Published in final edited form as:

J Neuroimmune Pharmacol. 2016 March ; 11(1): 26–35. doi:10.1007/s11481-015-9616-y.

Pattern of CXCR7 Gene Expression in Mouse Brain Under Normal and Inflammatory Conditions

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Abstract

The chemokine stromal cell-derived factor-1 (SDF-1)/CXCL12 acting via its G-protein coupled receptor (GPCR) CXCR4 has been implicated in neurogenesis, neuromodulation, brain inflammation, HIV-1 encephalopathy and tumor growth. CXCR7 was identified as an alternate receptor for SDF-1/CXCL12. Characterization of CXCR7-deficient mice demonstrated a role for CXCR7 in fetal endothelial biology, cardiac development, and B-cell localization. Despite its ligand binding properties, CXCR7 does not seem to signal like a conventional GPCR. It has been suggested that CXCR7 may not function alone but in combination with CXCR4. Here, we investigated the regional localization of CXCR7 receptors in adult mouse brain using CXCR7-EGFP transgenic mice. We found that the receptors were expressed in various brain regions including olfactory bulb, cerebral cortex, hippocampus, subventricular zone (SVZ), hypothalamus and cerebellum. Extensive CXCR7 expression was associated with cerebral blood vessels. Using cell type specific markers, CXCR7 expression was found in neurons, astrocytes and oligodendrocyte progenitors. GAD-expressing neurons exhibited CXCR7 expression in the hippocampus. Expression of CXCR7 in the dentate gyrus included cells that expressed nestin, GFAP and cells that appeared to be immature granule cells. In mice with Experimental Autoimmune Encephalomyelitis (EAE), CXCR7 was expressed by migrating oligodendrocyte progenitors in the SVZ. We then compared the distribution of SDF-1/CXCL12 and CXCR7 using bitransgenic mice expressing both CXCR7-EGFP and SDF-1-mRFP. Enhanced expression of SDF-1/CXCL12 and CXCR7 was observed in the corpus callosum, SVZ and cerebellum. Overall, the expression of CXCR7 in normal and pathological nervous system suggests CXCR4-independent functions of SDF-1/CXCL12 mediated through its interaction with CXCR7.

Keywords

Chemokine; Chemokine receptor; CXCR7; SDF-1/CXCL12; Nervous system; EAE

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Conflict of Interest Authors have no conflict of interest to disclose.

Introduction

The chemokine stromal cell-derived factor-1 (SDF-1)/CXCL12 acting via its G-protein coupled receptor (GPCR) CXCR4 has been implicated in neurogenesis, neuromodulation, brain inflammation, HIV-1 encephalopathy and tumor growth in adult brain (Stumm and Höllt 2007; Guyon and Nahon 2007; Miller et al. 2008; Guyon 2014).

Targeted deletion of SDF-1/CXCL12 and CXCR4 leads to similar phenotypes including abnormalities in the development of the cardiovascular system, hematopoietic system and numerous brain structures (Nagasawa et al. 1996; Ma et al. 1998; Zou et al. 1998; Lu et al. 2002). Thus, SDF-1/CXCL12 and CXCR4 for long has been thought to be a non-redundant chemokine/receptor system. Recently, RDC1, now termed CXCR7, was identified as a novel, alternate receptor for SDF-1/CXCL12 (Balabanian et al. 2005). Like CXCR4, CXCR7 serves as a co-receptor for some HIV-1 strains (Shimizu et al. 2000) and has also been shown to be involved in tumor growth (Miao et al. 2007). Characterization of CXCR7-deficient mice revealed a role for CXCR7 in fetal endothelial biology, cardiac development, and B-cell localization (Sierro et al. 2007). Furthermore, CXCR7 has been shown to be essential in the migratory properties of mouse cortical neurons (Sánchez-Alcañiz et al. 2011; Wang et al. 2011). In addition to SDF-1/CXCL12, CXCR7 binds to the CXCL11 chemokine but with a lower affinity (Burns et al. 2006). Despite its ligand binding properties, CXCR7 does not seem to signal like a conventional receptor and fails to couple to classic G-protein signaling pathways activated by chemokines (Sierro et al. 2007; Levoe et al. 2009; Rajagopal et al. 2010; Ehrlich et al. 2013). Indeed, it has been suggested that CXCR7 may not always function alone but may also modulate the function of CXCR4. In contrast to CXCR4, which is the best-characterized chemokine receptor in the nervous system, few studies have described the expression and function of CXCR7 in the brain (Schönemeier et al. 2008a, b; Shimizu et al. 2011). Knowledge of the precise expression pattern and characterization of cells expressing CXCR7 is required to further explore the function of CXCR7 in the brain. It is well established that SDF-1/CXCL12 is constitutively expressed by endothelial and neuronal cells in various regions of the adult rat brain (Tham et al. 2001; Stumm et al. 2002; Banisadr et al. 2003) and the expression patterns of SDF-1/CXCL12 and CXCR4 have been well established in structures associated with various functions such as their role in adult neurogenesis and neuroinflammation among other functions (Banisadr et al. 2002; Stumm et al. 2002; Tissir et al. 2004; Tran et al. 2007).

In order to further understand the role of CXCR7 in the brain and determine which brain structures express CXCR7 and how it may be involved in SDF-1/CXCL12 signaling, we analyzed the expression of CXCR7 using CXCR7-EGFP transgenic mice. Our experiments considered normal adult mouse brain as well as in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). We have recently established an important role for CXCR4 signaling in brain inflammation and oligodendrocyte progenitor cell (OPC)-mediated remyelination during MS (Banisadr et al. 2011). Other studies also indicate that SDF-1/CXCL12 regulates OPC-mediated remyelination (Carbajal et al. 2010; Patel et al. 2010, 2012). In vitro studies have revealed functional expression of CXCR7 in OPCs (Göttle et al. 2010), suggesting that it may regulate CXCR4 activation during OPC differentiation, as is observed for other neural

progenitors (Boldajipour et al. 2008). CXCR7 antagonism during EAE also helps preserving axonal integrity (Cruz-Orengo et al. 2011a). Williams et al. (2014) have shown that during a cuprizone model of myelin injury, CXCR7 antagonist treatment led to an increased level of SDF-1/CXCL12 resulting in CXCR4 activation and enhanced OPC differentiation within the demyelinated white matter. Here, we aim to further examine the regulation of CXCR7 in an animal model of MS.

In this study, we demonstrated that CXCR7 was expressed in various regions throughout the adult brain and its expression was enhanced in migrating OPCs during EAE in association with an upregulation of SDF-1/CXCL12 in the SVZ, suggesting that SDF-1/CXCL12 may have CXCR4-independent effects via CXCR7.

Materials and Methods

Animals

The following transgenic mice were used in this study: SDF-1-RFP (generated in our laboratory) and CXCR7-EGFP (The Gene Expression Nervous System Atlas project GENSAT; <http://www.gensat.org/index.html>; Memi et al. 2013). SDF-1-RFP/CXCR7-EGFP bitransgenic mice were made in our laboratory by crossing SDF-1-RFP mice with CXCR7-EGFP mice. SDF-1-RFP mice were generated by Dr. Hosung Jung as described by Jung et al. (2009). All of the procedures performed on animals within this study were conducted in accordance with the guidelines of Northwestern University Animal Care and Use Committee.

EAE Induction

Six to seven week old mice (Swiss Webster background) were immunized s.c. with 100 µl of an emulsion containing 200 µg of Mycobacterium tuberculosis H37Ra (Difco) and 50 µg proteolipid protein (residues 139–151, HSLGKWLGHDPKF) (PLP_{139–151}) distributed over three spots on the flank (Fuller et al. 2004). Clinical signs of EAE appeared typically after 10–14 days. Naïve mice were used as controls for EAE.

Clinical Evaluation of EAE

The animals were observed daily and clinical severity assessed in a blinded fashion on a 0–5 scale as follows: 0, asymptomatic; 1, loss of tail tonicity; 2, atonic tail and hind leg weakness; 3, hind limb paralysis; 4, hind limb paralysis and forelimb weakness; 5, moribund (Fuller et al. 2004). A relapse was defined as a sustained increase (more than 2 days) in at least one full grade in clinical score after the animal had improved previously at least a full clinical score and had stabilized for at least 2 days.

Antibodies

The following antibodies were used in this study: anti-GFAP (1:2000, product # G3893, Sigma, St. Louis, MO, USA) to characterize astrocytes, anti-NeuN (1:500, product # MAB377, Chemicon, Temecula, CA) to label neurons, anti-Olig2 (1:500, product # AB9610, Millipore, Billerica, MA, USA) oligodendrocyte lineage marker, anti-GAD67 (1:300, MAB5406, Millipore, Billerica, MA, USA) to label GABAergic neurons, anti-Nestin

(1:300, product # 556309, BD Bioscience, San Jose, CA, USA) to label neural progenitor cells and granule cells were identified by anti-Prox-1 (1:1000, product # AB5475, Chemicon, Temecula, CA).

Tissue Preparation

CXCR7-EGFP and SDF-1-RFP/CXCR7-EGFP mice were anesthetized and perfused transcardially with cold PBS, followed by a freshly prepared solution of 4 % paraformaldehyde (PFA) in PBS, pH 7.4. The brains were rapidly removed and post-fixed overnight in 4 % PFA at 4 °C. Forty-micrometer thick coronal sections were cut with a vibratome (Leica VT 1000S; Leica Biosystems, Nussloch, Germany) and collected in cold PBS. Sections were then processed for histology or immunohistochemistry. For vascular labeling, mice were injected intravenously with 0.1 ml of a 1 mg/ml solution of TxRed-labelled tomato lectin before perfusion.

Histology and Immunohistochemistry

In order to assess the regulation of SDF-1/CXCL12 and CXCR7 expression in EAE mice, sections of CXCR7-EGFP and SDF-1-RFP/CXCR7-EGFP mice induced with EAE as described above, were analyzed by a confocal microscope and compared to naïve mice.

To characterize the cell types expressing CXCR7, sections were processed for immunohistochemistry. Immunohistochemistry was performed on free-floating sections using the abovementioned primary antibodies. The appropriate isotype-specific secondary antibodies consisted of AlexaFluor 633 or 647-conjugated preparations (1:500; Molecular Probes, Grand Island, NY, USA).

Sections were incubated in PBS/4 % goat serum/0.1 % triton for 90 min. They were then incubated with primary antibodies diluted in PBS/2 % normal serum/0.1 % triton overnight at 4 °C. The sections were then washed with PBS and incubated with secondary antibodies for 1 h. Sections were washed with PBS, mounted on slides, and analyzed by confocal microscopy. Image acquisition software (Fluoview) was used. Adjacent sections were used as controls and incubated in the absence of primary antibodies to confirm the specificity of the staining.

Quantification

Olig2-expressing cells and CXCR7-EGFP positive cells were quantified in the SVZ of naïve and EAE mice. Result is expressed as percentage of CXCR7/Olig2-positive cells compared to the total number of CXCR7-EGFP cells in the SVZ ($n=6$). Data were reported as mean $\pm$ standard error of the mean.

Results

Expression of CXCR7 in Adult Mouse Brain

To map the regional distribution of CXCR7 in mouse brain, we analyzed coronal sections at rostrocaudal intervals of 240 μ m. CXCR7 was widely expressed in the mature mouse brain in various regions including the olfactory bulb, cerebral cortex, hippocampus, subventricular

zone, ventricular walls, hypothalamus, cerebellum and spinal cord (Fig. 1). Extensive CXCR7 expression was also associated with blood vessels throughout the brain (Fig. 2). In the telencephalon, the olfactory bulb exhibited CXCR7 expression mainly in the glomerular layer, ependymal and subependymal layer and olfactory ventricle (Fig. 1a). Scattered cells expressing CXCR7-EGFP were observed in the external plexiform layer and granular cell layer of the olfactory bulb. In the ventral striatum, CXCR7 was observed in the nucleus accumbens (Fig. 1b). CXCR7 was also expressed in the subventricular zone (SVZ) where cells showed the morphology of migrating oligodendrocyte progenitors (Fig. 1c). Strong expression of CXCR7 was also observed in the wall of the lateral ventricle (Fig. 1c). In the basal ganglia, CXCR7-expressing cell bodies were mainly evident in the globus pallidus which contained a dense network of CXCR7-positive fibers and dispersed small cell bodies expressing CXCR7 (Fig. 1d and e). Figure 1e shows these CXCR7-EGFP expressing cells at higher magnification. In the dorsal part of the striatum, however, CXCR7 expression was barely detectable. The cerebral cortex exhibited CXCR7-EGFP expression with variations in its laminar distribution (Fig. 1f). CXCR7 expressing cells were predominant in layer IV–V and to a lesser extent in layers II, III and VI of the parietal cortex. The same pattern was evident in the motor and primary somatosensory cortex. In the hippocampus, CXCR7 expression was confined to the subgranular layer, molecular layer, pyramidal layer, oriens layer and the hilus. Only few cells expressed CXCR7 in the granular layer (Fig. 1g). More posteriorly, the subiculum exhibited prominent CXCR7 expression (not shown).

In the diencephalon, CXCR7 was predominantly expressed in the hypothalamus. In the hypothalamus, CXCR7 expression was most prevalent in the ventromedial hypothalamic nucleus (Fig. 1h and i) and the supraoptic nucleus (Fig. 1j).

In the mesencephalon, very faint expression was detected in the substantia nigra (not shown).

In the cerebellum, CXCR7 was localized within the Purkinje cell layer (Fig. 1k). CXCR7 was also expressed in the ventral horn and dorsal horn of the spinal cord (Fig. 1l).

Vascular Expression of CXCR7 in Adult Mouse Brain

CXCR7 receptors are extensively expressed on blood vessels throughout the brain as demonstrated in Fig. 4. Blood vessels were visualized with tomato lectin. The colocalization of CXCR7-EGFP (Fig. 2a) and TxRed-labelled tomato lectin (Fig. 2b) is shown in the merged image (Fig. 2c).

Developmental Distribution of CXCR7 in the Hippocampus of Postnatal Mouse Brain

In order to study the expression of CXCR7 in the hippocampus during postnatal development in more detail, histology was carried out at 7 days, 3, 6 and 8 weeks post-natal (Fig. 3). At 7 days, CXCR7 was mostly localized in the hilus, molecular layer, only few CXCR7 expressing cells were observed in the subgranular layer (Fig. 3a and b). Later during development, stronger expression was observed in the subgranular layer of the dentate gyrus, molecular layer and pyramidal layer (Fig. 3c–h).

Characterization of CXCR7 Expressing Cells in CXCR7-EGFP Mouse Brain

We performed immunohistochemistry on CXCR7-EGFP transgenic mice to further characterize the types of cells expressing CXCR7 in adult mouse brain. Using cell type specific markers, CXCR7 expression was mainly found in neurons, astrocytes and endothelial cells. Moreover, CXCR7 was also expressed by neural stem cells. Figure 4a shows the colocalization of CXCR7-EGFP (green) and nestin (red) in the SGZ of CXCR7-EGFP adult mouse. Only few CXCR7-EGFP cells expressed the granule cell marker Prox-1 (Fig. 4b). CXCR7 colocalized with GAD67 in the DG (arrowheads in Fig. 4c) suggesting expression of CXCR7 by GABAergic interneurons. Figure 4d illustrates the colocalization of CXCR7 with GFAP, a marker for astrocytes and type I stem cells. Figure 3e and f confirm the neuronal expression of CXCR7 in the DG and cerebral cortex respectively. Arrowheads show cells expressing both CXCR7-EGFP (green) and the neuronal marker NeuN (red). Figure 7c shows the expression of CXCR7 in OPCs in the SVZ. No staining was observed in the absence of primary antibodies confirming the specificity of the labelling, as shown in the insert in Fig 4a.

Enhanced Expression of SDF-1/CXCL12 and CXCR7 During EAE

We then compared the expression pattern of SDF-1/CXCL12 and CXCR7 in EAE brain using SDF-1-RFP/CXCR7-EGFP dual transgenic mice (Fig. 5). SDF-1/CXCL12 was highly upregulated in the corpus callosum (Fig. 5b) and SVZ (Fig. 5d) which correlated with the enhanced expression of CXCR7. SDF-1/CXCR7 were upregulated in the cingulate cortex (Fig. 5e), cerebral blood vessels (Fig. 5f), cerebellum (Fig. 5g) and spinal cord (Fig. 5h). In the spinal cord, SDF-1/CXCL12 was highly expressed in the meninges. Panels A, C and inserts in E, F and G show the expression of SDF-1-RFP and CXCR7-EGFP in naïve mouse. Figure 6 shows the comparative expression of CXCR7 and SDF-1/CXCL12 in the corpus callosum (Fig. 6a) and SVZ (Fig. 6b) at higher magnification. Arrowheads show that SDF-1/CXCL12 containing vessels are expressed inside CXCR7-expressing cells, suggesting that the upregulation of CXCR7 may have triggered SDF-1/CXCL12 endocytosis, consistent with previous reports in the literature (Sánchez-Alcañiz et al. 2011; Mithal et al. 2013; Abe et al. 2014).

Cell-Selective Expression Pattern of CXCR7 During EAE

Changes in the cell-selective expression pattern of CXCR7 were analyzed at the peak of the disease (score 4). To identify which cell types upregulate CXCR7 expression after EAE, immunohistochemistry using cell-specific markers was carried out. We had noted that CXCR7 was expressed by some cells exhibiting the morphology of migrating OPCs in the posterior part of the SVZ (Fig. 1c). Immunostaining using an anti-Olig2 antibody demonstrated that these CXCR7-expressing cells also expressed Olig2, a transcription factor expressed by OPCs (Fig. 7).

Figure 7 shows that CXCR7 was upregulated in Olig2-expressing OPCs in the SVZ of EAE mouse brain, an enhanced vascular expression was also observed. As seen in the merged image (Fig. 7f), the majority of the CXCR7 expressing cells in the SVZ colocalized with Olig2 suggesting expression of CXCR7 by OPCs. Quantification of CXCR7 and Olig2

expressing cells showed that at the peak of EAE (score 4), 89.88 % $\pm$ 3.8 of CXCR7 expressing cells were Olig2 positive ($n=6$).

Discussion

The identification of CXCR7 as a second receptor for SDF-1/CXCL12 suggests that the effects of SDF-1/CXCL12 are not exclusively mediated via CXCR4. In the present study we demonstrated that CXCR7 was expressed in various regions in the mouse brain and its expression was upregulated in peptide-induced EAE, suggesting that in addition to CXCR4, CXCR7 has a role in SDF-1/CXCL12 signaling in normal and pathological conditions such as MS. Previous reports in the literature have indicated that CXCR7 transcripts are widely expressed throughout the rat brain (Schönemeier et al. 2008a) and developing mouse forebrain (Tiveron et al. 2010); Shimizu et al. (2011) have shown that CXCR7 is expressed in human adult neurons in normal and pathological conditions.

Indeed, our dual immunohistochemistry studies showed that CXCR7 expression was normally associated with astrocytes, neurons and neural stem cells in the DG and OPCs in the SVZ. In addition, CXCR7-EGFP was highly expressed in meningeal and parenchymal blood vessels. Distinct brain regions showed CXCR7 expression, these regions included the olfactory nuclei, cerebral cortex, accumbens nucleus, globus pallidus, hippocampus, SVZ, hypothalamus, cerebellum and spinal cord. In the hippocampus, CXCR7 was localized to a population of dentate gyrus neurons that have a GABAergic phenotype, presumably GABAergic interneurons, consistent with findings by Schönemeier et al. (2008a). In the dentate gyrus, we identified a major difference in the granule cell layer (GCL). The reporter labels the SGZ strongly and the outer part of the GCL weakly (Fig. 2f). In contrast, CXCR7 mRNA is uniformly expressed throughout the GCL in the adult rat (Schönemeier et al. 2008a). This could be due to species differences but could also reflect limitations of transgenic reporter mice. In many other brain structures, though, CXCR7-EGFP reporter and CXCR7 mRNA seem to have similar patterns.

Expression of CXCR7 in the hypothalamus suggests that CXCR7 might be involved in SDF-1/CXCL12 dependent modulation of hypothalamic functions. Particularly, the expression of CXCR7 in the supraoptic nucleus, known to contain vasopressin-expressing neurons is of interest as it was previously described that SDF-1/CXCL12 can modulate central vasopressin neuronal activity and release (Banisadr et al. 2002; Callewaere et al. 2006).

SDF-1/CXCL12 expressed in cerebral vascular endothelial cells is of particular importance in the development of tumors in the CNS (Zlotnik 2004; Savarin-Vuillat and Ransohoff 2007). Thus, the widespread expression of CXCR7 and SDF-1/CXCL12 in cerebral blood vessels demonstrated in this study may be relevant to their role in the development of brain tumors.

Importantly, SDF-1/CXCL12 is well known to play a key role in neurogenesis (Bhattacharyya et al. 2008; Kolodziej et al. 2008); we have previously shown that SDF-1/CXCL12 and CXCR4 are expressed in neurogenic regions of the mouse brain (Tran et al.

2007). Moreover, SDF-1/CXCL12 signaling is important for the migration of progenitors in the brain in response to different types of pathology (Stumm et al. 2002; Ceradini et al. 2004; Imitola et al. 2004; Ohab and Carmichael 2008; Robin et al. 2006). We found that CXCR7 was also expressed in neurogenic regions of the mouse brain including the olfactory bulb, SVZ and SGZ of the dentate gyrus. In the SGZ, an overlap of CXCR7 expression with progenitor cell marker, nestin, was evident. In addition, the expression of CXCR7 in the dentate gyrus included cells that express GFAP and few cells expressed Prox-1 as well. During postnatal development, CXCR7 expression is lower in mature granule cells but persists in progenitor cells and immature granule cells.

These recent observations support the involvement of CXCR7 in SDF-1/CXCL12 signaling on neural progenitor cells and perhaps adult neurogenesis. In the SVZ, we demonstrated that CXCR7 colocalized with OPC marker Olig2. Other in vitro studies on OPCs have revealed functional expression of CXCR7 where activation of CXCR7 promotes oligodendroglial cell maturation (Göttle et al. 2010). Generally speaking OPCs in the adult brain are found distributed in the parenchyma. However, migration of additional OPCs from the SVZ has been reported to occur in the context of syndromes such as EAE (Menn et al. 2006; Nait-Oumesmar et al. 2007) and is considered a major phase in the remyelination process during MS. Indeed, current treatment modalities for MS primarily rely on immune suppression, which can lead to unfavorable side effects. In this context, the use of a CXCR7 antagonist during demyelination has proven beneficial by controlling abluminal levels of SDF-1/CXCL12 thus influencing leukocyte entry into the CNS parenchyma (Cruz-Orengo et al. 2011b). The same group has reported that CXCR7 antagonists prevents axonal injury during EAE while qualitative myelin content and presence of oligodendrocytes were intact (Cruz-Orengo et al. 2011a). Our recent studies showed that SDF-1/CXCL12, synthesized by cells in the inflamed white matter, can induce the migration of transplanted OPCs to demyelinated white matter during EAE (Banisadr et al. 2011). The up-regulation of CXCR7 during demyelination, at the peak of EAE, and its colocalization by OPCs arguably suggests that CXCR7 can facilitate the migration of OPCs to sites of pathology, via directed expression of SDF-1/CXCL12 on the surface of astrocytes (Banisadr et al. 2014) and CNS endothelial cells, therefore, promote myelin repair. In a viral model of demyelination, CXCR4 signaling has been known indispensable for OPC proliferation and enhanced remyelination (Carbajal et al. 2011). One could also argue that the enhanced expression of CXCR7 may regulate extracellular SDF-1/CXCL12 and CXCR4 activation and have significant indirect implications for the treatment of MS. Indeed, a closer look at the colocalization of CXCR7 and SDF-1/CXCL12 in the corpus callosum and SVZ suggests that upregulation of CXCR7 may mediate the endocytosis of SDF-1/CXCL12; thus, reducing the availability of SDF-1/CXCL12 to CXCR4 as previously suggested (Sánchez-Alcañiz et al. 2011; Mithal et al. 2013; Abe et al. 2014); supporting prior findings that CXCR7 functions as a scavenger to remove extracellular SDF-1/CXCL12, thereby indirectly controlling CXCR4 signaling (Sánchez-Alcañiz et al. 2011; Abe et al. 2014). Altogether, the clear understanding of the function of CXCR7 associated with its distribution within specific regions of the brain and its regulation during pathologies opens new perspectives for development of more specific therapeutic approaches that include chemokine-based drugs. The present findings provide evidence that SDF-1/CXCL12 function in the CNS is not

exclusive to its signaling via CXCR4 and modulation of CXCR7 signaling might present a promising therapeutic approach to promote repair in demyelinating diseases such as MS.

Acknowledgments

This work was supported by funding from the National Institutes of Health.

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Fig. 1.

Regional distribution of CXCR7 in 7-week old CXCR7-EGFP mouse brain. **a:** Olfactory bulb (OB): CXCR7 was mainly expressed in the glomerular layer (Gl), ependymal and subependymal layer and olfactory ventricle (E/OV). (EPI: external plexiform layer, GrO: granular cell layer of the olfactory bulb). **b:** Accumbens nucleus (Acb). **c:** Subventricular zone (SVZ), strong expression of CXCR7 was also observed in the wall of the lateral ventricle (LV). **d, e:** Globus pallidus (GP): contained a dense network of CXCR7-positive fibers and dispersed small cell bodies expressing CXCR7. Panel E shows CXCR7

expression in the GP at higher magnification. **f**: Cortex: CXCR7 expressing cells were predominant in layer IV–V and to a lesser extent in layers II and VI of the primary somatosensory cortex (S1). **g**: Hippocampus: CXCR7 expression was confined to the subgranular layer (SGL), molecular layer (Mol), pyramidal layer (Py), oriens layer (Or) and hilus. (Gr: granular layer). **h–j**: Hypothalamus, CXCR7 expression was most prevalent in the ventromedial hypothalamic nucleus (VMH, panel **h**) and supraoptic nucleus (SO, panel **j**). Panel **i** shows cells expressing CXCR7 in the VMH at a higher magnification. **k**: Cerebellum: CXCR7 was localized within the Purkinje cell layer (PC). **l**: Spinal cord, CXCR7 was expressed by cells in the ventral horn (VH) and dorsal horn (DH). Scale bars in **a, d, f, g, h, k, l** = 200 μ m, scale bars in **b, c, e, i, j** = 50 μ m

Fig. 2.
Vascular expression of CXCR7 in adult mouse brain. For vascular labeling, mice were injected intravenously with 0.1 ml of a 1 mg/ml solution of TxRed-labelled tomato lectin before perfusion. CXCR7-EGFP (**a**) and TxRed-labelled tomato lectin (**b**) colocalize as shown in the merged panel (**c**) confirming the vascular expression of CXCR7. Scale bars = 100 μ m

Fig. 3. Developmental distribution of CXCR7 in the hippocampus of postnatal mouse brain. Histology was carried out at 7 days (**a, b**), 3 weeks (**c, d**), 6 weeks (**e, f**) and 8 weeks (**g, h**) postnatally. At 7 days, CXCR7 was mostly localized in the hilus, molecular layer (Mol), oriens layer (Or), only few CXCR7 expressing cells were observed in the subgranular layer (SGL) (**a, b**). Later during development, stronger expression was observed in the subgranular layer (SGL) of the dentate gyrus (DG), molecular layer (Mol) and pyramidal

layer (py) (**c–h**). cc: corpus callosum; Rad: stratum radiatum of the hippocampus; Gr: granular layer. Scale bars in **a, c, e, g** = 200 μm , scale bars in **b, d, f, h** = 50 μm

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Fig. 4. Characterization of CXCR7 expressing cells in CXCR7-EGFP adult mouse brain. Immunohistochemistry was carried out using cell specific markers. **a:** CXCR7 (*green*) colocalized with nestin (*red*) in the SGZ; insert corresponds to control section incubated in the absence of primary antibody. **b:** Few CXCR7-EGFP (*green*) cells expressed Prox-1 (*red*). **c:** CXCR7 colocalized with GAD67 (*arrowheads* in panel c). **d:** Expression of CXCR7 by GFAP expressing cells with astrocyte like characteristics, the ones in the SGZ are neural stem cells. **e, f:** neuronal expression of CXCR7 in the DG and cerebral cortex respectively.

Arrowheads show cells expressing both CXCR7-EGFP (*green*) and the neuronal marker NeuN (*red*). Scale bars = 50 μ m

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Fig. 5.

Comparative expression pattern of SDF-1/CXCL12 and CXCR7 during EAE. SDF-1/CXCL12 was highly upregulated in the corpus callosum (cc) (b) and subventricular zone (SVZ) (d) which correlated with the enhanced expression of CXCR7. SDF-1 and CXCR7 were both upregulated in the cingulate cortex (Cg) (e), cerebral blood vessels (f), cerebellum (g) and spinal cord (h). Panels a, c and inserts in e, f and g show the expression of SDF-1-RFP and CXCR7-EGFP in naïve mouse. Scale bars = 50 μ m

Fig. 6. Comparative cellular localization of SDF-1/CXCL12 and CXCR7 during EAE. We compared the relative expression pattern of SDF-1/CXCL12 and CXCR7 in EAE brain using SDF-1-RFP/CXCR7-EGFP dual transgenic mice. SDF-1/CXCL12 and CXCR7 are expressed by the same cells in the corpus callosum (**a**) and subventricular zone (**b**). Scale bars = 20 μm

Fig. 7.

CXCR7 expression in peptide-induced EAE. CXCR7 is expressed by cells exhibiting the morphology of migrating progenitors in the SVZ of naïve mouse brain (**a**). Immunostaining using an anti-Olig2 antibody (**b**) shows that these CXCR7-expressing cells (panel **a**, *green*) colocalize with Olig2 (panel **b**, *red*). Arrowhead in panel **c** shows the colocalization of CXCR7 and Olig2 in naïve mouse brain. Panels **d–f** show that the expression of CXCR7 (**d**) is upregulated in the SVZ in EAE mouse brain and corresponds to a higher expression by Olig2-labeled oligodendrocyte progenitors (**f**). Scale bars = 100 μm