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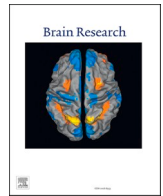
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A Disintegrin And Metalloprotease 10 expression within the murine central nervous system

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ABSTRACT

A Disintegrin And Metalloprotease 10 (ADAM10), is able to control several important physiopathological processes through the shedding of a large number of protein substrates. Although ADAM10 plays a crucial role in the central nervous system (CNS) development and function, its protein distribution in the CNS has not been fully addressed. Here, we described the regional and cellular ADAM10 protein expression in C57BL/6 mice examined by immunofluorescence 1) throughout the adult mouse brain, cerebellum and spinal cord *in vivo* and 2) in different cell types as neurons, astrocytes, oligodendrocytes and microglia *in vitro*. We observed ADAM10 expression through the whole CNS, with a strong expression in the hippocampus, in the hypothalamus and in the cerebral and piriform cortex in the brain, in the Purkinje and in granular cell layers in the cerebellum and in the spinal cord to a lower extent. *In vivo*, ADAM10 protein expression was mainly found in neurons and in some oligodendroglial cell populations. However, in primary cultures we observed ADAM10 expression in neurons, oligodendrocytes, astrocytes and microglia. Interestingly, ADAM10 was not only found in the membrane but also in cytoplasmic vesicles and in the nucleus of primary cultured cells. Overall, this work highlights a wide distribution of ADAM10 throughout the CNS. The nuclear localization of ADAM10, probably due to its intracellular domain, emphasizes its role in cell signalling in physiological and pathological conditions. Further investigations are required to better elucidate the role of ADAM10 in glial cells.

1. Introduction

A disintegrin and metalloproteases (ADAMs) are zinc-dependent endopeptidases that have a key role in cell adhesion and proteolytic processing of the ectodomains of diverse cell surface receptors, extracellular matrix proteins and signalling molecules (Edwards et al., 2008). ADAMs' members are type I transmembrane proteins with a large extracellular domain that are widely expressed. This family is now considered as the major proteinase family mediating the shedding of numerous proteins. Among all the ADAMs known so far, three of them are described as α -secretases, ADAM9, ADAM10 and ADAM17. These enzymes are involved in multiple cell processes such as migration, cell adhesion and proliferation but also inflammation and cell death (Yang et al., 2006; Koenen et al., 2009; Dreymueller et al., 2017; Smith et al.,

2020; Zhu et al., 2021). ADAM10, which was firstly described more than 30 years ago (Chantray & Glynn, 1990), is one of the most studied α -secretases belonging to the ADAMs family (Allinson et al., 2003). ADAM10 plays a key role in the central nervous system (CNS) development. In fact, ADAM10 KO mice are lethal at E9-E10 mainly because of heart and CNS development defects (Hartmann et al., 2002). Its role is also crucial in CNS repair as it promotes neural precursor cells migration towards demyelinated lesions (Klingener et al., 2014). In the CNS, neuronal ADAM10 regulates proliferation, migration, differentiation and survival of various cells, and is involved in axonal growth and myelination (Saftig & Lichtenthaler, 2015).

ADAM10 is first synthesised as a proenzyme and upon cleavage of its prodomain by furins, ADAM10 becomes catalytically active (summarised by Endres & Deller, 2017). This mature form of ADAM10 is

Abbreviations: ADAM, A Disintegrin And Metalloprotease; APP, Amyloid Precursor Protein; CaBP, Calbindin Protein; CNPase, 2'3'-Cyclic Nucleotide 3'-Phosphodiesterase; CNS, Central Nervous System; DIV, Days *in vitro*; E, Embryonic day; FGF, Fibroblast Growth Factor; GFAP, Glial Fibrillary Acidic Protein; ICD, Intracellular Domain; i.p., intraperitoneal; ISH, *in situ* Hybridization; MAG, Myelin-Associated Glycoprotein; NG2, Neuronal Glial antigen 2; OPC, Oligodendrocyte Precursor Cell; P, Postnatal day; PDGF, Platelet-Derived Growth Factor; RT, Room temperature.

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addressed at the cell membrane to promote substrate processing. Its structure is composed of different domains, including a metalloprotease domain, a disintegrin, a cysteine-rich, a transmembrane and an intracellular domain (Edwards et al., 2008; Vincent & Checler, 2012). These last years, numerous substrates of ADAM10 have been described, among them TNFR1 (Black et al., 1997; Zbodakova et al., 2021), PrP (Altmepfen et al., 2015), L1CAM (Yang et al., 2011; Linneberg et al., 2019) or Notch (Pan & Rubin, 1997; Caolo et al., 2020). The most well-known substrate of ADAM10 is the amyloid precursor protein (APP). While the cleavage of APP by the β - and γ -secretases lead to the amyloidogenic pathways and the formation of the neurotoxic peptide A β , the APP cleavage by α -secretases release the sAPP α peptide, thus inhibiting the A β formation through the non-amyloidogenic pathway (summarised by Müller et al., 2017; Vitória et al., 2022). Furthermore, ADAM10 is involved in Notch processing, and Notch deficient mice showed a very similar phenotype to the ADAM10 deficient mice, assuming a central role of the ADAM10-Notch pathway during development (Hartmann et al., 2002; Vincent, 2016).

Moreover, ADAM10 is also processed by other enzymes, such as ADAM9 and ADAM15, releasing an extracellular fragment (soluble ADAM10), and by γ -secretases releasing an intracellular domain (ICD) (Tousseyn et al., 2009). Interestingly, the ICD of ADAM10 can translocate to the nucleus and it has been found in nuclear speckles, suggesting a role for this fragment in gene regulation (Tousseyn et al., 2009). Finally, ADAMs and α -secretase modulation is now considered as a valuable therapeutic strategy in multiple diseases such as Alzheimer's disease, Parkinson's disease, prion disease or cancers (Wetzel et al., 2017; Smith et al., 2020; Harrison et al., 2021; Vitória et al., 2022; Mohammadi et al., 2023).

Although ADAM10 is shown to be critical for CNS development and function, ADAM10 protein distribution in the brain and its cellular expression has not been fully studied. The protein was first isolated from the myelin membrane of bovine brains (Chantry & Glynn, 1990). *Adam10* mRNA localization was described in the mouse brain during pre- and post-natal development and in adulthood by *in situ* hybridization (ISH), as well as in the human brain by Northern blot (Marcinkiewicz & Seidah, 2000). In the chicken brain, *Adam10* mRNA was found in the developing blood vessels, oligodendrocytes, and subsets of neurons (Lin et al., 2008). In the perinatal mouse cortex, expression of ADAM10 was suggested in neurons and glial cells in the ventricular zone and the cortical plate (Ma et al., 2013). In the adult mouse CNS, *Adam10* mRNA was predominantly described in the neurons of the cerebral cortex, hippocampus, thalamus and cerebellar granular cells (Kärkkäinen et al., 2000). More recently, a widespread distribution of *Adam10* mRNA was reported in the rodent brain, with a particularly high expression in the cerebellum, but also in the cerebral cortex, olfactory bulb, hippocampus and thalamus (Guo et al., 2016). Data mainly indicated that ADAM10 is expressed in neurons and astrocytes. However, some studies have also suggested the expression of ADAM10 in mature and precursors of oligodendrocytes (Lin et al., 2008; Sakry et al., 2014). Thus, detailed information regarding the cellular expression of the enzyme is not complete. Furthermore, most of the previous studies investigated ADAM10 at the mRNA level, and they focused on embryonic or post-natal expression. To the best of our knowledge, no data are available regarding ADAM10 protein expression in the adult CNS.

Thus, the aims of our study were (1) to describe the ADAM10 expression in different regions of the mouse adult brain, the spinal cord and in the cerebellum, and (2) to precisely pinpoint the expression of the protein and its subcellular localization within different cell types such as neurons, astrocytes, oligodendrocytes and microglia, better revealed through *in vitro* studies.

2. Results

2.1. ADAM10 is widely expressed throughout the mouse brain

Immunohistochemistry performed on the adult mouse brain showed that ADAM10 was widely expressed throughout the brain. The distribution of ADAM10 immunoreactivity has been summarised in Table 1. ADAM10 staining was found predominantly in the hippocampus, some olfactory areas and, with a lesser degree of intensity, in thalamic and hypothalamic nuclei. However, we did not observe any staining in areas such as the layer I in the brain cortex, the stratum oriens in the hippocampus, and in fiber tracts such as the stria medullaris.

2.1.1. Cerebral cortex

Different ADAM10 staining intensities were observed depending on the cortical layer (Fig. 1A). Layer I, the outermost layer, showed some scattered small cell bodies that did not seem to express ADAM10. In layer II/III, we observed a large number of small neuronal somas with an

Table 1

Distribution of ADAM10 immunoreactivity in the mouse brain. Intensity of ADAM10 labeling was scored as intense (+++), moderate (++) , weak (+), or background level (–) by comparing with internal standards as follows: Stratum pyramidale of CA3 in the hippocampus (+++), lateral dorsal nucleus of the thalamus (++) , fimbria (+) and the stratum radiatum of CA3 in the (–).

Brain		Hippocampus	
Olfactory areas		CA1	
Piriform cortex		Stratum lacunosum moleculare	+
Molecular layer	–	Stratum radiatum	–
Pyramidal layer	+++	Stratum pyramidale	++
Polymorph layer	–	Stratum oriens	–
Nucleus of the lateral olfactory tract	+++		
Cerebral neocortex		CA2	
<i>In somatosensory cortex</i>		Stratum lacunosum moleculare	+
Layer I	–	Stratum radiatum	–
Layer II/III	++	Stratum pyramidale	+++
Layer IV	+	Stratum oriens	–
Layer V	+++	CA3	
Layer VIa	++	Stratum lacunosum moleculare	–
Layer VIb	+	Stratum radiatum	–
Epithalamus		Stratum lucidum	++
Medial habenula	+	Stratum pyramidale	+++
Lateral habenula	+	Stratum oriens	–
Thalamus		Dentate gyrus	
Lateral dorsal nucleus	++	Polymorph layer	+++
Ventral medial nucleus	+	Granule cell layer	+
Ventral anterior-lateral complex	+	Molecular layer	–
Ventral posteromedial nucleus	+	Fiber tracts	
Ventral posterolateral nucleus	++	Fornix system	
Reticular nucleus	++	Alveus	–
Hypothalamus		Dorsal fornix	–
Ventromedial hypothalamic nucleus	+++	Fimbria	+
Arcuate hypothalamic nucleus	+++	Corpus callosum	+
Globus pallidus	+++	Internal capsule	++
Oculomotor nucleus	++	Stria medullaris	–
Red nucleus	++		
Cortical amygdalar area	++		
Substantia nigra	+++		
Cerebellum		Spinal cord	
Molecular layer	+	Ventral horn	++
Purkinje cell layer	+++	Dorsal horn	++
Granular layer	+++		
White matter	++		

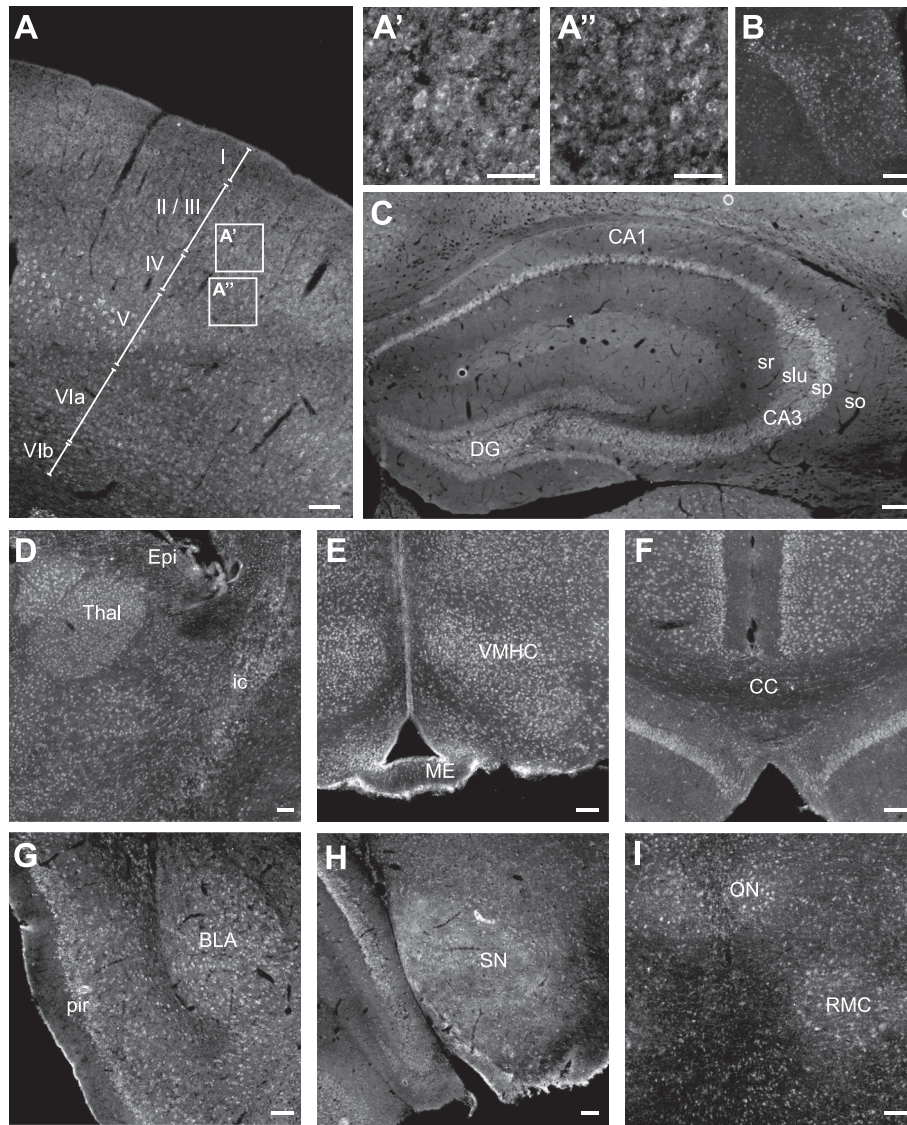


Fig. 1. ADAM10 expression throughout the brain of adult mice. Brain coronal sections were immunostained for ADAM10. Regions shown are (A) cortex, (A') and (A'') higher magnification of cortical layers II/III and IV, respectively, (B) the fimbria, (C) the hippocampus, (D) thalamus, epithalamus and internal capsule, (E) medial eminence and ventromedial hypothalamic nuclei, (F) corpus callosum, (G) piriform cortex and amygdala, (H) substantia nigra and (I) oculomotor and red nuclei. BLA: Basolateral amygdaloid nucleus, CA1/3: Cornu amonis 1/3, CC: Corpus callosum, DG: Dentate gyrus, Epi: Epithalamus, ic: Internal capsule, ME: Medial eminence, ON: Oculomotor nucleus, pir: Piriform cortex, RMC: Red nucleus (magnocellular part), slu: Stratum lucidum, SN: Substantia nigra, so: Stratum oriens, sp: Stratum pyramidale, sr: Stratum radiatum, Thal: Thalamus, VMHC: Ventromedial hypothalamic nucleus. Scale bars: 40 μm in (A') and (A'') and 100 μm in other panels.

intense ADAM10 immunoreactivity (Fig. 1A and 1A'). Layer IV presented fewer small neural somas than layer II/III (Fig. 1A and 1A''). We found large neuronal cell bodies in layer V, which were intensely stained, making this layer the most ADAM10 immunoreactive in the cortex. Layer VIa contained small neurons that were ADAM10 immunoreactive. Finally, layer VIb contained fewer neurons than VIa and they were also less immunoreactive than their counterparts in the VIa layer.

2.1.2. Hippocampus

In the CA1 and CA2 regions of the hippocampus, ADAM10 immunoreactivity was present in the pyramidal and lucidum strata, but absent in oriens and radiatum strata (Fig. 1C). In the CA3, we observed an intense ADAM10 immunostaining in the stratum pyramidale and in the stratum lucidum (Fig. 1C and 2A). In the dentate gyrus, we observed an ADAM10 immunostaining which was intense in the polymorph layer, moderate in the granule cell layer and almost absent in the molecular layer (Fig. 1C and 2B). ADAM10 staining mostly colocalized with NeuN

staining (neuron marker, Fig. 2A, arrowheads) in the hippocampus but not with GFAP staining (astrocyte marker, Fig. 2).

2.1.3. Epithalamus, thalamus and hypothalamus

Weak ADAM10 immunoreactivity was observed in the medial and lateral habenula of the epithalamus (Table 1 and Fig. 1D). In the thalamus, most of the thalamic nuclei showed weak ADAM10 staining. However, ventral thalamic nuclei presented a slightly higher ADAM10 staining (Fig. 1D). The hypothalamic nuclei showed higher immunoreactivity than the thalamic ones with intense ADAM10 immunostaining in the ventromedial nucleus (Table 1 and Fig. 1E).

2.1.4. Fiber tracts

ADAM10 was absent in most of the fiber tracts of the brain as their immunostaining intensity was comparable to background levels (Table 1). In the corpus callosum we could find some ADAM10 positive immunostaining (Fig. 1F). The rest of the fiber tracts did not seem to

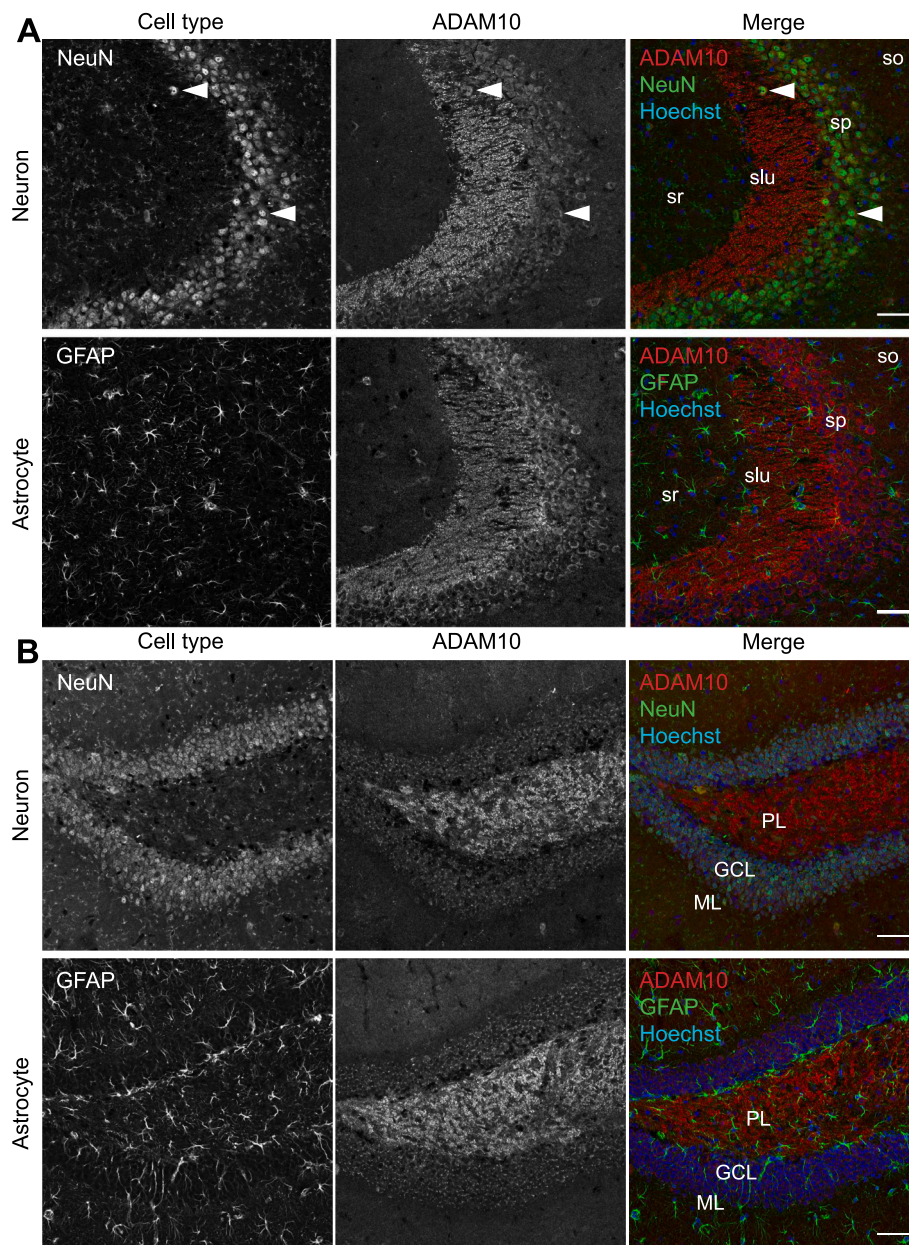


Fig. 2. ADAM10 expression in the hippocampal region of adult mice. Brain coronal sections were immunostained for ADAM10 and specific cell markers were used such as NeuN and GFAP for neurons and astrocytes, respectively. Regions depicted are (A) the CA2-CA3 and (B) the dentate gyrus. GCL: Granule cell layer, GFAP: Glial fibrillary acidic protein, Hoechst: nuclear staining, ML: Molecular layer, NeuN: Neuronal nuclear antigen, PL: Polymorph layer, slu: Stratum lucidum, so: Stratum oriens, sp: Stratum pyramidale, sr: Stratum radiatum. Scale bars: 50 μ m.

present ADAM10 staining except for the fimbria (Fig. 1B) and the internal capsule (Fig. 1D), which presented mild staining.

2.1.5. Other brain regions

Strong ADAM10 immunostaining was detected in the piriform cortex (Fig. 1G), located in the ventrolateral cortical region next to the basolateral amygdala. We also observed intense and moderate ADAM10 staining in the substantia nigra (Fig. 1H) and in the oculomotor and red nucleus (Fig. 1I), respectively.

2.2. ADAM10 is mainly expressed in granular and Purkinje cells in the cerebellum

ADAM10 immunostaining in the cerebellum showed different expression patterns among the cerebellar layers (Fig. 3A, B and C). We

observed weak ADAM10 immunostaining in the white matter region, mostly formed by myelinated axons (Fig. 3D). Conversely, the granular layer presented moderate ADAM10 immunostaining corresponding to the cytoplasm of the granule cells (ADAM10/NeuN co-staining). Interestingly, we found intense ADAM10 immunoreactivity in the Purkinje cell layer corresponding to the cell bodies and in their dendrites present in the molecular layer (ADAM10/CaBP co-staining, Fig. 3D). ADAM10 staining was not colocalized with GFAP staining (Fig. 3D).

2.3. ADAM10 is expressed in the grey matter in the spinal cord

In the spinal cord, we mainly observed ADAM10 immunostaining in the grey matter (Fig. 4A, B and C). Conversely, the white matter did not present ADAM10 staining, as fluorescence levels were similar to the negative immunohistochemistry control conditions. In the grey matter,

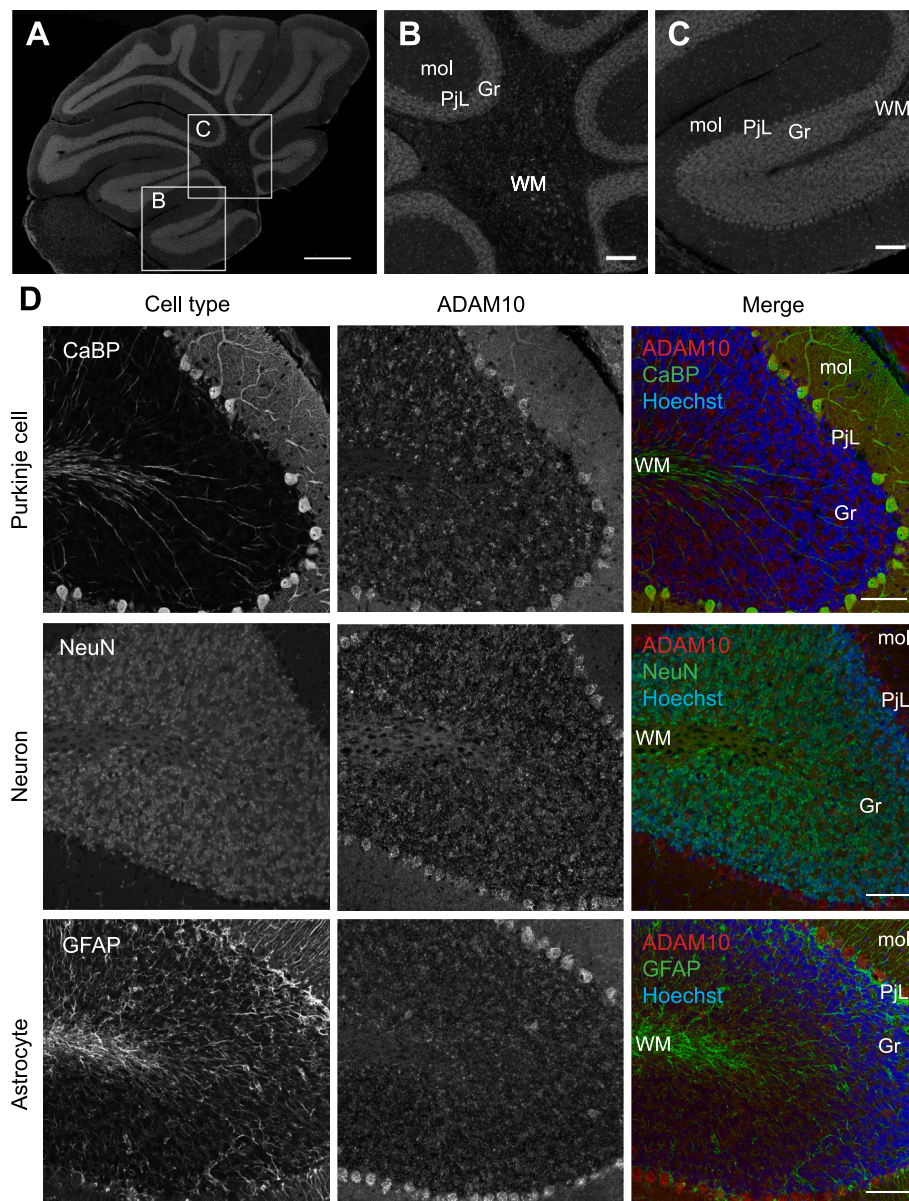


Fig. 3. ADAM10 expression in the cerebellum of adult mice. Sagittal cerebellum sections were immunostained for ADAM10. Regions shown are the whole cerebellum (A) with zoomed-in images of (B) the central part of the cerebellum and (C) the end of one of the lobules. (D) Cerebellum sagittal sections were immunostained for ADAM10 and specific cell markers were used such as NeuN for granular neurons, CaBP for Purkinje neuronal cell bodies and GFAP for astrocytes. CaBP: Calbindin protein, GFAP: Glial fibrillary acidic protein, Gr: Granular layer, Hoechst: nuclear staining, NeuN: Neuronal nuclear antigen, mol: Molecular layer, PjL: Purkinje cell layer, WM: White matter. Scale bars: 500 μm for (A), 100 μm for the zoomed-in panels (B) and (C) and 50 μm for (D).

we found ADAM10 immunopositive cells corresponding to the sensory (Fig. 4D, arrowheads) and motoneuron (Fig. 4E, arrowheads) cell bodies and membranes (ADAM10/NeuN co-staining) present in both ventral and dorsal horns. However, ADAM10 immunostaining did not colocalize with GFAP (Fig. 4D and E).

2.4. ADAM10 expression varies between cell types in the central nervous system

To assess which cell types expressed ADAM10 *in vivo*, we imaged neurons, astrocytes, oligodendrocytes and microglia at higher magnification, and evaluated colocalization between ADAM10 and specific cell markers (NeuN, GFAP, Olig2 or Iba1, Fig. 5). We depicted representative regions where each cell type was clearly expressed. In NeuN⁺ neurons, ADAM10 immunostaining was more intense in the membrane and in the perimembranous area (arrowheads, Fig. 5A). Astrocytes did not show

ADAM10 colocalization with either GFAP (Fig. 5B) or S100 β staining (not shown). Oligodendroglial cells were stained with Olig2 and some populations colocalized with ADAM10 immunostaining (arrowhead, Fig. 5C), where the labelling was cytoplasmic and nuclear. Microglia Iba1⁺ did not present any colocalization with ADAM10 immunostaining (Fig. 5D).

2.5. ADAM10 expression varies between cell types in *in vitro* primary cultures

In hippocampal neurons, ADAM10 expression was mainly localized in the cell body, the nucleus and the perinuclear region (Fig. 6A). ADAM10 staining was also present in the axon and, to a lesser extent, in the dendrites (arrowheads, Fig. 6A). The intracellular localization of ADAM10 was not homogenous but revealed a punctiform staining, an aggregate-like pattern suggesting a vesicular transport.

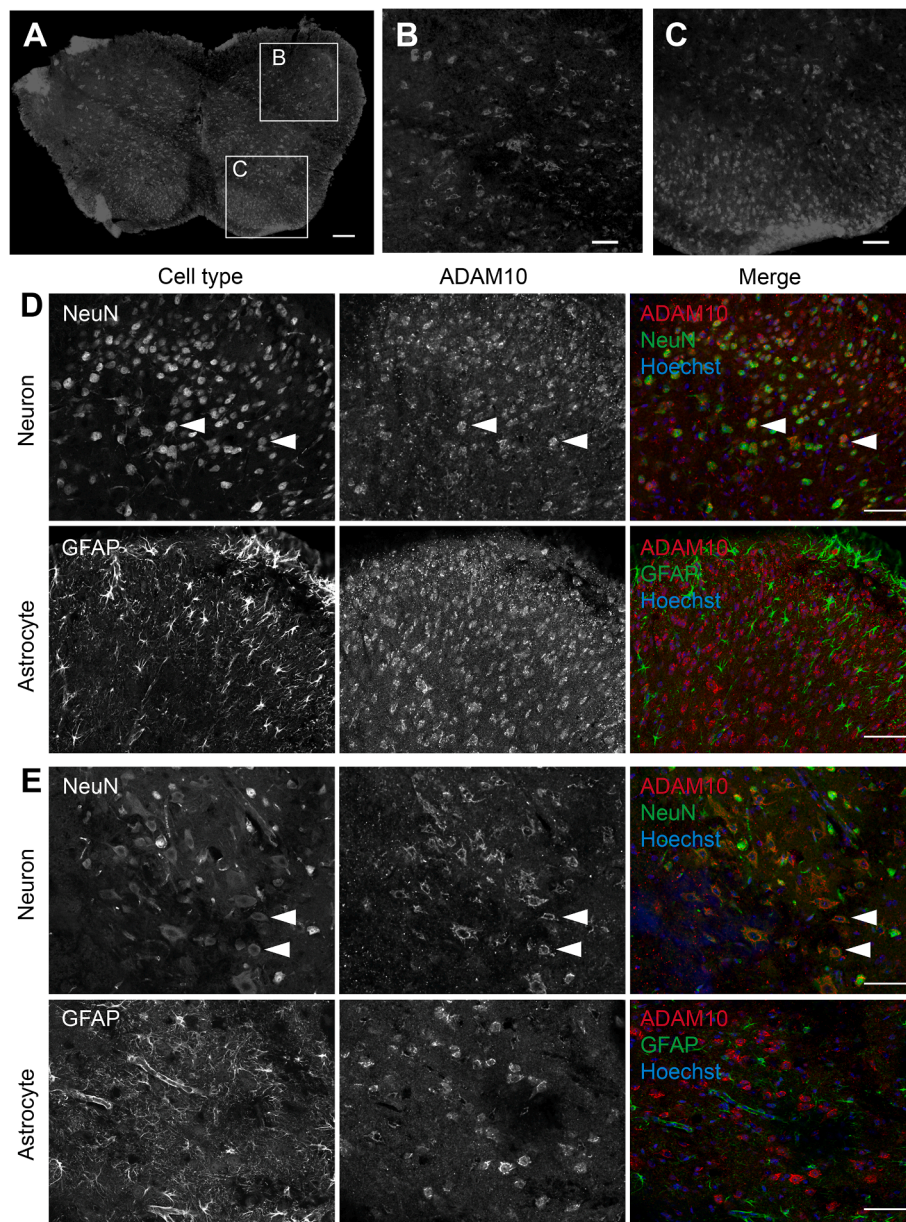


Fig. 4. ADAM10 expression in the spinal cord of adult mice. Transversal spinal cord sections were immunostained for ADAM10. Regions shown are whole spinal cord (A) with zoomed-in images of the dorsal (B) and ventral (C) horns. (D) and (E) spinal cord transversal sections from adult mice were immunostained for ADAM10 and specific cell markers were used such as NeuN and GFAP for neurons and astrocytes, respectively. Regions depicted are (D) the posterior or sensory horn (E) and the anterior or motor horn. Hoechst: nuclear staining, NeuN: Neuronal nuclear antigen, GFAP: Glial fibrillary acidic protein. Scale bars: 200 μm for (A), 100 μm for the zoomed-in panels (B) and (C) and 50 μm for (D) and (E).

Astrocytes cultured *in vitro* presented a less ramified morphology than *in vivo* (Fig. 5B). We could observe a strong ADAM10 immunostaining inside the nucleus, in aggregates in the perinuclear region and throughout the cytoplasm (Fig. 6B).

The ADAM10 immunostaining in mature MAG^+ oligodendrocytes was intense in the cell body and inside the nucleus. In myelin processes, we were able to observe dotted ADAM10 staining in the membrane extensions (arrowheads, Fig. 6C). ADAM10 presented a similar expression pattern in less differentiated oligodendrocytes (less branched cells) although due to their reduced surface, ADAM10 staining was more concentrated in the cellular body (not shown).

Microglia presented a non-branched flattened morphology *in vitro* (Fig. 6D) compared to *in vivo* conditions (Fig. 5D). Results showed that microglia presented predominantly nuclear dotted ADAM10 staining (Fig. 6D), although we could find some ADAM10 labelling in the

cytoplasm (vesicular staining) and at the cell membrane.

3. Discussion

In this study, we described the expression of ADAM10 in different regions of the brain, the cerebellum and in the spinal cord of adult mice, with a predominant neuronal expression. We also described the cellular localization of ADAM10 in neurons, astrocytes, oligodendrocytes and microglia *in vitro*, where ADAM10 staining was observed at the membrane, in the cytoplasm and in the nucleus of neurons and glial cells. To control the specificity of the ADAM10 labelling and to strengthen our observations, we also used a second antibody raised against an intracellular epitope of ADAM10 and a third antibody raised against an extracellular epitope of ADAM10. We obtained the same CNS immunolabeling results as with the first antibody, *in vitro* and *in vivo*, such as

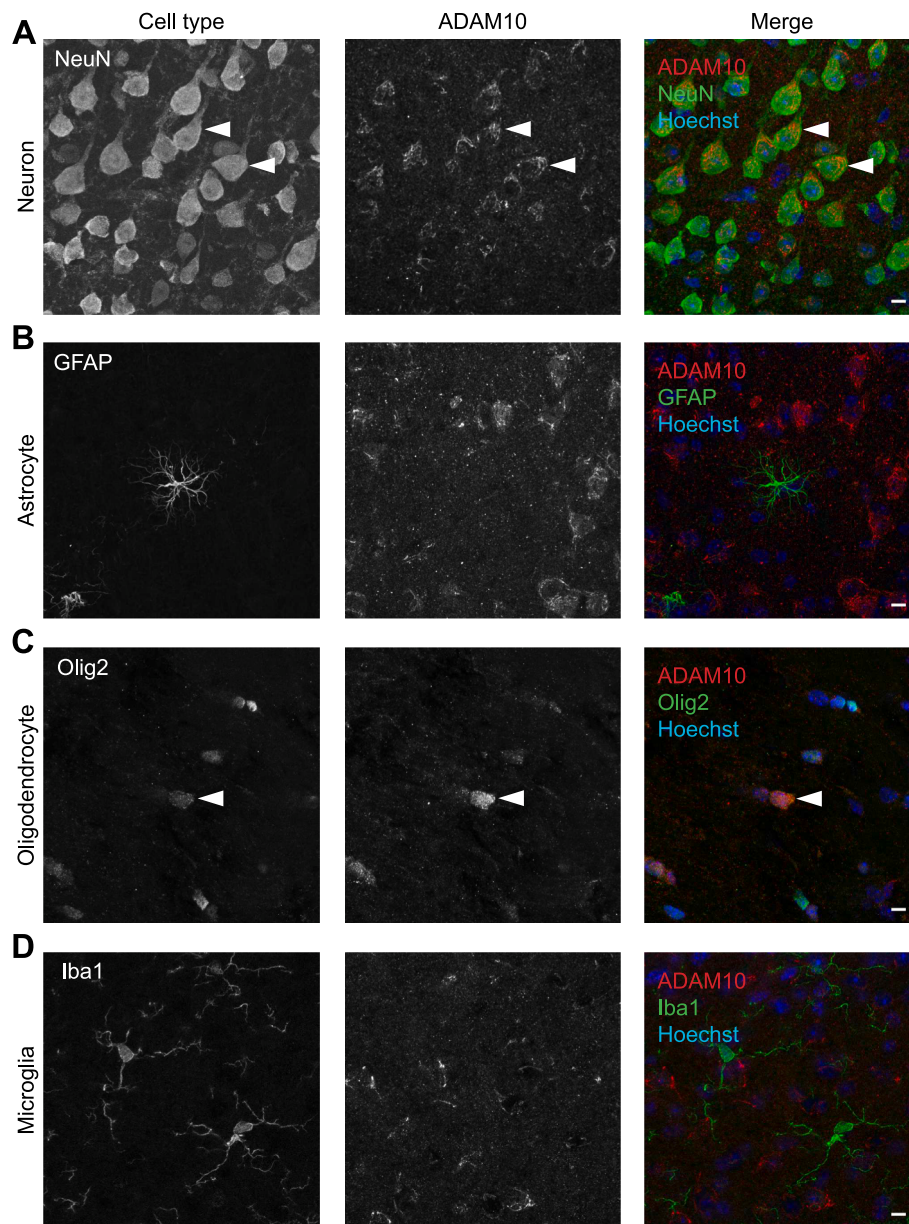


Fig. 5. ADAM10 expression in different cell types in the brain of adult mice. Brain coronal sections were immunostained for ADAM10 and specific cell markers were used such as (A) NeuN for neurons, (B) GFAP for astrocytes, (C) Olig2 for oligodendrocytes and (D) Iba1 for microglia. Confocal images were taken from regions where each cell type was clearly defined: thalamus for neurons, fimbria for oligodendrocytes, hippocampus for astrocytes and cortex for microglia. Hoechst: nuclear staining, NeuN: Neuronal nuclear antigen, GFAP: Glial fibrillary acidic protein. Scale bars: 20 μ m.

shown here for the hippocampus as a representative labelled region (Fig. S1 and S2).

The subcellular localization of ADAM10 showed it at the plasma membrane, since this protein is described as a transmembrane enzyme (Vincent, 2016). In astrocytes, the membrane staining was not obvious compared to other cell types. This could be explained by the shape of astrocytes in culture, as their large membrane surface could make the membrane staining challenging to highlight. We also observed a cytoplasmic punctiform staining in neurons and glial cells, highlighting a vesicular staining and therefore the intracellular trafficking of the enzyme throughout the cell. Indeed, ADAM10 is synthesised and undergoes maturation in the Golgi before being addressed to the plasma membrane (Endres & Deller, 2017). In addition, the nuclear staining could also come from the intracellular processing of ADAM10, as our antibody is able to label the released intracellular fragment, ADAM10 ICD. ADAM10 can be cleaved by other proteases such as ADAM9 or

ADAM15, which liberate a soluble ADAM10 (Hsia et al., 2019). The presenilin/ γ -secretase complex can cleave the remaining ADAM10 to release the ICD fragment, which will be transported to the nucleus (Tousseyn et al., 2009). Hence, the ICD trafficking probably corresponds to the cytoplasmic and nuclear staining we observed in the different cell types. As the ICD cleavage and trafficking might be different within the different cell types, the staining varies. Further investigations are required to understand better the role of ADAM10 ICD signalling in CNS glial cells.

Regarding ADAM10 distribution throughout the CNS, our observations indicate strong ADAM10 expression in the hippocampus, the cerebellum, the piriform cortex and, to a lower extent, in the cerebral cortex. These observations are in line with previous studies investigating *Adam10* mRNA expression by ISH (Ma et al., 2013; Guo et al., 2016; Endres & Deller, 2017), confirming thus our observations at the level of ADAM10 protein distribution.

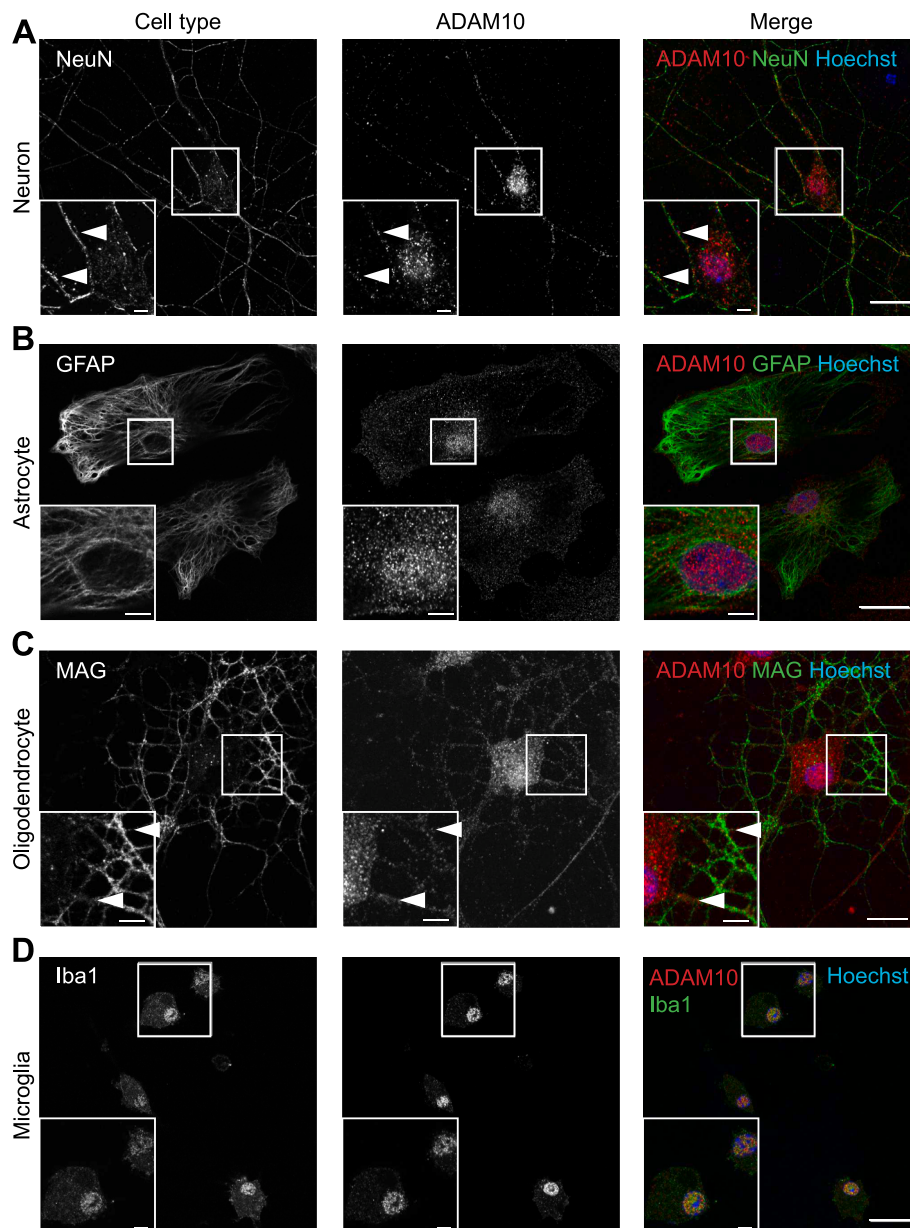


Fig. 6. ADAM10 expression in primary cultures of neurons, astrocytes, oligodendrocytes and microglia. Confocal images of primary cell cultures immunostained for ADAM10 and specific cell markers were used such as (A) NeuN for neurons, (B) GFAP for astrocytes, (C) Olig2 for oligodendrocytes and (D) Iba1 for microglia. Hoechst: nuclear staining. GFAP: Glial fibrillary acidic protein, MAG: Myelin associated glycoprotein, NeuN: Neuronal nuclear antigen. Scale bars: 20 μm for main images and 5 μm for zoomed-in images.

ADAM10 expression in neurons is fully described in the literature, while the expression of the enzyme at the protein level in oligodendrocyte has not been investigated or only suggested so far (Marcinkiewicz & Seidah, 2000; Lin et al., 2008; Guo et al., 2016). In this study, we observed ADAM10 protein expression mainly in neurons and sparsely in few oligodendrocytes *in vivo*. Since heterogeneous oligodendroglial populations have been described, distinct gene expression patterns could be observed (Marques et al., 2016). Marques and colleagues, using single-cell RNA sequencing in mice, found that *Adam10* mRNA is expressed at E13.5 in oligodendrocytes and its level is decreased from P7 to adults (Marques et al., 2018 and <https://ki.se/en/mbb/oligointerno>). The same group has also shown that *Adam10* is present in mature oligodendrocytes (Marques et al., 2016 and <https://linnarssonlab.org/oligodendrocytes/>), in line with our observations.

Although we did observe ADAM10 protein staining in microglia and astrocytes in primary cultures, we were not able to detect ADAM10

protein in these cells of adult mouse CNS *in vivo*. Differences between embryonic stages (primary cells obtained from postnatal mice) and adult life (12-week old mice, brain sections) may explain these discrepancies. Indeed, *Adam10* mRNA expression has been shown to be decreased with age (Endres & Deller, 2017; Marques et al., 2018), and since neurons strongly express ADAM10, it might be difficult to detect glial cells labelling in tissues. Besides, in human CNS tissues, ADAM10 protein is not detected in neurons or glial cells by immunohistochemistry, but found in endothelial cells (Uhlén et al., 2015). However, data were obtained from human tissues and cell type markers to perform co-staining were not used, which could explain the discrepancy with our data. The Human Protein Atlas also reported ADAM10 expression in the U251 human glioblastoma cell line, with an intracellular punctiform staining suggesting a vesicular transport of the protein consistent with our findings. Furthermore, Allen brain cell atlas provides single cell transcriptomics data, but, to the best of our knowledge, no information

regarding ADAM10 expression at the protein levels. However, recent transcriptomic data carried out in mouse and human brain confirms that ADAM10 is expressed in microglia and astrocytes (Pan et al., 2020; Sjöstedt et al., 2020). Thus, the results of our study supplement available data from Atlas or large-scale maps.

While our study showed extensive data, some limitations can be stated. First, longitudinal and developmental study could be carried out on embryonic, perinatal, juvenile and adult mouse CNS tissues to assess the expression of ADAM10 throughout ages. Second, further studies are needed to improve the semi-quantitative scoring, such as absolute quantification of immunofluorescence or western blot analysis. Furthermore, and as mentioned before, non-neuronal staining appears challenging to reveal *in vivo* alongside neurons. While our study was carried out throughout the mouse adult brain on different anteroposterior regions, cerebral blood vessels were not highlighted. Our mapping study was not exhaustive and we cannot exclude that other non-neuronal cells may also express ADAM10, such as endothelial or ependymal cells. Finally, data were obtained from mouse tissues and mapping profile or cellular expression in other species should be carried out to achieve greater accuracy on ADAM10 expression.

In conclusion, this study highlights ADAM10 protein expression in neurons, oligodendrocytes, astrocytes and microglia by immunostaining. Furthermore, the ADAM10 protein distribution within the different regions of the CNS is consistent with previous studies performed on mRNA localisation, with a strong ADAM10 expression in the cerebral cortex, hippocampus, hypothalamus and cerebellum. Considering the implication of ADAM10 in multiple cellular processes and pathological mechanisms, it could be interesting to investigate the modulation of ADAM10 expression during development, and in pathological situations like Alzheimer's disease or demyelinating diseases like multiple sclerosis.

4. Experimental procedures

4.1. Animals

Adult C57BL/6 male mice (12-week old, Janvier, Le Genet St Isle, France) were used for tissue section preparation. For primary cultures, E16 mice were used for neurons, P1 mice for microglia and P4 mice for oligodendroglia and astrocytes. Animals were housed in a controlled temperature environment ($22 \pm 2^\circ\text{C}$), under a 12 h light/dark cycle. Animal care and experiments were approved by Université Paris Cité Animal Ethics Committee, adhering to the French regulations and the European Communities Council Directive on the protection of animals used for scientific purposes.

4.2. Cell culture

Neurons: Neuronal culture were kindly provided by Dr D. Carrel (Synaptic Trafficking group, CNRS UMR 8250) and obtained as previously described with an adapted protocol of rat embryo neuronal culture (Carrel et al., 2011). Briefly, hippocampi of mouse embryos were dissected at E16. After trypsinization, tissue dissociation was achieved with a Pasteur pipette. Cells were plated on poly-D-lysine-coated (P7280, Sigma) coverslips at a density of 60 000–75 000 cells per 15 mm coverslip and cultivated in supplemented Neurobasal medium (21103–049, Life Technologies) that had been conditioned for 24 h on a confluent cortical glia layer (70–80 % confluency). Before incubation, the medium was supplemented with 2 % B27 (17504–044, Life Technologies), 0.5 mM of L-glutamine (Glutamax™, 35050038, Gibco), 10 U/ml of penicillin G, and 10 mg/ml of streptomycin (15070–063, Life Technologies). Neurons were incubated at 37°C in an atmosphere of humidified 5 % CO_2 and were fixed at 14 DIV with paraformaldehyde 4 % (PFA, EM-15710 - Euromedex) to perform immunocytochemistry experiments.

Oligodendrocyte precursor cells: P4 mice were sacrificed by

decapitation and brain and cerebellum were collected in HBSS media (14170–088, Gibco). Hemispheres and cerebellum of each mouse were dissociated in a gentleMACS™ C-tube (130–096–334, Miltenyi Biotech) using the Neural Tissue Dissociation Kit with papain (130–092–628, Miltenyi Biotech) and the pre-recorded '37C_NTDK_1' program of the gentleMACS™ dissociator (Miltenyi Biotech). After dissociation, the cell suspension was filtered using a $70\ \mu\text{m}$ strainer to eliminate dead cells and DNA aggregates and the filtered cells were counted. Cell number was then used to determine the volumes for cell resuspension in PBS-BSA 0.1 % for magnetic labelling according to the manufacturer's instructions using the CD140a/PDGFR α antibody kit (130–101–502, Miltenyi Biotech). Cells were blocked for 10 min at 4°C using the FcR Blocking reagent and then immunolabeled with the CD140/PDGFR α antibody coupled to paramagnetic beads for 15 min at 4°C . After a wash and resuspension in PBS-BSA 0.1 %, cells were applied to a LS magnetic column (130–122–729, Miltenyi Biotech), placed in the MultiMACS™ Cell24 Separator and the POSSEL-SCA program was followed. Magnetic labelled cells (PDGFR α^+ cells, OPCs) were retained by the column and flow-through was discarded. After three PBS-BSA 0.1 % washes, the column was placed in the elution chamber of the MultiMACS™ Cell24 Separator where cells were recovered. Labelled cells (OPCs) were seeded in 24-well plates containing one glass coverslip per well (ϕ 14 mm, Knittel glass) previously coated with $20\ \mu\text{g}/\text{mL}$ of poly-L-lysine (P5899, Sigma). Cells were cultured with a medium containing 10 ng/mL of human PDGF-AA (315–17, Peprotech), 10 ng/mL of human FGF (F0291, Sigma), 2 mM of L-glutamine, 0.1 U/mL of penicillin, 0.1 $\mu\text{g}/\text{mL}$ of streptomycin and 2 % MACS® NeuroBrew® supplement (130–093–566, Miltenyi Biotech) diluted in MACS® NeuroMedium (130–093–570, Miltenyi Biotech). Cells were incubated at 37°C in an atmosphere of humidified 5 % CO_2 and were fixed at 8 DIV with PFA 4 % to perform immunocytochemistry experiments.

Astrocytes: Non-labeled cells (flow-through) collected from the oligodendrocyte MACS® cultures were cultured in 24-well plates containing one glass slide per well (ϕ 14 mm, Knittel glass), previously coated with $20\ \mu\text{g}/\text{mL}$ of poly-L-lysine (P5899, Sigma) in medium containing 10 % Fetal Bovine Serum (FBS, Invitrogen), 2 mM of L-glutamine, 1 mM of sodium pyruvate (11360070, Life Technologies), 0.5 $\mu\text{g}/\text{mL}$ of fungizone (15290026, Life Technologies), 0.1 U/mL of penicillin and 0.1 $\mu\text{g}/\text{mL}$ of streptomycin diluted in DMEM (11510–416, Life Technologies). In this medium, astrocytes are able to grow faster than other cell populations that the flow-through might contain such as microglia. The medium was changed after 5 DIV and then after 2 days. Cells were incubated at 37°C in an atmosphere of humidified 5 % CO_2 and were fixed at 7 DIV with 4 % PFA to perform immunocytochemistry staining.

Microglia: Microglial cultures obtained from mouse P1 pups were kindly provided by Dr A. Roumier (INSERM UMR-S 839) that were prepared as previously described (Kolodziejczak et al., 2015). Briefly, mice cortices were dissected and dissociated by mechanical dissociation after incubation with trypsin 0.25 %. Cells were then plated into Petri dishes previously coated with poly-ornithine (15 $\mu\text{g}/\text{mL}$) and grown in DMEM (Invitrogen) supplemented with 10 % fetal bovine serum (Bio-West). Microglia were detached from the astrocytic layer between 12 and 14 DIV by gentle shaking and settled on 12-well plates. Microglial cells were then fixed the day after with 4 % PFA for further immunostaining.

4.3. Histological preparation for immunohistochemistry

Mice were deeply anesthetized with pentobarbital (60 mg/kg, i.p.) and perfused through the heart (2 ml/min) with PBS 0.1 M (pH 7.4), and then 4 % PFA solution before tissue removal. Brain, spinal cord and cerebellum were extracted and stored into a 30 % sucrose solution for 2–3 days at 4°C . Samples were then cut at $20\ \mu\text{m}$ with a frozen microtome in order to obtain brain coronal sections (from bregma 2.10 mm to -3.80 mm), transversal sections of the lumbar spinal cord and cerebellar

sagittal sections (vermis, from lateral 0.36 mm to 0.48 mm) (Franklin & Paxinos, 2008). Sections were stored at 4 °C in a PBS 0.1 M solution with 0.01 % azide to prevent contamination.

4.4. Immunohistochemistry

Brain, cerebellar and spinal cord sections were washed with PBS 0.1 M, incubated 1 h with L-lysine (0.1 M, L5626, Sigma) in a PBS solution containing 0.25 % Triton-X100, 0.2 % gelatine, and 0.1 % sodium azide (PBS-GTA). Then, the sections were incubated overnight at 4 °C with the following primary antibodies diluted in PBS-GTA: ADAM10 (1:500, ab39177, Abcam), Olig2 (1:1 000, AF2418, R&D), NeuN (1:1 000, MAB377, Chemicon), GFAP (1:500, ab4674, Abcam) and Iba1 (1:500, ab178846, Abcam), MAG (1:1 000 MAB1567, Millipore), CaBP (1:1 000, CB300, Swant), CNPase (1:200, ab6319, Abcam). To control the specificity of the ADAM10 labelling, we also used a second antibody raised against a different intracellular epitope of ADAM10 (1:500, ab1997, Abcam) and a third antibody raised against an extracellular epitope of ADAM10 (1:200, sc25578, Santa Cruz). Sections were then washed for 30 min and incubated with a PBS-GTA containing the appropriate secondary antibodies 2 h at room temperature (RT): donkey anti-rabbit Alexa Fluor™ 555 (1:1 000, Invitrogen, A31572), donkey anti-chicken Alexa Fluor™ 488 (1:500, 703–545-155, Jackson ImmunoResearch), donkey anti-goat Alexa Fluor™ 488 (1:500, Jackson ImmunoResearch, 705–545-147) and goat anti-mouse Alexa Fluor™ 488 (1:2 000, A21121, Invitrogen). After 3 washes, slices were incubated for 5 min with 1 % Hoechst 33258-PBS (1:30 000, 14530, Sigma). Finally, sections were washed and mounted with Permafluor (TA-030-FM, ThermoScientific).

4.5. Immunocytochemistry

Fixed cells were washed with PBS 0.1 M for 30 min and then incubated with a blocking solution containing 0.1 M of L-lysine (L5626, Sigma) in PBS-GTA or 1 h at RT. Afterwards, coverslips were incubated (overnight, 4 °C) with a PBS-GTA solution containing the same primary antibodies used for immunohistochemistry. Coverslips were then washed with PBS and incubated with PBS-GTA containing secondary antibodies for 30 min at RT. After washes, coverslips were incubated with a 1 % Hoechst 33258-PBS (1:30 000, 14530, Sigma) solution for 5 min, washed with PBS and mounted with Permafluor.

4.6. Imaging: immunocyto/histochemistry analysis

Images were acquired with a confocal microscope Zeiss LSM 710 with a 20x objective for the brain, cerebellar and spinal cord slices and a Plan-Apochromat 63x/1.4 oil objective for the primary cells. Confocal configurations were adapted for 488 and 555 nm excitations corresponding to Alexa Fluor™ 488 and Alexa Fluor™ 555 fluorochromes, respectively. Images were then processed with ZEN software (version 2.3). Four different coverslips were imaged for each cell type. For the brain, we imaged at least 8 bregma regions ranging from 2.80 mm to –3.80 mm of 4 different adult mice to perform the mapping of ADAM10. For the spinal cord and cerebellum, at least 8 slices from each animal (2 for each staining) were imaged. Distribution of ADAM10 labelling was scored as followed: Intense (+++), moderate (++) , weak (+), or background level (–) by comparing with internal standards as follows: Stratum pyramidale of CA3 in the hippocampus (+++), lateral dorsal nucleus of the thalamus (++) , fimbria (+) and the stratum radiatum of CA3 in the (–). Background level (–) was determined in immunohistochemistry control conditions performed without primary antibodies.

CRedit authorship contribution statement

Aida Padilla-Ferrer: Writing – original draft, Methodology, Investigation. **Alex Carrete:** Methodology, Investigation. **Anne Simon:**

Methodology, Investigation, Writing – review & editing. **Delphine Meffre:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Mehrnaz Jafarian-Tehrani:** Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.brainres.2024.148888>.

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