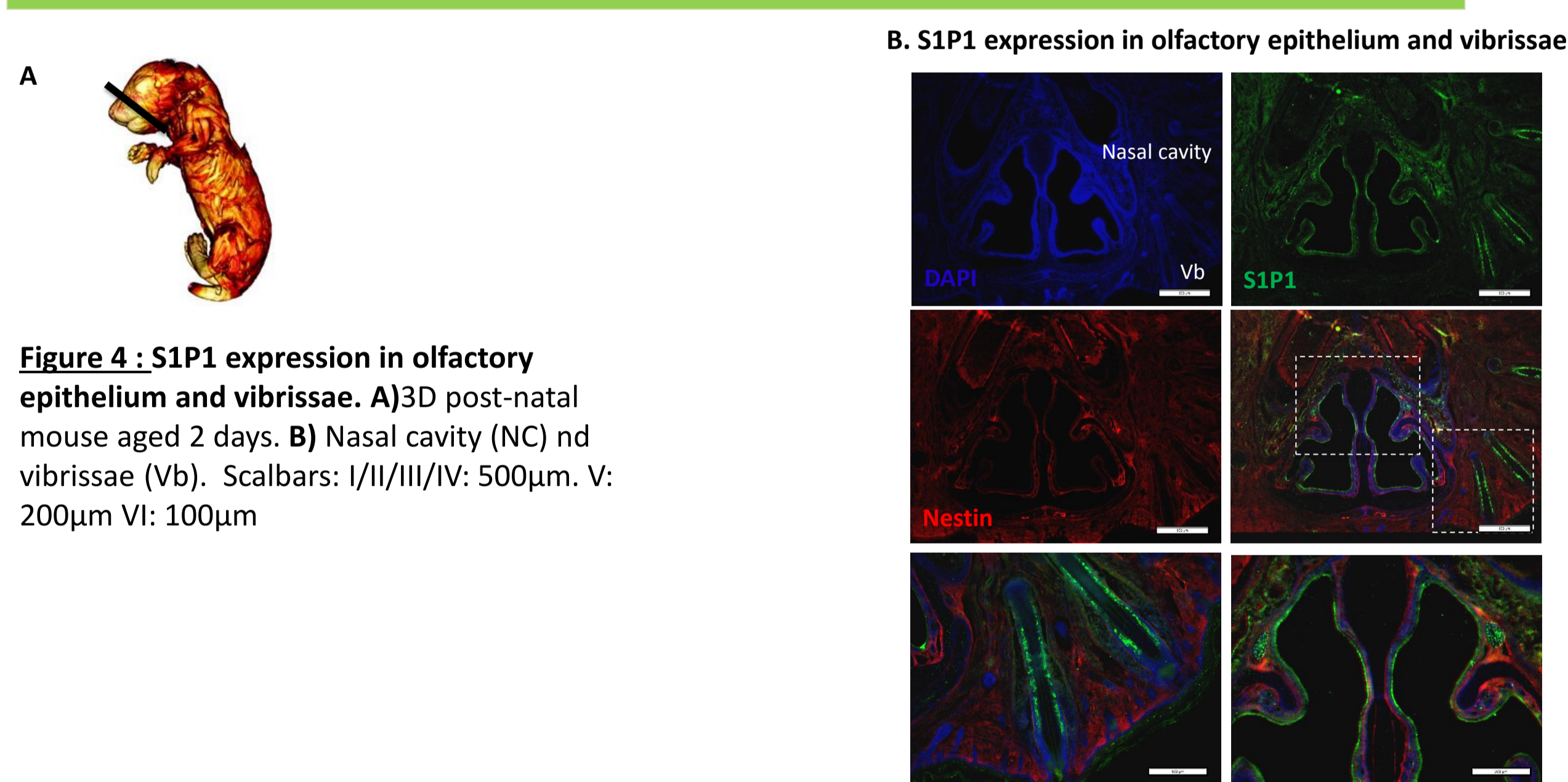


Figure 2 : S1P1 expression on the midline of the hindbrain, otic vesicle, olfactory epithelium, choroid plexus and growing bone.

A. 3D embryo aged 15.5 days [1] B. I/II/III/IV: The midbrain (Mb) with the 3d and the 4th ventricles (3V&4V). V & VI : 3V & 4V. Scalbars : I/II/III/IV: 500μm V&VI: 100μm. C. I/II/III/IV : The midbrain (Mb), otic vesicle (OV) and the eye (E). V & VI : E and OV. Scalbars : I/II/III/IV: 500μm. V & VI: 200μm. D. I/II/III/IV : The nasal cavity (NC) and olfactory bulb (OB). V & VI : NC & OB. E. I. Cortex (C), II. C. III. Bucal cavity (BC) IV. spinal cord (SC) V. mandibule with immunostaining and VI. haematoxyline-éosine. Scalbars : I, III & VI: 500μm. II & IV : 200μm. V: 100μm

Conclusion : E 15.5 is a pivotal age in the development and S1P1 expression seems more important at this age, especially for the bone development and olfactory epithelium. At this age this is the first time that we see S1P1 expression on choroid plexus. Finally, S1P1 Expression on the midline of the hindbrain seems to decrease with the development.

S1P1 expression in the postnatal brain: P2



Conclusion : S1P1 expression is observed at this age only in the olfactory epithelium and around the vibrissae. This age marks the end of the nervous system development. The expression of S1P1 seems to be more important during the developmental milestones.

Conclusions

We demonstrated that the S1P1 receptor is expressed during the nervous system and the head development. It is seemed to be important for bone, olfactory, choroid plexus development and the midline of the hindbrain. The simultaneous staining of S1P1 and typical structure of different types of cells allow us to exclude the idea that this receptor is expressed by neurones, radial glial cells and microglia. The use of other markers would be to consider to determine the cellular type involved in the pattern observed. On the other side, S1P1 expression seems to colocalize with forse 1, which is known to be expressed in the olfactory neuroepithelium. It would therefore be interesting to use confocal microscopy to determine its reliability.

References

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