Parvalbumin interneuron-derived tissue-type plasminogen activator shapes perineuronal net structure

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Abstract

Background: Perineuronal nets (PNNs) are specialized extracellular matrix structures mainly found around fast-spiking parvalbumin (FS-PV) interneurons. In the adult, their degradation alters FS-PV-driven functions, such as brain plasticity and memory, and altered PNN structures have been found in neurodevelopmental and central nervous system disorders such as Alzheimer’s disease, leading to interest in identifying targets able to modify or participate in PNN metabolism. The serine protease tissue-type plasminogen activator (tPA) plays multifaceted roles in brain pathophysiology. However, its cellular expression profile in the brain remains unclear and a possible role in matrix plasticity through PNN remodeling has never been investigated.

Result: By combining a GFP reporter approach, immunohistology, electrophysiology, and single-cell RT-PCR, we discovered that cortical FS-PV interneurons are a source of tPA in vivo. We found that mice specifically lacking tPA in FS-PV interneurons display denser PNNs in the somatosensory cortex, suggesting a role for tPA from FS-PV interneurons in PNN remodeling. In vitro analyses in primary cultures of mouse interneurons also showed that tPA converts plasminogen into active plasmin, which in turn, directly degrades aggrecan, a major structural chondroitin sulfate proteoglycan (CSPG) in PNNs.

Conclusions: We demonstrate that tPA released from FS-PV interneurons in the central nervous system reduces PNN density through CSPG degradation. The discovery of this tPA-dependent PNN remodeling opens interesting insights into the control of brain plasticity.

Keywords: Tissue-type plasminogen activator, Plasminogen, Parvalbumin interneurons, Perineuronal nets, Aggrecan
chondroitinase ABC (ChABC) strongly affects neuronal excitability and influences brain plasticity [2, 11, 30, 42, 62, 70, 76]. In the brain, endogenous proteases such as A Disintegrin And Metallloproteinase with Thrombospondin Motifs (ADAMTS) or matrix metalloproteinases (MMPs) may regulate PNN functions through the cleavage of their CSPGs, including aggrecan, neurocan, versican, and brevican [34, 86]. Paradigms of sensory deprivation have demonstrated the critical implication of PNN remodeling during periods of heightened plasticity, as well as the involvement of endogenous MMP9 in PNN plasticity [37, 59, 60, 66]. Interestingly, it has been shown that the expression and activity of the serine protease, tissue-type plasminogen activator (tPA) increase after sensory deprivation. Furthermore, tPA is thought to participate in plasticity-related mechanisms [16, 52, 53, 58]. However, a direct link between tPA-driven plasticity and PNNs remains unestablished.

In the (neuro-)vascular clinical community, tPA is well known as being the major enzymatic activator of plasminogen, thus promoting vascular fibrinolysis. Besides, tPA also has prominent effects in the central nervous system (CNS). As reviewed recently [87], many actions of tPA in the CNS rely on its proteolytic activity. Some functions occur independently of plasmin(ogen), via different receptors and binding partners which include laminin, low-density lipoprotein receptor-related proteins (LRPs), N-methyl-D-aspartate receptor (NMDAR), or some growth factor precursors. Via these multiple targets, tPA is a key player in CNS physiological and pathological processes (for recent reviews see [87, 98]). For instance, during development, tPA controls neuronal migration [64] and promotes axonal growth/synaptic plasticity [4, 67, 68, 82]. Later in life, tPA modulates learning and memory processes [5, 32, 48] and anxiety-related behaviors [54, 65]. Under pathological conditions, tPA controls neuronal death [44, 45, 61, 94], neuro-inflammation, and blood–brain barrier permeability [47, 55, 77, 81].

Consensual descriptions of the cellular distribution of tPA in the CNS are lacking. So far, most literature agrees that tPA can be released in the extracellular space by endothelial cells, oligodendrocytes, and hippocampal neurons [43, 46, 80]. Microglia and astrocytes are uncertain sources of tPA under physiological conditions but might be under pathological conditions [1, 91]. In the CNS, tPA is easily detected (mRNA and protein) in hippocampal mossy fibers [46, 74, 85], but its immunohistological detection in cortical neurons is only available through its somatic accumulation after the blockade of axo-dendritic transport with colchicine. Interestingly, immunohistological (after colchicine injection), electrophysiological, and transcriptional analyses revealed the expression of tPA in a subset of excitatory neurons [46, 84]. tPA expression has also been suggested in some GABAergic interneurons in different brain areas [17, 28, 80].

In the present study, using a viral reporter construct, electrophysiology, and single-cell RT-PCR in mice, we found that cortical GABAergic neurons, mainly FS-PV interneurons, express tPA. Using conditional mice presenting a depletion of tPA restricted to PV cells, as well as primary culture of interneurons, we show that tPA originating from PV cells controls PNN turnover through plasmin. This regulation occurs through the cleavage of a component crucial for PNN structural integrity, the CSPG aggrecan.

**Results**

tPA is expressed by cortical fast-spiking parvalbumin interneurons in vivo

We sought to identify the cell subtypes expressing tPA in the somatosensory cortex of adult mice. We used a viral reporter construct encoding for green fluorescent protein (GFP) under the control of the human tPA (Plat) promoter (AAV-Plat-GFP, Fig. 1A). This construct was first validated in the dentate gyrus (DG; Additional file 1: Fig. S1A-B), a brain structure in which tPA expression is well described [46, 80]. After injection of the AAV-Plat-GFP in the somatosensory cortex, immunohistological analyses revealed that the GFP reporter was only detected in NeuN-positive neuronal cells but neither in GFAP-positive astrocytes nor in Iba-1-positive microglia (Fig. 1A; these three cell types are potential targets of AAV9-driven infection [50, 56]). Even under inflammatory conditions (lipopolysaccharide treatment), neither reactive astrocytes nor reactive microglia expressed GFP, excluding the expression of tPA in these cell types (Additional file 1: Fig. S2).
Fig. 1 (See legend on previous page.)
Confirming our previous findings [46], a subset of tPA-GFP+ neurons were excitatory glutamatergic neurons, as they expressed Tbr1 (Additional file 1: Fig. S3). In addition, we found that 36.9 % of Plat-GFP+ neurons were positive for GABA (Fig. 1B,C). Among these Plat-GFP GABAergic interneurons, the majority (72.7 %) were parvalbumin+ (Fig. 1B,C) and 76.1 % of these parvalbumin+ neurons were enwrapped with PNNs (stained with WFA; Fig. 1D,E). In situ hybridization confirmed the presence of tPA mRNA in PV interneurons, and more precisely those enwrapped by PNNs (Additional file 1: Fig. S4).

We also performed electrophysiological and molecular characterizations of 28 neurons from deeper layers (IV-V) of the mouse primary visual cortex by combining patch-clamp recordings and single-cell RT-PCR [13]. The PCR protocol was designed to probe the expression of different cell type-specific markers (VGLUT1, GAD65 and GAD67, PV, S100β, Sst, APC, and Akr1c18), plasminogen activators (Plat: tPA gene and Plau: urokinase), and extracellular matrix-related genes (Ncan, Ptprz1, Ptprr, Sema3a, Sdc4, and Mybpc1; Additional file 1: Table S1). Pyramidal cells (n=14) were identified by the triangular shape of their soma and their regular spiking firing pattern characterized by broad spikes and marked frequency adaption. FS-PV interneurons of G42 mice (PV-expressing reporter mouse) [15] were characterized by low input resistance and high rheobase currents [36]. FS-PV interneurons fired short-duration action potentials with sharp post-hyperpolarizing potentials and were able to sustain a high firing rate with little or no spike frequency adaptation (Fig. 2A).

The molecular analysis of the FS-PV interneuron shown in Fig. 2B confirmed the expression of PV and GAD65/67, as well as SST, APC, Akr1c18, Plat, Ncan, Ptprr, and Sema3a.

Analyzed neurons were grouped together according to their overall molecular similarity using Ward’s clustering [14], yielding two main groups (Fig. 2C). All neurons in the left cluster were pyramidal cells expressing VGLUT1 but none in either cluster expressed GADs or PV. By contrast, all neurons in the right cluster expressed PV and were identified as FS-PV interneurons. A sub-cluster of two FS-PV interneurons (dotted box, Fig. 2C) displayed a similar molecular profile with expression of Sst, Plat, Ncan, Plat, and Ptprr. Plat mRNA was detected in 14.3 % of FS-PV cells (n=2 of 14 cells, Fig. 2D), a proportion similar to that observed in pyramidal cells (21.4 %, n=3 of 14 cells, p=0.663) and confirming our previous observations [46].

tPA produced by PV interneurons degrades surrounding PNNs in a plasmin-dependent manner (in vivo and in vitro investigations)

To assess the effect of tPA expressed by PV cells on the remodeling of PNNs, we generated a conditional knock-out mouse strain lacking tPA in PV cells by crossing tPAFlox+/+ mice with PV-Cre+/− mice (tPAFlox−/−; PV-Cre+/− mice were used as controls, Additional file 1: Fig. S5). In both genotypes, the total number of PV interneurons, as well as the number of PV interneurons enwrapped with PNNs, was similar (Fig. 3A–C). However, the overall intensity of WFA was higher in cKO mice than in WT mice (+14.4 %, Fig. 3D). A semi-quantitative analysis based on a qualitative grading PNN staining intensity around PV soma, from very low to very high WFA staining (Fig. 3E), revealed a slightly higher percentage of neurons enwrapped with the highest degree of PNN coverage in cKO mice (Fig. 3F). Overall, these observations support the idea that tPA released from PV interneurons can contribute to PNN turnover in vivo.

To dissect the potential underlying mechanism of this effect, we used a model of primary murine interneuron cultures [27; Fig. 4]. Immunocytochemistry confirmed the presence of PNN-like structures (displaying WFA labelling and expressing aggrekan and hyaluronic acid, two major components of PNN; Additional file 1: Fig. S6A) that were sensitive to ChABC (Additional file 1: Fig. S6B). We assessed PNN integrity in response to treatment with tPA alone or in combination with its conventional substrate plasminogen, plasminogen alone, or active plasmin (Fig. 4A,B). After 24h exposure, we observed a significant PNN degradation with plasmin treatment (~87.3%) or with the combination of plasminogen+tPA (~87.6%), while tPA alone did not alter PNN turnover (Fig. 4A,B). This suggests that tPA might not directly promote PNN degradation. Accordingly, aprotinin, a blocker of plasmin proteolytic activity, prevented plasminogen + tPA-induced PNN degradation (Additional file 1: Fig. S7A-B) demonstrating that tPA promotes PNN degradation via a plasmin-dependent mechanism. We found that plasminogen alone could also induce PNN degradation (~88.4 %, Fig. 4A,B), suggesting that endogenous tPA released by interneurons converts extracellular plasminogen into plasmin. To confirm this, a fluorescent assay of plasmin activity revealed the actual conversion of plasminogen in active plasmin in the absence of exogenous tPA treatment (Additional file 1: Fig. S7C-D). We also exposed cultured interneurons from tPA Null mice to plasminogen, plasmin, or plasminogen + tPA during 24 h (Fig. 4C,D). Although plasmin and plasminogen + tPA significantly reduced WFA staining (~88.2 % for plasmin; ~91.5 % for plasminogen + tPA), plasminogen alone did not (Fig. 4C,D), confirming that PNN degradation depends on endogenous tPA produced by interneurons. We also found that plasmin-dependent degradation of PNNs in vitro was not sensitive to TIMP3 [90], suggesting that MMPs or ADAMTS are not downstream effectors in this pathway (Additional file 1: Fig. S8). To understand how plasmin promotes PNN
Fig. 2  Electrophysiological and molecular characterization of tPA expressing FS-PV interneurons. A, B Characterization of Plat-expressing FS-PV cells. A Current-clamp recordings of a Plat-expressing FS-PV interneuron. Upper traces show voltage responses to current steps (bottom traces). This neuron typically displayed a low input resistance, a high rheobasic current (120 pA, black trace first depolarizing current step) and fired short-duration action potentials with fast and large after hyperpolarizing potentials. A strong depolarizing current (380 pA) evoked a high and sustained firing rate with little or no spike frequency adaptation (shaded trace). B Agarose gel analysis of the RT-PCR products of the same FS-PV neuron showing expression of VGlut1, GAD65, GAD67, PV, Akr1c18, Sst, APC, Ncan, Plat, Ptprr, and Sema3a. C Ward’s clustering based on the expression of 16 genes performed on 28 cortical neurons (upper panel). The x-axis represents individual cells and the y-axis the average Euclidian within-cluster linkage distance. Pyramidal (grey) and FS-PV (magenta) cells were segregated into two first-order clusters as suggested by Thorndike procedure (dotted line). Gene expression profile of genes expression across the different cell clusters (bottom panel). For each cell, colored and white squares indicate presence and absence of genes, respectively. Note the presence of VGlut1 in pyramidal cells and of PV in FS-PV cells. Plat-expressing FS-PV neurons (dotted box) displayed a similar gene expression profile and were segregated together. D Histograms depicting a similar occurrence of Plat in pyramidal and FS-PV cells.

Fig. 3  Specific deletion of tPA in PV interneurons affects PNNs morphology. A Representative images of PV cells (magenta) and PNNs (yellow) in the somatosensory cortex of adult tPAFlx+/−; PV-Cre+/− mice (cKO) and their control littermates (Ctrl), tPAFlx−/−; PV-Cre+/− mice (Scale bar: 100 μm). Quantitative analysis of the density of PV cells (B) and PNN-containing PV cells (C) in both genotypes. D Average WFA intensity was quantified in both groups. E PNNs were assigned to different categories based on the intensity of WFA staining (Scale bar: 10 μm). The percentage of each PNN subtype (F) and the graph show mean ± sem (N=5 animals; n=4 coronal sections per animal). Mann–Whitney test; *: p<0.05
Fig. 3 (See legend on previous page.)
degradation, we incubated a fragment of the aggrecan core protein (corresponding to G1-IGD-G2) with plasminogen, plasmin, tPA alone or in combination, and with or without aprotinin (Fig. 4E–G). ADAMTS-4 was used as a positive proteolytic control. Aggrecan core protein was efficiently cleaved by plasmin (51% loss in the signal of the core protein) and plasminogen + tPA (28.7 % loss in the signal of the core protein), leading to two fragments (55 and 65 kDa) which were similarly obtained with the control treatment with ADAMTS4. (−66%). Plasmin treatment also leads to two products of 90 and 30 kDa due to a second cleavage site. Moreover, as observed with WFA staining, a significant decrease of aggrecan staining around PV cells in vitro revealed that tPA-dependent activation of plasminogen degrades this perineuronal CSPG in situ (Fig. 4H,I).

**Discussion**

The present study provides the first demonstration that tPA expressed by FS-PV interneurons regulates PNN remodeling, through the conversion of plasminogen into plasmin. This remodeling results from the cleavage of a major component of PNNs, the CSPG aggrecan.

tPA expression was previously reported in excitatory pyramidal neurons using in situ hybridization techniques, immunohistochemistry, and single-cell PCR coupled with patch-clamp recordings [46, 75]. Sparse and inconsistent studies have also reported tPA expression in some subtypes of GABAergic interneurons. Immunohistochemical studies [28] have reported tPA expression in VIP-positive, but not in SST-positive perivascular interneurons. Using a reporter mouse line, Stevenson and Lawrence discovered the specific expression of tPA in SST-positive oriens-lacunosum moleculare interneurons, a subpopulation of hippocampal interneurons responsible for synaptic plasticity in Shaffer collateral synapses [80]. Furthermore, the presence of tPA in PV-positive neurons but not in SST-positive neurons has been proposed in the somatosensory cortex, although the specificity of the staining is not entirely clear [17]. One of our main findings is the demonstration by molecular, electrophysiological, and single-cell RT-PCR approaches that tPA is expressed by GABAergic interneurons, including FS-PV interneurons. These FS-PV interneurons are fundamental for several behavioral responses and their dysfunction participates in several diseases, including schizophrenia and autism [35, 73]. Interestingly, their activity is at least in part under the control of a specialized ECM that surrounds them, the PNNs. PNNs are dynamic structures, not only during experience-driven plastic developmental periods [51, 83, 96], but also in adults. For instance, modification of PNN density with the diurnal-nocturnal cycle, or their enzymatic degradation may have a potential impact on memory processing [30, 63, 78]. In line with this, memory-related diseases (Alzheimer’s disease, drug addiction), but also many other pathological conditions (schizophrenia, epilepsy, depression, multiple sclerosis, stroke, Huntington’s disease) may to some extent involve or impact PNN integrity and function [12, 23, 86, 93].

CSPGs, the major components of PNNs, are sensitive to endogenous processing by proteases of the MMP and ADAMTS families [34, 86]. FS-PV interneurons and pyramidal cells express an array of proteases that could thus process PNNs from both sides [21, 71]. Previous studies have shown that the tPA/plasmin system can degrade several CSPGs found in PNNs, such as neurocan, versican, and phosphacan [38, 95] and can activate pro-MMPs and pro-ADAMTS4 into their mature form [19, 41]. Interestingly, the tPA/plasmin system can also increase ChABC-mediated axonal regrowth after spinal cord injury through CSPG degradation [9]. However, the direct evidence of PNN degradation by the tPA/plasmin system has never been provided. Here, we show that the conditional deletion of tPA in FS-PV interneurons in vivo leads to a moderate increase in PNN density in the
Fig. 4 (See legend on previous page.)
somatosensory cortex, which is indicative of a cell-au-
tonomous regulation of PNN plasticity under physiological
conditions. Thus, the histological modifications observed
in vivo under basal conditions could reflect modifications
of the dynamic balance between CSPG synthesis and their
degradation. However, using in vitro experiments, by combining cell imaging and pharmacology, we demonstrate that tPA indeed promotes PNN degradation
around PV interneurons through plasminogen conver-
sion into plasmin. The latter can be blocked by aprotinin
and is independent of MMP or ADAMTS activation,
since a broad-range inhibitor (TIMP3) did not reverse
the effects of plasmin. Overall, our study shows that tPA/
plasmin can exert a cell-autonomous effect on PNN de-
gradation when expressed by PV interneurons. Moreover,
since tPA was described to be induced as an immediate
gene early following seizure or long-term potentiation
[68], we can hypothesize that its expression may be also
triggered in PV cells under certain conditions to promote
PNN remodeling. In addition, considering that tPA is
also expressed in the brain by various cell types includ-
ing pyramidal cells [46] or oligodendrocytes [43], it could
also probably drive PNN degradation through a non-cell
autonomous manner when released in the extracellular
space.

The consequence of PNN degradation on FS-PV cell
function has been the subject of several studies, some-
times with contradictory results [2, 22, 24, 25, 31]. For
instance, PNN digestion induced by chondroitinase ABC
was shown to reduce [2] or increase the excitability of
interneurons [22]. However, these discrepancies could be
explained by the experimental approaches used. Indeed,
genetic removal of specific PNN components might affect
the development of neuronal networks, which is not the
case when PNN are acutely digested in the adult brain.
Additionally, in vitro models cannot reflect the comp lex-
ity of neuronal networks. It would be relevant to perform
fine electrophysiological studies to determine the impact
of tPA-driven degradation of PNN on PV cell functions.
Furthermore, it has been described that the activity of PV
cells and the density of PNNs influence fear memory and
social behaviors [3, 6, 8, 18, 69, 79]. Therefore, it would
also be interesting to investigate the consequence of PNN
degradation by the tPA/plasmin, using PV-Cre x tPA flox
mice, on these behavioral responses.

Finally, we also demonstrate that tPA-mediated PNN
degradation can occur through the cleavage of the core
protein of aggrecan. However, we cannot exclude that the
plasminogen activator system may also promote PNN
degradation through the cumulative cleavage of differ-
ent CSPG as previously mentioned [38, 95]. Neverthe-
less, given that aggrecan is one of the main component
of PNNs, we can hypothesize that plasmin is more likely
to influence PNN remodeling by acting on this particu-
lar CSPG. Indeed, aggrecan was shown to play a crucial
role in PNN structure since its conditional knockout in
the brain results in complete PNN loss in the cortex and
the reactivation of juvenile plasticity [72]. Interestingly,
both tPA and plasmin are also involved in plasticity-
related mechanisms such as ocular dominance shift and
dendritic spine remodeling during the critical period [52,
53]. In view of these results, we hypothesize that PNN
degradation by the tPA/plasmin system could promote
the restoration of juvenile plasticity under pathological
conditions.

Conclusions
In summary, our findings show that in the presence of
plasminogen, tPA released from FS-PV cells reduces
PNN density through CSPG degradation. This could
affect several PNN properties, including balancing
GABAergic and glutamatergic neurotransmissions [2,
22, 42, 70, 76, 78], being a physical protective barrier
[10, 29, 57], a contributor to pathogenic pathways
[40, 49, 62, 97], and a driver of synaptic plasticity and behavioral out-
come [11, 29, 70, 76]. Overall, we provide unanticipated
mechanistic insights in the regulation of PNNs with rel-
evance to neuronal function, which could translate into
new targets to promote plasticity/recovery under pathological
conditions.

Methods
Animals
All experiments were conducted in accordance with
the French ethical law (Decree 2013-118) and the Euro-
pean Communities Council guidelines (2010/63/EU).
Protocols were approved by our local ethics commit-
tee dependent on the French Ministry of Research and
Higher Education (agreement numbers Cenomexa
#25267 and Ce5/2012/062). All applicable international,
national, and/or institutional guidelines for the care
and use of animals were followed. Electrophysiological
experiments were performed on G42 transgenic mice (Jack-
son laboratories #007677, GAD67-GFP, 50). Histological
analyses were performed on 8-week-old male Swiss mice,
tPAFlox<sup>+/−</sup> mice, tPAFlox<sup>++</sup> mice; PV-Cre<sup>−/−</sup> and their
control (tPAFlox<sup>−/−</sup>; PV-Cre<sup>++/−</sup>) littermates (20-25g).
Pregnant tPA Null mice and their WT littermates at gesta-
tional day 14 were used for in vitro neuronal cultures.
Animals were housed with a 12-h light/12-h dark cycle
with free access to water and food.

tPA Null mice were generated by the Mouse Clini-
cal Institute (ICS, Illkirch, France). Briefly tPAFlox<sup>++</sup>
mice (on a C57BL6N-Tac genetic background) in which
exon 3 is flanked by loxP sites (see 3) were crossed with
Rosa<sup>26</sup>-Cre mice [7] to induce Cre-mediated excision
of the third exon in germline. Mice were genotyped by PCR analysis and southern blots, using tail genomic DNA samples, to detect the presence/absence of loxP sequences. PV-Cre+/- female mice (Jackson laboratory # 008069, Pvaltmt1(cre)Arbr, [33]) were crossed with tPAFlox+/+ male mice or tPAFlox−/− male mice to generate tPAFlox+/−; PV-Cre+/− (cKO) and their control littermates tPAFlox−/−; PV-Cre−/−. Mice were genotyped by PCR for the presence of loxP sites and Cre transgene.

**Whole-cell recordings in acute slices**

G42 mice were deeply anesthetized with isoflurane. After decapitation, brains were quickly removed and placed into ice-cold oxygenated artificial cerebrospinal fluid (aCSF) containing in mM: 126 NaCl, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, 1 MgCl2, 26 NaHCO3, 10 glucose, 15 sucrose, and 1 kynurenic acid (Sigma). Coronal slices (300 μm thick) containing the primary visual cortex were cut with a vibratome (VT1000S; Leica) and allowed to recover at room temperature for at least 30 min in aCSF saturated with O2/CO2 (95 %/5 %). Patch pipettes (4–6 MΩ) pulled from borosilicate glass were filled with 8 μl of RNase-free internal solution containing in mM: 144 K-gluconate, 3 MgCl2, 0.5 EGTA, 10 HEPES, pH 7.2 (285/295 mOsm). Whole-cell recordings were performed at room temperature using a patch-clamp amplifier (MultiClamp 700B, MDS). Membrane potentials were not corrected for liquid junction potential. Data were filtered at 10 kHz and digitized at 20 kHz using an acquisition board (Digidata 1440, MDS). Cells were set at −60 mV by continuous current injection and submitted to current pulses (800 ms, from −100 to 280 pA with 20 pA increments).

**Cytoplasm harvesting and scRT-PCR**

At the end of the whole-cell recording, lasting <15 min, the cytoplasmic content was harvested in the recording pipette. The content of the pipette was expelled into a test tube and RT was performed in a final volume of 10 μL, as described previously [39]. The scRT-PCR protocol was designed to probe simultaneously the expression of neuronal markers and key molecular elements including tissue-type plasminogen activator gene, Plat. Two-step amplification was performed essentially as described [13, 20]. Briefly, cDNAs present in the 10 μL reverse transcription reaction were first amplified simultaneously using all external primer pairs (Additional file 1: Table S1). Taq polymerase (2.5 U; Qiagen) and 20 pmol of each primer were added to the buffer supplied by the manufacturer (final volume, 100 μL) and 20 cycles (94°C, 30 s; 60°C, 30 s; and 72°C, 35 s) of PCR were run. Second rounds of PCR were performed using 1 μl of the first PCR product as a template. In this second round, each amplicon was individually re-amplified using its specific nested primer pair (Additional file 1: Table S1) by performing 35 PCR cycles as described above. Ten microliters of each individual PCR product was run on a 2 % agarose gel stained with ethidium bromide using ΦX174 digested by HaeIII as a molecular weight marker.

**Unsupervised clustering**

To classify cells, unsupervised clustering was performed using 16 molecular parameters (VGlut1, Gad65, Gad67, PV, Akr1c18, Sst, S100β, APC, Mybpc1, Ncan, Plat, Plau, Ptprr, Ptprz1, Sdc4, Sema3a). The presence of a given gene was digitized by 1 and its absence was digitized by 0. Ward-linkage hierarchical clustering [92] was performed and plotted as a dendrogram using “scipy.cluster.hierarchy” functions on Python 3.7. The final number of clusters was suggested by the Thorndike procedure [88] as described previously [14].

**Viral production**

Viral particles were provided by Gilles Bonvento and Alexis Bemelmans (INSEERM U1169/MIRCen CEA, Fontenay aux Roses 92265, France).

The clone encoding for a GFP under the control of a 1.2-kb sequence of the Human Plat promoter (spanning from −1035 to +207 bp from the transcription start codon) was purchased from GeneCopoeia (pPlat-GFP; Catalog No.: HPRM12655-PF02). The pPlat-GFP was then subcloned in the pDONR221 for AAV production. All the constructs were amplified in Escherichia coli JM109 cells and purified by a Nucleobond endotoxin-free plasmid DNA PC 2000 kit (Macherey-Nagel) according to the manufacturer’s instructions. The vector used for this study was an AAV9 serotype. Self-complementary AAV vectors expressing the Plat-GFP construct were produced by transfecting HEK293 cells with the adenovirus helper plasmid (pXX6-80), the AAV packaging plasmid carrying the rep2 and the cap8 genes, and the AAV2 shuttle plasmid containing the Plat-GFP transgene in a sc genome. Recombinant vectors (rAAV) were purified by ultracentrifugation on a discontinuous iodixanin density gradient followed by dialysis against the formulation buffer of the vector stocks, 0.5 mmol/l MgCl2 and 1.25 mmol/l KCl in phosphate-buffered saline (PBSMK; five buffer changes, 3 h per round of dialysis). Physical particles were quantified by real-time PCR. Vector titers are expressed as viral genomes per milliliter (vg/mL).

**Stereotoxic injection of AAV virus**

Animals were deeply anesthetized with isoflurane 5 % and maintained with 2 % isoflurane in a 70 %/30 % mixture of NO2/O2 in a stereotoxic frame (Havard Apparatus). AAV9-Plat-GFP (1.25.1013 vp/mL) were injected
through a glass micropipette in the right hemisphere in a volume of 0.5 μL at a rate of 0.2 μL/min. Coordinates (relative to bregma) according to the Paxinos Mouse Brain Atlas were as follows: AP: −0.25 mm; ML: −3.4 mm; DV: −0.4 mm and −0.8 mm for the somatosensory cortex. The needle was left in position for a further 5 min and then removed slowly from the brain. After recovery from surgery, mice were left undisturbed for 3 weeks for effective and stable transgene expression.

Antibodies
The following antibodies, lectins, and biotinylated protein were used: mouse anti-NeuN (1:800, MAB377, Merck); anti-GFP (rabbit, 1:1000, ab6556, abcam or chicken, 1:1000, ab13970, abcam); chicken anti-Glial Fibrillary Acidic Protein (GFAP) (1:2000, ab4674, abcam); rabbit anti-ionized calcium-binding adapter molecule-1 (Iba-1) (1:1000, 019-19741, Fujifilm); rabbit anti-GABA (1:500, A2052, Sigma Aldrich); anti-parvalbumin (rabbit, 1:3000, ab 11427, abcam or guinea pig, 1:500, GP72, Swant); rabbit anti-aggrecan (1:1000, AB1031, Merck); or Biotinylated Wisteria floribunda Agglutinin (WFA) (1:1000, L1516, Sigma Aldrich).

Immunohistochemistry
Mice were deeply anesthetized with isoflurane 5 % in 70 %/30 % mixture of NO2/O2. A transcardial perfusion was performed with ice-cold 0.9 % NaCl with 3 % heparin followed by 150 ml of fixative solution containing 4 % paraformaldehyde (in PBS 0.1M, pH 7.4). Brains were removed and cryoprotected in 20 % sucrose solution (in PBS 0.1M, pH 7.4) for 24 h and frozen in Tissue-Tek (Miles Scientific). Cryostat sections of 10 μm were collected on Poly-Lysine slides and stored at −80°C. Sections were incubated overnight at room temperature with primary antibodies. Corresponding Fab’2-conjugated secondary antibodies were diluted at 1:800 (Jackson Immunoresearch). Images were acquired using Leica DM6000 microscope-coupled CoolSnap camera, visualized with Metamorph 7.0 software (Molecular Devices), and further processed were realized using ImageJ software.

For 3D reconstruction, images were acquired using Leica TCS SP8 Confocal/STED 3 × microscope with an oil-immersion ×40, 1.44-N.A. objective at a resolution of 512×512 pixels with 572 Hz speed and a step size of 0.22μm.

Histological analysis
To estimate the percentage of each Plat-GFP cell, all transfected cells were quantified (1090.3 ± 58.9 Plat-GFP cells/brain, from four sections per brain, 3 WT mice). For PNN/PV cells quantification, 2 images per section were acquired to cover all layers of the somatosensory cortex with a good resolution (four sections per brain, five mice per condition). PNN intensity was calculated as the intensity of WFA staining through the entire section of the somatosensory cortex. The overall intensity of WFA staining considers the high WFA staining around the soma and proximal dendrites and low WFA staining found around dendrites. PNN morphology was evaluated using a qualitative classification, ranging from High+ (strong WFA staining) to Low— (faint WFA staining). Only cells of which the soma was in the focal plane were classified. Analyses were performed blind to genotype.

Primary cultures of interneurons
GABAergic interneurons derived from the medial ganglionic eminence (MGE) were prepared from fetal mice (embryonic day 14) as previously described [27]. Medial ganglionic eminences were dissected in HBSS (Hanks’ Balanced Salt Solution, Gibco) /HEPES 10mM, incubated 15 min at 37°C in HBSS/HEPES with trypsin (0.05 %, Gibco) and DNAse I (100 μg/mL, Worthington) and mechanically dissociated through a glass pipette. Then, cells were plated (425,000 cells/mL) on glass bottom microwell dishes (MatTek Corporation) or on 24-well plates (500,000 cells/mL) previously coated with poly-d-lysine (0.1 mg/mL, Sigma Aldrich, P6407-5MG) and laminin (0.02 mg/mL, Gibco) in Neurobasal medium (Life Technologies) containing 2 % B27 supplement (Life Technologies) and 1 % Glutamax (Life Technology). Cytosine β-D-arabinofuranoside hydrochloride (5 μM, Sigma Aldrich) was added at DIV3, and fresh medium was added at DIV7.

In vitro treatments
Cells were treated at DIV14 with either 100 nM Human Glu-plasminogen (Enzyme Research Laboratories, HPg 2001), 200 nM Human Plasmin (Enzyme Research Laboratories, HPlasmin), or 10 nM or 200 nM Human tPA (Actilyse®, Boehringer Ingelheim) for 24 h.

Immunocytochemistry
Cells were fixed during 10 min in 0.1 M PBS containing 4 % paraformaldehyde. After PBS washes, cells incubated for 1 h in PBS–Triton 0.25 % containing 1 % BSA and then incubated overnight at 4°C in blocking buffer containing primary antibodies. Confocal images were acquired with a Leica TCS SP8 Confocal/STED 3 × microscope with an oil-immersion ×40, 1.44-N.A. objective. Confocal images were taken at a resolution of 1024×1024 pixels with 400 Hz speed and a step size of 0.45μm. Laser intensity, gain, and offset were maintained constant in each analysis.
PNN quantification in vitro
Confocal images were analyzed with the ImageJ software. Random PV-positive cells were imaged with 160 × 160 × 15 μm z-stack with 0.45 μm step size. A threshold was applied to the WFA channel or aggrecan channel (range 0–255), and the area was quantified in 40 neurons from 4 independent experiments.

Enzymatic degradation assays
Recombinant human aggrecan core protein G1-IGD-G2 (156 nM) (Biotechne) was incubated at 37°C overnight with either 7.8 nM recombinant human ADAMTS4 protein (Biotechne), purified human plasmin (Enzyme Research Laboratories), Human Glu-plasminogen (Enzyme Research Laboratories), and/or tPA (Actilyse) in a buffer containing 50 mM Tris, 10mM CaCl2, 150 mM NaCl, and 0.05 % Brij35. Then, 78 nM Aprotinin treatment was applied with either Glu-plasminogen + tPA treatment or plasmin treatment. Digestion products were analyzed by SDS-PAGE on 10 % Tris-Glycine gel stained with GelCode Imperial Blue Stain Reagent (Pierce).

Statistics
For sc-PCR analyses, between-group comparisons were performed using Mann–Whitney nonparametric test. Comparison of the occurrence of expressed genes between cell types was determined using Fisher’s exact test. A p-value below 0.05 was considered statistically significant.

Otherwise, experiments were analyzed by investigators blinded to group allocation. Unblinding was made after completion of statistical analysis. All statistical analyses were performed using the GraphPad Prism 8 software. Comparisons between more than two independents samples were performed using two-tailed Kruskal-Wallis test and appropriate post hoc test (Dunn’s test). Mann–Whitney U test was used for the comparisons of two independent samples. Plasmin activity was analyzed using 2-way Anova (two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli). Recombinant aggrecan degradation was analyzed using one-way ANOVA followed by Fisher’s LSD test. The intensity of the 120-kDa band was compared between Agg G1-G2 and protease-treated conditions. A threshold of p < 0.05 was defined as statistically significant. All graph data are presented as the mean standard error mean (SEM). Sample sizes are indicated in each corresponding legend.

Abbreviations
AAV: Adeno-associated viruses; ADAMTS: A Disintegrin And Metalloproteinase with Thrombospondin Motifs; CSPGs: Chondroitin sulfate proteoglycans; ECM: Extracellular matrix; F5-PV: Fast-spiking parvalbumin; GABA: Gamma-aminobutyric acid; LRP: Low-density lipoprotein receptor–related proteins; MMPs: Matrix metalloproteinases; NMDAR: N-methyl-D-aspartate receptor; PNN: Perineuronal nets; PV: Parvalbumin; TIMP3: Tissue inhibitor of metalloproteinases-3; tPA: Tissue-type plasminogen activator; VIP: Vasointestinal peptide; WFA: Wisteria floribunda agglutinin.

Supplementary Information
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Authors’ contributions
DV and CA designed and supervised the study. ML, SD, GD, and PP performed the data analysis. ML, SD, GD, PP, SL, CR, EP, JP, YH, LL, and EM performed the experiments. ML, SD, GD, PP, CA, BL, BC, VA, and DV wrote the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article and its supplementary information files. All data supporting our results are available from the corresponding author upon reasonable request.

Declarations
Ethics approval and consent to participate
All experiments were conducted in accordance with the French ethical law (Decree 2013-118) and the European Communities Council guidelines (2010/63/EU).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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