Intermediate Progenitors Facilitate Intracortical Progression of Thalamocortical Axons and Interneurons through CXCL12 Chemokine Signaling

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Glutamatergic principal neurons, GABAergic interneurons and thalamocortical axons (TCAs) are essential elements of the cerebrocortical network. Principal neurons originate locally from radial glia and intermediate progenitors (IPCs), whereas interneurons and TCAs are of extrinsic origin. Little is known how the assembly of these elements is coordinated. C-X-C motif chemokine 12 (CXCL12), which is known to guide axons outside the neural tube and interneurons in the cortex, is expressed in the meninges and IPCs. Using mouse genetics, we dissected the influence of IPC-derived CXCL12 on TCAs and interneurons by showing that *Cxcl12* ablation in IPCs, leaving meningeal *Cxcl12* intact, attenuates intracortical TCA growth and disrupts tangential interneuron migration in the subventricular zone. In accordance with strong CXCR4 expression in the forming thalamus and TCAs, we identified a CXCR4-dependent growth-promoting effect of CXCL12 on TCAs in thalamus explants. Together, our findings indicate a cell-autonomous role of CXCR4 in promoting TCA growth. We propose that CXCL12 signals from IPCs link cortical neurogenesis to the progression of TCAs and interneurons spatially and temporally.

Key words: cortical development; CXCL12/CXCR4; intermediate/basal progenitors; interneuron; thalamocortical axons; thalamus

Significance Statement

The cerebral cortex exerts higher brain functions including perceptual and emotional processing. Evolutionary expansion of the mammalian cortex is mediated by intermediate progenitors, transient amplifying cells generating cortical excitatory neurons. During the peak period of cortical neurogenesis, migrating precursors of inhibitory interneurons originating in subcortical areas and thalamic axons invade the cortex. Although defects in the assembly of cortical network elements cause neurological and mental disorders, little is known how neurogenesis, interneuron recruitment, and axonal ingrowth are coordinated. We demonstrate that intermediate progenitors release the chemotactic cytokine CXCL12 to promote intracortical interneuron migration and growth of thalamic axons via the cognate receptor CXCR4. This paracrine signal may ensure thalamocortical connectivity and dispersion of inhibitory neurons in the rapidly growing cortex.

Introduction

The cerebral cortex exerts perceptual, motor, and cognitive functions including emotional processing. Cortical neurons approx-

imately consist of 80% glutamatergic principal neurons (PNs) and 20% GABAergic interneurons (Parnavelas, 2000). Thalamocortical axons (TCAs) terminating on layer IV PNs convey sensory information to the cortex and represent its major excitatory input. Neurodevelopmental defects that disturb the balance of these excitatory and inhibitory systems are thought to underlie epilepsy and mental disorders including schizophrenia, person-

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ality disorders, and autism spectrum disorders. Thus, elucidating the mechanisms that govern the assembly of the cortical network is central to understanding causes and pathogenesis of mental illnesses (Rubenstein, 2011).

Cortical PNs are generated locally, whereas cortical interneurons are of subpallial origin. In mice, most interneurons enter the cortex after embryonic day (E)12. They migrate preferentially on characteristic routes including the cortical subventricular zone (SVZ) and marginal zone (MZ) (Wonders and Anderson, 2006). Individual interneurons spend days migrating in the cortex and perform random movements. This unusual behavior is thought to disperse the cells before they integrate into the network (López-Bendito et al., 2008; Tanaka et al., 2009). Like interneurons, TCAs travel long distances before reaching their destination. Murine TCAs navigate through the ventral telencephalon for 2 d and reach the cortex ~E14. Until E15.5, they traverse the cortex in the SVZ and intermediate zone (IZ) to form contacts with the subplate, where they pause for several days before growing into the cortical plate (CP) (López-Bendito and Molnár, 2003; Mire et al., 2012). Thus, the peak of cortical neurogenesis, taking place from E12 to E16 in mice (Hevner et al., 2004), coincides with intracortical TCA growth and interneuron dispersion.

Cortical basal/intermediate progenitor cells (IPCs) generate a significant proportion of PN (Kowalczyk et al., 2009; Vasistha et al., 2014) and enable evolutionary expansion of the cortex by amplification of cell numbers (Martínez-Cerdeño et al., 2006). Sufficient interneurons and TCAs have to be integrated into the fast growing cortex, demanding signal mechanisms that coordinate PN production with progression of invading elements. Several findings suggest that the chemokine CXCL12 (stromal cell-derived factor-1) via its receptors CXCR4 and CXCR7 acts as such a coordinating signal. First, in addition to the meninges, CXCL12 is highly expressed in IPCs and guides migrating interneurons in the cortex (Stumm et al., 2003; Tiveron et al., 2006; Stumm and Holtt, 2007; Li et al., 2008; Lysko et al., 2011; Sánchez-Alcañiz et al., 2011; Wang et al., 2011; Abe et al., 2014). Second, we observed that CXCR4 is highly expressed in the forming thalamus and TCAs. In analogy to the CXCL12/CXCR4 axis defining the initial trajectories of motor axons growing out of the neural tube (Lieberman et al., 2005), CXCL12 might similarly be involved in the formation of the thalamocortical projection.

Despite the well documented role of CXCL12 in cortical development, the specific contribution of IPC-derived CXCL12 has not been dissected. In this report, we used explant experiments and a wealth of histochemical and fluorescent dye labeling technology in a series of mouse genetic models including conditional *Cxcl12* ablation in IPCs. We thus provide conclusive evidence

Table 1. Primary antibodies

Primary antibody	Concentration	Source
Rabbit anti-GFP	1:500	Synaptic Systems #132002
Chicken anti-GFP	1:500	Aves Labs #GFP-1020
Chicken anti-neurofilament	1:10,000; enhanced*	Aves Labs #NFM (160 kDa)
Goat anti-SOX2	1:200	R&D Systems #AF2018
Rabbit anti-L1	1:400	provided by Dr Fritz Rathjen (Berlin)
Rat anti-CXCR4 (2B11)	1:1000; enhanced*	BD Bioscience #551968
Rabbit anti-CXCR4 (UMB-2)	1:2; enhanced*	provided by Dr Stefan Schulz (Jena)

*Biotin-tyramine amplification.

Figure 1. Patterns of *Cxcr4*, *Cxcr7*, and *Cxcl12* in the early thalamus. **A–O**, Darkfield micrographs of emulsion-dipped coronal sections show the thalamus after *in situ* hybridization with ³⁵S-labeled probes for *Cxcr4* (**A, F, K**), *Cxcl12* (**B, G, L**), *Cxcr7* (**C, H, M**), and *Gbx2* (**D, I, N**) at E12.5 (**A–E**), E13.5 (**F–J**), and E14.5 (**K–O**). **E, J, O**, Overlays of false color displays of the adjacent sections shown in **A, D, F, I**; and **K, N, A, E, F, J, K, O**, *Cxcr4* is expressed in the progenitor domain along the midline and in the *Gbx2*⁺ differentiation area of the thalamus. Arrowheads in **E, J**, and **O** point to *Cxcr4*-expressing lateral thalamic nuclei. **B, G, L**, *Cxcl12* is present in the meninges covering the thalamus. **C, H, M**, *Cxcr7* is expressed in the progenitor domain along the midline. **A, C, F, H**, Arrows point to the *zona limitans intrathalamica* for orientation. **P–P''**, Confocal images show immunostained GFP and SOX2 in a coronal section of an E14.5 *Cxcr4*-GFP reporter mouse. 3V, Third ventricle; Hi, hippocampal anlage; Th, thalamus. Scale bars: **D, I, N, P'**, 500 μm.

that CXCL12, emanating from IPCs, promotes intracortical TCA growth and interneuron migration in the SVZ.

Materials and Methods

Animals. Animal procedures were in accordance with German and EU guidelines. *Cxcr4*-GFP and CXCL12-RFP mice (Bhattacharyya et al., 2008) were on CD1 background; *Cxcl12*^{+/-} (Nagasawa et al., 1996), *Cxcl12*^{LoxP} (Tzeng et al., 2011), and *Tbr2*^{+/-Cre} mice (Costello et al., 2011)

Figure 2. Transient *Cxcr4* expression in the thalamic mantle zone. **A, B, D**, Dual *in situ* hybridization for *Cxcr4/Sox2* (**A, B**) and *Cxcr4/Gbx2* (**D**) at E13.5 (**A**) and E14.5 (**B, D**). **A, B, D**, Bright-field views and **A', B', D'** are dark-field views of the same specimens. **A, B**, Horizontal and **D** is a coronal section. *Cxcr4* was detected with a ^{35}S -labeled probe (white signal in **A', B', D'**); *Sox2* and *Gbx2* were detected with digoxigenin (DIG)-labeled probes (**A, B, D**, blue signal). *Cxcr4* is expressed in the *Sox2* $^{+}$ and *Gbx2* $^{+}$ thalamic mantle zone. **C**, The H&E-stained coronal section shows the diencephalon, the box corresponds to the thalamic area shown in **D**. **E–G**, Boxes in the H&E-stained coronal sections correspond to the thalamic area shown in **H–P**. Images show the thalamus at rostral (top), mid (middle), and caudal levels (bottom). **H–P**, Dark-field micrographs of emulsion-dipped coronal sections after *in situ* hybridization with ^{35}S -labeled probes for *Cxcr4* (**H–J**), *Cxcl12* (**K–M**), and *Cxcr7* (**N–P**). 3V, Third ventricle; IC, internal capsule; dLG, dorsal lateral geniculate; GE, ganglionic eminence; Hi, hippocampus; HTh, hypothalamus; Th, thalamus. Scale bars: **A, B, D**, 200 μm ; **C, H–J**, 500 μm ; **E–G**, 1 mm.

were on C57BL/6J background. All embryos examined in this study were collected regardless of sex.

Histology and DiI tracing. Established *in situ* hybridization and immunohistochemical procedures with previously characterized probes and antibodies were used (Stumm et al., 2002; Sánchez-Alcañiz et al., 2011; Suzuki-Hirano et al., 2011). A *Sox2* probe (GI:127140985, nucleotides 418–1681) was generated by RT-PCR cloning. Primary antibodies are listed in Table 1. Axon tracing was performed as described previously (Molnár et al., 1998).

Thalamic explant cultures. Thalami were prepared from E13.5 *Cxcr4*-GFP $^{+}$ brains and cut into $300 \times 300 \mu\text{m}^2$ pieces using a McIlwain tissue chopper. Explants were placed onto collagen-coated coverslips, embedded in collagen (3 mg/ml, BD Biosciences) and cultured in Neurobasal Medium (Thermo Fisher Scientific) supplemented with 2 mM glutamine, 5% FBS, and 100 U/ml Pen-Strep (all PAA). CXCL12 (Peprotech) and AMD3100 (Sigma-Aldrich) were added immediately after plating. Native GFP was imaged after 48 h in fixed explants.

Image acquisition and analysis. Confocal images were captured with an LSM510 Meta; other micrographs were taken using an Axio Imager A1 (Zeiss). Throughout the study, *Cxcl12* $^{-/-}$ mice were compared with control littermates (*Cxcl12* $^{+/+}$ or *Cxcl12* $^{+/-}$). All measurements (thalamus area, intracortical TCA length, and cell counts) were normalized to mean values of litter controls. The area of the developing thalamus was determined based on *Gbx2*, *Sox2*, and *Slc17a6* patterns using ImageJ. Intracortical TCA length was determined using ImageJ as illustrated in Results (see Fig. 6F). Quantification of interneuron distribution in the cortex has been described (Abe et al., 2014). Axon outgrowth from thalamus explants was quantified by measuring the 20 longest axons in each explant with ImageJ using the Neuron plug-in (illustrated in Fig. 6G). In each experimental run, axon length was normalized to control mean of the respective run. Statistics were calculated and plotted with GraphPad Prism and IBM SPSS software. Adobe Photoshop and InDesign CS6 were used to process and arrange figures.

Results

Meninges are the primary CXCL12 source for early CXCR4-expressing thalamic neurons

Little is known about the expression of the *Cxcl12*, *Cxcr4*, and *Cxcr7* genes in the developing thalamus. We thus analyzed spatiotemporal expression profiles of these genes by highly sensitive *in situ* hybridization with radiolabeled probes, focusing on the *Gbx2* $^{+}$ domain in the developing diencephalon. This domain corresponds to the thalamic mantle zone (tMZ) and is composed of postmitotic glutamatergic neurons, which project to the cortex and constitute the nuclear complex that is traditionally viewed as the thalamus (Chatterjee and Li, 2012). First, we examined E12.5, E13.5, and E14.5 embryos (Fig. 1A–O), because most thalamic neurons are generated during this period (Suzuki-Hirano et al., 2011). Adjacent sections revealed strong expression of *Cxcr4* and *Cxcr7* in the progenitor domain along the third ventricle at E12.5 and E13.5. In contrast, the tMZ expressed *Cxcr4* but not *Cxcr7* at this early stage of thalamic development (Fig. 1A, C, E, F, H, J). At E14.5, the lateral tMZ still exhibited contiguous strong *Cxcr4* signal and virtually no *Cxcr7* signal, whereas the medial tMZ contained scattered *Cxcr4* $^{+}$ cells and uniform faint *Cxcr7* signal (Fig. 1K, M, O). *Cxcl12* was expressed in the meninges covering the thalamus but, apart from some forming blood vessels, was not detected within the thalamus during E12.5–E14.5 (Fig. 1B, G, L). We confirmed localization of *Cxcr4* in the thalamus by dual labeling with the thalamic marker SOX2 (Vue et al., 2007). Specifically, dual immunofluorescence for GFP and SOX2 showed that the entire SOX2 $^{+}$ domain was *Cxcr4*-GFP $^{+}$ at E14.5 (Fig. 1P–P'). Because of its slow turnover, GFP traces back brain structures that expressed the reporter during the preceding days. Thus, the *Cxcr4*-GFP pattern at E14.5 corresponds to the finding that vir-

Figure 3. CXCL12 activates CXCR4 receptors in the developing thalamus but is dispensable for thalamus formation. **A–E**, Confocal images show immunohistochemistry for inactive/nonphosphorylated CXCR4 (CXCR4^{inact}; **A, B, D, E**) and total CXCR4 (CXCR4; **C**) in coronal thalamus sections. **A**, Overlay of immunostained GFP, CXCR4^{inact}, and DAPI in an E13.5 *Cxcr4*-GFP reporter mouse shows CXCR4 in the thalamocortical projection (arrowheads). Note that the lateral thalamus (asterisk) contains only sparse CXCR4^{inact} signal despite being *Cxcr4*-GFP⁺. **B, C**, The lateral thalamus (asterisk) shows scant signal for CXCR4^{inact} (**B**) but strong signal for CXCR4 (**C**). Sections were counterstained with DAPI. **D, E**, Immunohistochemistry for CXCR4^{inact} in an E14.5 *Cxcl12*^{+/+} (control; **D**) and a *Cxcl12*^{-/-} littermate (**E**) demonstrates that CXCR4^{inact} increases in the lateral thalamus (asterisks) in the absence of CXCL12. **F, G**, Dark-field micrographs of emulsion-dipped coronal sections through the thalamus of an E14.5 *Cxcl12*^{+/+} and a *Cxcl12*^{-/-} littermate after *in situ* hybridization with a ³⁵S-labeled probe for *Sox2*. **H**, Matching thalamus sections, cut at five rostrocaudal sectional levels in E14.5 control (*n* = 3) and *Cxcl12*^{-/-} littermates (*n* = 3), were hybridized for *Gbx2* (sectional levels 1, 3, and 5) and *Sox2* (sectional levels 2 and 4) as thalamus markers. The marker⁺ area was determined in micrographs of emulsion-dipped sections after setting a threshold as shown in the insets to the right (signal above threshold appears black). **I, J**, Confocal images show SOX2-immunoreactivity in the thalamus of an E16.5 *Cxcl12*^{+/+} (**I**) and a *Cxcl12*^{-/-} littermate (**J**). **K**, The thalamus area was determined based on SOX2-immunoreactivity at a rostral and at a caudal sectional level in E16.5 control and *Cxcl12*^{-/-} littermates (*n* = 5 each). **L, M**, X-ray autoradiographs show *Slc17a6* (*Vglut2*) mRNA in hybridized coronal head sections of an E18.5 *Cxcl12*^{+/+} (**L**) and a *Cxcl12*^{-/-} littermate (**M**). **N**, The *Slc17a6* mRNA-positive area corresponding to thalamus and epithalamus was determined in x-ray autoradiographs at a rostral and at a caudal sectional level in E18.5 *Cxcl12*^{+/+} and *Cxcl12*^{-/-} littermates (*n* = 4 each). **H, K, N**, The thalamus area is not altered in *Cxcl12*^{-/-} mice (data are mean ± SEM). Th, Thalamus. Scale bars: **A, C, E, G, I**, 200 μm; **L, M**, 500 μm.

tually the entire forming thalamus expresses *Cxcr4* mRNA at E12.5 and E13.5. Dual *in situ* hybridization showed that at E14.5 *Cxcr4* mRNA was still expressed in *Sox2*⁺ and *Gbx2*⁺ parts of the thalamus (Fig. 2*A–D*).

Next, we examined stages during which thalamic nuclei begin to differentiate (E16.5, E17.5, P0; demonstrated for E17.5 in Fig. 2*E–P*). We observed that *Cxcr4* was progressively downregulated while *Cxcl12* and *Cxcr7* were upregulated in the thalamus. Moderate to faint contiguous *Cxcr4* signal could be detected until E17.5 in dorsolateral nuclei. Scattered, strongly *Cxcr4*⁺ cells were present throughout the thalamus at all stages.

Our gene expression analyses suggest that CXCR4 is the predominantly expressed CXCL12 receptor in early postmitotic thalamocortical neurons and that the meninges are the primary diencephalic CXCL12 source. To test this assumption, we studied CXCR4 receptor activation, making use of UMB-2 and 2B11 anti-CXCR4 antibodies. UMB-2 recognizes its epitope within the amino acid residues 343–352 only when the serine cluster S346–8 is unphosphorylated. As this cluster becomes immediately phosphorylated upon CXCL12 stimulation (Mueller et al., 2013), UMB-2 binds only inactive CXCR4 receptors (Fig. 3*A, B, D, E*; CXCR4^{inact}). 2B11 is directed against the CXCR4 N terminus and recognizes CXCR4 receptors independently of their activation

state (Fig. 3*C*; CXCR4). By applying these antibodies to adjacent sections from E13.5 *Cxcr4*-GFP animals, we found that 2B11 and UMB-2 generated staining medially in the *Cxcr4*-GFP⁺ thalamus, whereas only 2B11 produced staining laterally in the *Cxcr4*-GFP⁺ thalamus (Fig. 3*A–C*; asterisks identify the lateral thalamus). To prove that lack in UMB-2 signal reflects CXCR4 activation in the lateral thalamus, we compared thalamic UMB-2 staining in E14.5 *Cxcl12*^{-/-} and control littermates. In the absence of CXCL12, there was a profound increase in UMB-2 signal in the lateral thalamus (Fig. 3*D, E*, asterisks). These findings indicate active signaling of the CXCL12/CXCR4 pathway in early thalamic neurons.

Development of the thalamus in *Cxcl12*^{-/-} mice

We then asked whether CXCL12 is required for thalamus formation. We thus examined thalamic size and shape in *Cxcl12*^{-/-} mice at different developmental stages. At E14.5, we alternately hybridized five sections at different rostrocaudal planes for *Gbx2* and *Sox2* to identify the boundaries between thalamus and epithalamus (*Gbx2*) and thalamus and prethalamus (*Sox2*; Vue et al., 2007). The thalamic area was defined as shown in Figure 3*H*, insets. We found that size and shape of the *Gbx2* and *Sox2* expression domains were not altered in *Cxcl12*^{-/-} mice (Fig. 3*F–H*).

Figure 4. *Cxcr4*-GFP identifies TCAs. **A–B''**, Confocal images show immunostained GFP and the axonal marker L1 in an E14.5 *Cxcr4*-GFP reporter mouse. *Cxcr4*-GFP labels TCAs identified by L1. Presumptive GFP⁺ interneurons (**B**, arrowheads) are L1-negative. **C**, Confocal image of a *Cxcr4*-GFP reporter mouse shows that GFP⁺ TCAs have traversed the internal capsule (IC) and reached the cortical SVZ at E13.5. **D, E**, Dark-field micrographs of emulsion-dipped coronal sections at E13.5 (**D**) and E14.5 (**E**) after *in situ* hybridization with a ³⁵S-labeled probe for *Cxcl12*. *Cxcl12* is expressed in the cortical SVZ, subplate (SP) and meninges (Mn) covering thalamus and cortex. **F**, Schematic summarizing patterns of *Cxcr4*-GFP and *Cxcl12* mRNA at E13.5 and E14.5. Arrows indicate putative cortical and arrowheads putative meningeal stimulation sites of *Cxcr4*⁺ TCAs and thalamic neurons, respectively. **G**, Dissociated thalamic neurons were immunostained for SOX2 and CXCR4 and counterstained with DAPI. Note CXCR4 in the neurite and the growth-cone. 3V, Third ventricle; Ctx, cortex; GE, ganglionic eminence; Hi, hippocampal anlage; latV, lateral ventricle; Th, thalamus; VZ, ventricular zone. Scale bars: **A', B', C–E**, 200 μ m; **G''** 20 μ m.

The thalamic boundaries were sharply delineated in the mutants and appeared similar to controls (shown for *Sox2* in Fig. 3*F, G*). We then immunostained for SOX2 at E16.5 (Fig. 3*L–K*) and hybridized for vesicular glutamate transporter 2 (*Vglut2/Slc17a6*; Fig. 3*L–N*) at E18.5. Again, we found the labeled areas to be of normal size and shape in *Cxcl12*^{−/−} embryos, suggesting that the thalamus forms independently of CXCL12. However, because *Gbx2*, *Sox2*, and *Vglut2* do not resolve thalamic subnuclei, these findings do not preclude the possibility that genesis of distinct thalamic nuclei might require CXCL12.

CXCR4⁺ TCAs traverse a *Cxcl12*-expressing domain in the cortical SVZ

Having identified CXCR4 in neurons forming thalamocortical projections, we wondered whether CXCR4 is functionally involved in TCA development. To address this question, we used the *Cxcr4*-GFP reporter for visualization of TCAs emerging from *Cxcr4*-expressing neurons. Coimmunostaining for GFP and the axonal marker L1 revealed *Cxcr4*-GFP/L1⁺ axons originating in

the thalamus and extending through the internal capsule toward the cortex (Fig. 4*A–B''*). GFP patterns in the E14.5 cortex (Fig. 4*B–B''*) were consistent with presence of *Cxcr4*-GFP in axons, interneurons, and Cajal–Retzius cells, and absence of *Cxcr4*-GFP from cortical PN (Stumm et al., 2003). These findings establish that *Cxcr4*-GFP specifically labels TCAs and rule out the possibility that corticothalamic axons are labeled.

Next, we examined where along the TCA trajectory *Cxcl12* is expressed during E12.5–E14.5 by comparing patterns of *Cxcr4*-GFP and *Cxcl12* mRNA. Areas in the ventral thalamus, prethalamus, internal capsule, and ventral telencephalon were traversed by *Cxcr4*-GFP⁺ TCAs before E13.5 but exhibited only very sparse *Cxcl12* signal (shown for E13.5 in Fig. 4*C, D*). By E13.5, *Cxcr4*-GFP⁺ TCAs reached the pallial–subpallial boundary and started to enter the *Cxcl12*-expressing domain in the cortical SVZ (Fig. 4*C, D, F*). Between E13.5 and E14.5, *Cxcr4*-GFP⁺ TCAs crossed the *Cxcl12*-expressing SVZ and progressed considerably within the cortex, covering more than one-half of its lateromedial extent (Fig. 4*A–F*). TCA growth dynamics observed with *Cxcr4*-GFP are consistent with findings by DiI tracing (Auladell et al., 2000).

A prerequisite for a direct influence of CXCL12 on TCAs is the presence of CXCR4 receptors in the axonal compartment. We observed prominent CXCR4 immunolabeling in *Cxcr4*-GFP⁺ TCAs entering the internal capsule (Fig. 3*A*, arrowheads). The receptor could also be identified in the distal axonal compartment (data not shown) but the signal was less intense than proximally, where TCAs are bundled. To confirm that CXCR4 is targeted toward the growth cone of neu-

rites extending from thalamic neurons, we immunostained CXCR4 and SOX2 in thalamic cultures. This revealed CXCR4 presence at the soma, neurite, and growth cone of SOX2⁺ thalamic neurons (Fig. 4*G–G''*). Together, these findings prompt to suggest that the thalamic meninges and the cortical SVZ are CXCL12 sources that influence TCA development through somatic and axonal CXCR4 stimulation, respectively.

CXCL12 promotes intracortical TCA elongation

To directly test whether CXCL12 is involved in TCA development, we traced the trajectories by DiI placement in control and *Cxcl12*^{−/−} E14.5 and E16.5 thalami (Fig. 5*A–H*). No misrouting of DiI-labeled TCAs was observed in the ventral thalamus, internal capsule, and ventral telencephalon of *Cxcl12*^{−/−} mice. However, labeled axons appeared less developed in the cortex of these mutants. Measurement of the intracortical length of DiI-labeled TCAs confirmed significantly delayed TCA growth in *Cxcl12*^{−/−} embryos compared with control littermates (E14.5, $p = 0.0003$ and E16.5, $p = 0.009$ for genotype, two-way ANOVA; Fig. 5*I, J*).

Figure 5. Deletion of *Cxcl12* attenuates intracortical TCA growth. **A–H**, Thalamic Dil labeling at E14.5 (**A, B, E, F**) and E16.5 (**C, D, G, H**) shown in coronal sections at a rostral and a mid-sectional level in control (**A–D**) and *Cxcl12*^{-/-} embryos (**E–H**). Arrowheads point to positions of the most advanced Dil-labeled TCAs. **I, J**, Mean and SEM of relative intracortical length of Dil-labeled TCAs in E14.5 (**I**) and E16.5 (**J**) control embryos and *Cxcl12*^{-/-} littermates at a rostral and mid-sectional level. **K–P**, Confocal images show immunostained GFP in control (**K–M**) and *Cxcl12*^{-/-} (**N–P**) E14.5 *Cxcr4*-GFP reporter mice at rostral (**K, N**), mid (**L, O**), and caudal sectional planes (**M, P**). Arrowheads point to positions of the most advanced GFP⁺ TCAs. **Q**, Mean and SEM of relative intracortical length of *Cxcr4*-GFP⁺ TCAs in control and *Cxcl12*^{-/-} E14.5 *Cxcr4*-GFP reporter mice at rostral, mid, and caudal sectional planes. Statistics in **I, J, Q**: ^{SSS} $p < 0.001$, ^{SS} $p < 0.01$ for genotype (two-way ANOVA). ^{**} $p < 0.01$, ^{*} $p < 0.05$ (Bonferroni's *post hoc* test). Numbers of analyzed embryos are indicated. GE, Ganglionic eminence; Hi, hippocampal anlage; latV, lateral ventricle. Scale bars: **H, M**, 200 μ m.

Length reductions by 55.0% at the rostral sectional level at E14.5 ($p < 0.01$) and by 31.0% at the mid-sectional level at E16.5 ($p < 0.05$) were significant in the *post hoc* test.

To focus specifically on *Cxcr4*⁺ TCAs, we crossed *Cxcr4*-GFP into the *Cxcl12*^{+/-} mouse line and generated *Cxcr4*-GFP; *Cxcl12*^{-/-} mutant embryos and *Cxcr4*-GFP control littermates (Fig. 5*K–P*). Length measurement of GFP⁺ fibers in the E14.5 cortex at three rostrocaudal sectional levels showed an overall growth reduction in the mutants compared with control littermates ($p = 0.0004$ for genotype, two-way ANOVA; Fig. 5*Q*). Length reduction by 31.8% at the rostral sectional level reached significance in the *post hoc* test ($p < 0.01$). The delayed growth was not accompanied by misrouting of *Cxcr4*-GFP⁺ TCAs in *Cxcr4*-GFP; *Cxcl12*^{-/-} embryos.

Next, we tested whether CXCL12 overexpression accelerates TCA development. We thus generated double-transgenic mice expressing *Cxcr4*-GFP and a CXCL12-RFP fusion protein under control of the *Cxcl12* promoter (CXCL12-RFP). In CXCL12-RFP mice, additional *Cxcl12* gene copies result in increased *Cxcl12* mRNA expression (Fig. 6*A, B*), and hence, in an increase of functional CXCL12. Immunofluorescent colabeling of *Cxcr4*-GFP and neurofilament (NF) as a TCA marker in E15.5 *Cxcr4*-GFP control and CXCL12-RFP; *Cxcr4*-GFP double-transgenic mice revealed accelerated TCA growth following CXCL12 overexpression (Fig. 6*C, D*). Remarkably, in an area corresponding to the visual cortex of CXCL12-RFP embryos, TCAs entered the CP prematurely and even targeted the MZ (Fig. 6*D*, asterisks). Measurement of intracortical TCA length at seven rostrocaudal sectional planes confirmed increased TCA length in CXCL12-RFP animals ($p = 0.0001$ for genotype, two-way ANOVA; Fig. 6*E*). An

increase in intracortical TCA length by 28% in the second sectional plane was significant in the *post hoc* test ($p < 0.05$). These gain and loss of function experiments establish that CXCL12 promotes intracortical progression of TCAs.

CXCR4 mediates a growth-promoting effect of CXCL12 on TCAs

Defective CXCL12/CXCR4 signaling affects the development of cortical cell populations including interneurons and Cajal-Retzius cells (Stumm and Holtt, 2007). Thus, impaired intracortical TCA growth in *Cxcl12*^{-/-} mice may be caused indirectly by defects in cortical development or reflect absence of growth-promoting CXCR4 signals in TCAs. To discriminate between these possibilities, we examined axon outgrowth in E13.5 *Cxcr4*-GFP⁺ thalamic explant cultures (Fig. 6*G–K*). Stimulation with CXCL12 dose-dependently increased outgrowth of *Cxcr4*-GFP⁺ axons ($p < 0.0001$, ANOVA; Fig. 6*L*). Outgrowth was increased by 15.9% after 20 nM ($p > 0.05$) and by 33.7% after 40 nM CXCL12 ($p < 0.001$) versus the nonstimulated control. The CXCR4 antagonist AMD3100, added at 6 μ M, blocked the growth-promoting effect of 40 nM CXCL12 ($p < 0.01$) but had no significant effect when applied alone. These findings suggest that a CXCL12/CXCR4 pathway exerts a direct growth-promoting effect on TCAs.

IPC-derived CXCL12 ensures efficient TCA growth and interneuron dispersion

We then asked, whether SVZ-derived CXCL12 is sufficient to promote TCA growth. To address this, we deleted *Cxcl12* selectively in IPCs using *Thr2*^{Cxcl/+}-mediated excision of the CXCL12-

Figure 6. A CXCL12/CXCR4 pathway accelerates intracortical TCA growth. **A, B**, Dark-field views of the cerebral cortex at E14.5 after *in situ* hybridization with a ³⁵S-labeled probe for *Cxcl12*. Comparison of a control (**A**) and a CXCL12-transgenic littermate overexpressing CXCL12 under the *Cxcl12* promoter (**B**) reveals increased *Cxcl12* mRNA expression in the transgenic animal. **C, D**, Confocal images show a *Cxcr4*-GFP transgenic control (**C**) and a *Cxcr4*-GFP;CXCL12-RFP transgenic littermate at E15.5 (**D**). Immunostained NF is white in **C** and **D**, and red in **C'**, **C''**, **D'**, and **D''**. GFP is green in **C'**, **C''**, **D'**, and **D''**. DAPI is shown in blue in **C, D, C'**, and **D'**. Arrowheads point to the location of the most advanced stained TCAs; asterisks point to TCAs crossing the CP prematurely (**D, D'**). **E**, Mean and SEM of relative intracortical length of *Cxcr4*-GFP⁺ TCAs in *Cxcr4*-GFP and *Cxcr4*-GFP;CXCL12-RFP E15.5 mice in seven sectional planes along the rostrocaudal axis. **F**, Scheme illustrating measurement of intracortical TCA length. **G**, Scheme illustrating experimental procedure of explant culturing. Thalamus explants were prepared from E13.5 *Cxcr4*-GFP mice and cultured for 2 d in medium supplemented with vehicle (control), 20 nM CXCL12, 40 nM CXCL12, or 6 μM CXCR4 antagonist AMD3100. In each explant, length (l) of the 20 longest axons was measured, normalized to control and expressed as mean ± SEM. **H–K**, Representative *Cxcr4*-GFP⁺ thalamus explants receiving vehicle (**H**), 40 nM CXCL12 (**I**), AMD3100 (**J**), and 40 nM CXCL12 + AMD3100 (**K**). **L**, CXCL12 dose-dependently stimulates axonal growth in thalamus explants (for each group, the number of analyzed explants is given in the corresponding bar). Statistics, **E**: ^{§§§}*p* < 0.001 for genotype (two-way ANOVA), **p* < 0.05 (Bonferroni's *post hoc* test); **L**: ****p* < 0.001, ***p* < 0.01 (one-way ANOVA and Tukey's *post hoc* test). Data are mean ± SEM. Ctx, Cortex; GE, ganglionic eminence; Hi, hippocampal anlage; latV, lateral ventricle; Mn, meninges; Th, thalamus. Scale bars: **A, C** (inset), **C', C'', D** (inset), **H, I**, 200 μm.

encoding exon2 in *Cxcl12*^{LoxP/−} mice (*Cxcl12cKO*^{Tbr2-Cre}; Costello et al., 2011; Tzeng et al., 2011). *In situ* hybridization with an exon2-selective probe confirmed that *Cxcl12* expression was indeed disrupted in the SVZ and intact in the meninges in E14.5 *Cxcl12cKO*^{Tbr2-Cre} mice (Fig. 7*A, B*). To visualize *Cxcr4*-expressing TCAs in these mutants, we generated E14.5 *Cxcr4*-GFP;*Cxcl12cKO*^{Tbr2-Cre} mice by crossbreeding *Tbr2*^{Cre} driver, *Cxcr4*-GFP reporter and *Cxcl12*^{LoxP/−} mice (Fig. 7*C, D*). Intracortical length of *Cxcr4*-GFP⁺ TCAs, determined at three rostrocaudal sectional levels, was reduced in the conditional mutants compared with littermate controls (*p* < 0.0001 for genotype, two-way ANOVA; Fig. 7*E*). The *post hoc* test identified that TCAs were significantly shorter by 28, 21, and 25% at the rostral (*p* <

0.001), medial (*p* < 0.05), and caudal (*p* < 0.01) level in the conditional mutants.

Having shown that IPC-derived CXCL12 promotes elongation of TCAs in the SVZ, we wondered whether IPCs have a similar effect on interneurons. It is well established that the CXCL12/CXCR4/CXCR7 signaling module regulates interneuron migration in the cortex, but the respective contributions of meningeal- and IPC-derived CXCL12 to this process has not been dissected by targeting the *Cxcl12* gene selectively in one of these tissues. We thus examined interneuron positioning in *Cxcl12cKO*^{Tbr2-Cre} mice. As GFP⁺ TCAs mask the position of weakly GFP-stained interneurons in the cortex of *Cxcr4*-GFP mice, we hybridized for *Lhx6*, a marker of medial ganglionic

Figure 7. IPC-derived CXCL12 ensures efficient intracortical TCA growth and interneuron dispersion. **A, B**, Dark-field micrographs of emulsion-dipped E14.5 coronal brain sections after *in situ* hybridization with a ³⁵S-labeled probe corresponding to exon 2 of the *Cxcl12* gene (exon2 is loxP-flanked in *Cxcl12*^{loxP} mice). *Cxcl12* is expressed in the cortical SVZ of a *Tbr2*^{Cre/+}; *Cxcl12*^{loxP/+} (control, **A**) but not in the SVZ of a *Tbr2*^{Cre/+}; *Cxcl12*^{loxP/-} mouse (*Cxcl12cKO*, **B**). *Cxcl12* expression is intact in the meninges (Mn) of the *Cxcl12cKO*. **C, D**, Confocal images of immunostained GFP in the cortex at mid-rostral-caudal sectional level in E14.5 *Cxcl12cKO* and control mice carrying the *Cxcr4*-GFP reporter. Arrowheads pointing to the most advanced GFP⁺ TCAs demonstrate that GFP⁺ TCAs are more advanced in the control (**C**) than in the *Cxcl12cKO* (**D**). **E**, Mean and SEM of relative intracortical length of *Cxcr4*-GFP⁺ TCAs in control (*n* = 8) and *Cxcl12cKO* embryos (*n* = 7). **F, G**, Schemes illustrating the TCA phenotype in the *Cxcl12cKO*. **H, I**, Dark-field micrographs of emulsion-dipped coronal sections through the E14.5 cortex after *in situ* hybridization with a ³⁵S-labeled probe for *Lhx6*. Fewer *Lhx6*⁺ interneurons populate the SVZ (arrows) in the *Cxcl12cKO* (**I**) than in the control (**H**). Note *Lhx6*⁺ interneurons are absent in the dorsomedial SVZ in the *Cxcl12cKO* (**I**, bracket). **J, K**, Confocal images of immunostained CXCR4 (white) and DAPI (blue) in coronal cortical sections of E14.5 control (**J**) and *Cxcl12cKO* mice (**K**). Cortical bins are shown in **K**. There are fewer CXCR4⁺ interneurons in the SVZ (bins 5–8) and more CXCR4⁺ interneurons in upper cortical layers (bins 1–3) of the *Cxcl12cKO* (**K**) than in the corresponding areas of the control (**J**). More CXCR4⁺ interneurons exhibit oblique orientation in the *Cxcl12cKO* (**K**, arrowheads) than in the control, indicating abnormal, surface-directed migration of these cells. **L**, Distribution of CXCR4⁺ cells in the cortex of E14.5 control (*n* = 8) and *Cxcl12cKO* mice (*n* = 7). Data are mean ± SEM of CXCR4⁺ cells per area in percentage of all CXCR4⁺ cells. **M**, Scheme illustrating the interneuron phenotype in the *Cxcl12cKO*. Statistics: ^{§§§}*p* < 0.0001 for genotype (**E**) and bin/genotype interaction (**L**; two-way ANOVA). ****p* < 0.001, ***p* < 0.01, **p* < 0.05 (Bonferroni's *post hoc* test compared with control); *n* numbers as indicated. GE, ganglionic eminence; latV, lateral ventricle; Mn, meninges; SP, subplate; Th, thalamus; VZ, ventricular zone. Scale bars: **A**, 500 μm; **C, H, J**, 200 μm.

eminence-derived interneurons. In the two examined E14.5 *Cxcl12cKO*^{Tbr2-Cre} mice, the lateral SVZ contained fewer *Lhx6*⁺ cells than the corresponding region in control littermates (Fig. 7H,I, arrows). The medial SVZ of the conditional mutants was devoid of *Lhx6*⁺ cells, whereas it was readily populated by *Lhx6*⁺ cells in the controls (Fig. 7H,I, brackets). To confirm these observations, we immunostained cortices of E14.5 *Cxcl12cKO*^{Tbr2-Cre} mice for CXCR4 and counted stained cells in 10 cortical bins in a midcortical area (Fig. 7J–L). In *Cxcl12cKO*^{Tbr2-Cre} mice, there was a significant reduction in CXCR4⁺ cells in bins 5–8 corresponding to the SVZ/IZ (*p* < 0.001) and a significant increase in CXCR4⁺ cells in bin 1 (*p* < 0.001) and bins 2/3 (*p* < 0.01) corresponding to the MZ and CP, respectively (two-way ANOVA and *post hoc* test). Leading processes of CXCR4-immunoreactive

interneurons migrating in the SVZ/IZ in *Cxcl12cKO*^{Tbr2-Cre} mice often exhibited an oblique orientation (Fig. 7K, arrowheads), suggesting that these cells were heading toward the MZ. In contrast, CXCR4-immunoreactive interneurons migrating in the SVZ/IZ of control mice had a more tangential orientation. In conclusion, CXCL12 produced by IPCs ensures efficient TCA growth and interneuron dispersion in the SVZ/IZ (Fig. 7F,G,M).

Discussion

Although considerable progress has been made in identifying molecules that guide migrating cortical interneurons and the forming thalamocortical tract (Hernández-Miranda et al., 2010; Kolodkin and Tessier-Lavigne, 2011; Grant et al., 2012; Price et al., 2012), little is known about mechanisms ensuring that PNs,

Figure 8. Schematic summarizing CXCL12/CXCR4 signaling for TCA and interneuron progression. **A**, *Cxcl12*-expressing structures are shown in red and *Cxcr4*-expressing structures in green. **B**, **C**, *Cxcr4*-GFP⁺ TCAs reach the cortical SVZ at E13.5 and traverse most of the cortex between E13.5 and E14.5. **D**, **E**, Intracortical growth of *Cxcr4*-GFP⁺ TCAs is reduced in *Cxcl12*-deficient mice (*Cxcl12* KO; **D**) and in conditional mutants lacking CXCL12 in IPCs (*Cxcl12* cKO; **E**). **F**, Orthotopic CXCL12 overexpression promotes intracortical growth of *Cxcr4*-GFP⁺ TCAs and leads to premature CP invasion and abnormal targeting of the MZ. **G**, CXCR4-positive interneurons migrate in the MZ, subplate, and SVZ. **H**, In *Cxcl12*-deficient mice, interneurons accumulate in the CP/SP area. **I**, After *Cxcl12* ablation in IPCs, interneurons leave the SVZ and seek upper cortical layers. Mn, Meninges; SP, subplate; Th, thalamus.

produced in large numbers in the rapidly growing mammalian cortex, are sufficiently supplied with interneurons and TCAs. A large proportion of cortical PNs arises from IPCs residing in the SVZ (Kolodkin and Tessier-Lavigne, 2011; Vasistha et al., 2014). Intriguingly, the SVZ is traversed by migrating interneurons and ingrowing TCAs during the peak of cortical neurogenesis (Hevner et al., 2004; Wonders and Anderson, 2006; Mire et al., 2012). Findings in the present study are consistent with a role of IPC-derived CXCL12 in promoting intracortical TCA growth through a CXCR4 pathway. To our knowledge, this is the first report to provide evidence that CXCL12 regulates axonal growth within the developing mammalian brain. In addition, we demonstrate that lack of IPC-derived CXCL12 disrupts tangential migration of interneurons in the SVZ/IZ. Our findings imply that while amplifying cortical neurons (Martínez-Cerdeño et al., 2006), IPCs enable efficient progression of TCAs and interneurons by releasing CXCL12 (Fig. 8).

CXCL12 promotes axonal growth in the mammalian brain

Although CXCL12 effects on axonal growth and axonal guidance have been studied *in vitro* (Arakawa et al., 2003; Chalasani et al., 2003; Pujol et al., 2005; Heskamp et al., 2013), CXCL12 effects on axonal development within the mammalian brain were not previously resolved. We thus analyzed expression and function of the CXCL12/CXCR4/CXCR7 signaling module in the embryonic thalamus and the forming thalamocortical projection, one of the major intracerebral axonal tracts. We found that CXCR4 receptors are widely present in cell bodies and growing axons of thalamocortical projection neurons. The *Cxcr7* gene is tran-

scribed in the progenitor domain but not in the differentiation field of the forming thalamus. Examination of the CXCR4 activation state showed that CXCL12, most likely emanating from the meninges covering the thalamus, signals through CXCR4 to the cell bodies of thalamic neurons. Regions in the thalamus, diencephalon, prethalamus, and internal capsule that are traversed by TCAs are largely *Cxcl12*-negative, suggesting that CXCL12 is not involved in TCA guidance in these regions. Consistently, misrouting of TCAs is not observed in embryos lacking CXCL12. Instead, we present several lines of evidence that CXCL12 stimulates TCA growth. First, CXCL12 enhances axonal growth in thalamus explants through CXCR4. Second, orthotopic CXCL12 overexpression in CXCL12-RFP-transgenic mice leads to increased intracortical TCA growth. Third, intracortical TCA growth is severely attenuated in *Cxcl12*^{−/−} mice. As the thalamic *Gbx2* and *Sox2* expression domains, which give rise to thalamocortical relay neurons (Chatterjee and Li, 2012), appear normal in *Cxcl12*^{−/−} embryos, it seems unlikely that reduced TCA growth is due to defective thalamus formation. A previous study addressing the role of CXCL12 in axonal development in a mammalian system demonstrated that mesenchymal CXCL12 guides CXCR4⁺ motor axons once they grow out of the neural tube (Lieberman et al., 2005). Our finding that CXCL12 promotes intracerebral growth of long-ranged axons connecting the thalamus to the late-developing cortex thus unveils a new aspect of CXCL12 function.

Dynamic changes in *Cxcr4* expression in the forming thalamus

Our *in situ* hybridization results showed that virtually all neurons in the tMZ express *Cxcr4* during the early stage of thalamic development (E12.5–E13.5; Fig. 1A,F). This finding is consistent with a prominent GFP signal observed in the entire tMZ (Fig. 1P) and most intracortical TCAs (Fig. 6C',C'') in E15.5 *Cxcr4*-GFP mice, where accumulated GFP protein reflects *Cxcr4* expression of the preceding days. Also, pioneer axons emerging from the E12.5 thalamus were clearly *Cxcr4*-GFP⁺. Thus, it is conceivable that the vast majority of TCAs crossing the *Cxcl12*-expressing SVZ between E13.5 and E14.5 express CXCR4 and may respond to the CXCL12 signal they encounter there. This assumption is supported by our finding that selective ablation of *Cxcl12* in the SVZ results in retarded intracortical TCA growth at E14.5.

Between E14.5 and E17.5, *Cxcr4* expression ceases in the medial thalamus but is sustained in the lateral thalamus, suggesting that CXCL12 may continuously influence TCAs emerging from lateral nuclei including the dorsal lateral geniculate. In support of this, elevated CXCL12 levels in E15.5 CXCL12-RFP mice cause TCAs to abnormally seek the CP and MZ only in cortical areas that are innervated by lateral thalamic nuclei (Fig. 6D).

It was reported that the growth speed of TCAs is intrinsically regulated by spontaneous activity of thalamic neurons: during the early stage of thalamic development (E12.5–E14.5) high neuronal activity maintains high growth speed, whereas reduced neuronal activity correlates to slower TCA growth during the late stage (E14.5–E16.5; Mire et al., 2012). The E14.5 time point, when TCAs switch from fast to slower growth, corresponds to the time point when *Cxcr4* expression decreases in the tMZ. Given that CXCR4 is highly activated in the tMZ between E12.5 and E17.5, a link between CXCR4 signaling, spontaneous activity of thalamic neurons and TCA growth seems worth considering.

IPC-derived CXCL12 supports tangential migration in the SVZ

The MZ and SVZ/IZ are major routes of interneurons migrating tangentially in the cortex (Wonders and Anderson, 2006). *Cxcl12* mRNA is expressed in the meninges, in the SVZ, and to lesser extent, in the subplate (Stumm et al., 2003, 2007; Abe et al., 2014). The chemokine is attractant for interneurons and supports tangential migration in the cortex. Both CXCL12 receptors, CXCR4 and CXCR7, are involved in this process (Sánchez-Alcañiz et al., 2011; Wang et al., 2011; Abe et al., 2014). The prominent *Cxcl12* mRNA signal in the SVZ is localized in IPC (Tiveron et al., 2006). This is a unique constellation because *Cxcl12* expression in neuronal progenitors has not been reported in other regions of the embryonic brain. It has been proposed that CXCL12 from IPCs provides a molecular link between IPCs and interneurons (Tiveron et al., 2006; Sessa et al., 2010). This hypothesis was based on the observation that *Pax6* and *Tbr2* mutants exhibit reduced *Cxcl12* expression in the SVZ and abnormal interneuron migration. However, there are severe defects in cortical development in *Tbr2* and *Pax6* mutants and absence of these crucial factors affects expression of a large set of genes other than *Cxcl12*. It was therefore not clear whether perturbed interneuron migration in *Tbr2* and *Pax6* mutants is indeed caused by defective *Cxcl12* expression in IPCs (Tiveron et al., 2006; Arnold et al., 2008; Sessa et al., 2010; Georgala et al., 2011; Elsen et al., 2013). We now show that in mice with a selective deletion of *Cxcl12* in IPCs, interneurons deviate from their normal migration in the SVZ to seek the remaining meningeal CXCL12 signal, thus providing proof that IPC-derived CXCL12 is essential for interneuron migration in the SVZ.

Conclusion

Evolution of the cortex is characterized by dramatic expansion of cortical size in mammals. This is enabled by IPC-mediated amplification of cortical neurons. Our study demonstrates that IPC-derived CXCL12 promotes TCA growth and interneuron migration, providing evidence that paracrine signaling mechanisms exist, which coordinate neuronal amplification and invasion of the cortex by extrinsic cell and axon elements.

References

- Abe P, Mueller W, Schütz D, MacKay F, Thelen M, Zhang P, Stumm R (2014) CXCR7 prevents excessive CXCL12-mediated downregulation of CXCR4 in migrating cortical interneurons. *Development* 141:1857–1863. [CrossRef Medline](#)
- Arakawa Y, Bito H, Furuyashiki T, Tsuji T, Takemoto-Kimura S, Kimura K, Nozaki K, Hashimoto N, Narumiya S (2003) Control of axon elongation via an SDF-1alpha/Rho/mDia pathway in cultured cerebellar granule neurons. *J Cell Biol* 161:381–391. [CrossRef Medline](#)
- Arnold SJ, Huang GJ, Cheung AF, Era T, Nishikawa S, Bikoff EK, Molnár Z, Robertson EJ, Groszer M (2008) The T-box transcription factor Eomes/Tbr2 regulates neurogenesis in the cortical subventricular zone. *Genes Dev* 22:2479–2484. [CrossRef Medline](#)
- Auladell C, Pérez-Sust P, Supèr H, Soriano E (2000) The early development of thalamocortical and corticothalamic projections in the mouse. *Anat Embryol* 201:169–179. [CrossRef Medline](#)
- Bhattacharyya BJ, Banisadr G, Jung H, Ren D, Cronshaw DG, Zou Y, Miller RJ (2008) The chemokine stromal cell-derived factor-1 regulates GABAergic inputs to neural progenitors in the postnatal dentate gyrus. *J Neurosci* 28:6720–6730. [CrossRef Medline](#)
- Chalasani SH, Sabelko KA, Sunshine MJ, Littman DR, Raper JA (2003) A chemokine, SDF-1, reduces the effectiveness of multiple axonal repellents and is required for normal axon pathfinding. *J Neurosci* 23:1360–1371. [Medline](#)
- Chatterjee M, Li JY (2012) Patterning and compartment formation in the diencephalon. *Front Neurosci* 6:66. [CrossRef Medline](#)
- Costello I, Pimeisl IM, Dräger S, Bikoff EK, Robertson EJ, Arnold SJ (2011) The T-box transcription factor Eomesodermin acts upstream of *Mesp1* to specify cardiac mesoderm during mouse gastrulation. *Nat Cell Biol* 13:1084–1091. [CrossRef Medline](#)
- Elsen GE, Hodge RD, Bedogni F, Daza RA, Nelson BR, Shiba N, Reiner SL, Hevner RF (2013) The protomap is propagated to cortical plate neurons through an Eomes-dependent intermediate map. *Proc Natl Acad Sci U S A* 110:4081–4086. [CrossRef Medline](#)
- Georgala PA, Carr CB, Price DJ (2011) The role of Pax6 in forebrain development. *Dev Neurobiol* 71:690–709. [CrossRef Medline](#)
- Grant E, Hoerder-Suabedissen A, Molnár Z (2012) Development of the corticothalamic projections. *Front Neurosci* 6:53. [CrossRef Medline](#)
- Hernández-Miranda LR, Parnavelas JG, Chiara F (2010) Molecules and mechanisms involved in the generation and migration of cortical interneurons. *ASN Neuro* 2:e00031. [CrossRef Medline](#)
- Heskamp A, Leibinger M, Andreadaki A, Gobrecht P, Diekmann H, Fischer D (2013) CXCL12/SDF-1 facilitates optic nerve regeneration. *Neurobiol Dis* 55:76–86. [CrossRef Medline](#)
- Hevner RF, Daza RA, Englund C, Kohtz J, Fink A (2004) Postnatal shifts of interneuron position in the neocortex of normal and reeler mice: evidence for inward radial migration. *Neuroscience* 124:605–618. [CrossRef Medline](#)
- Kolodkin AL, Tessier-Lavigne M (2011) Mechanisms and molecules of neuronal wiring: a primer. *Cold Spring Harb Perspect Biol* 3:a001727. [CrossRef Medline](#)
- Kowalczyk T, Pontious A, Englund C, Daza RA, Bedogni F, Hodge R, Attardo A, Bell C, Huttner WB, Hevner RF (2009) Intermediate neuronal progenitors (basal progenitors) produce pyramidal-projection neurons for all layers of cerebral cortex. *Cereb Cortex* 19:2439–2450. [CrossRef Medline](#)
- Li G, Adesnik H, Li J, Long J, Nicoll RA, Rubenstein JL, Pleasure SJ (2008) Regional distribution of cortical interneurons and development of inhibitory tone are regulated by Cxcl12/Cxcr4 signaling. *J Neurosci* 28:1085–1098. [CrossRef Medline](#)
- Lieberman I, Agalliu D, Nagasawa T, Ericson J, Jessell TM (2005) A Cxcl12-CXCR4 chemokine signaling pathway defines the initial trajectory of mammalian motor axons. *Neuron* 47:667–679. [CrossRef Medline](#)
- López-Bendito G, Molnár Z (2003) Thalamocortical development: how are we going to get there? *Nat Rev Neurosci* 4:276–289. [CrossRef Medline](#)
- López-Bendito G, Sánchez-Alcañiz JA, Pla R, Borrell V, Picó E, Valdeolmillos M, Marín O (2008) Chemokine signaling controls intracortical migration and final distribution of GABAergic interneurons. *J Neurosci* 28:1613–1624. [CrossRef Medline](#)
- Lysko DE, Putt M, Golden JA (2011) SDF1 regulates leading process branching and speed of migrating interneurons. *J Neurosci* 31:1739–1745. [CrossRef Medline](#)
- Martínez-Cerdeño V, Noctor SC, Kriegstein AR (2006) The role of intermediate progenitor cells in the evolutionary expansion of the cerebral cortex. *Cereb Cortex* 16:i152–161. [CrossRef Medline](#)
- Mire E, Mezzera C, Leyva-Díaz E, Paternain AV, Squarzone P, Bluy L, Castillo-Paterna M, López MJ, Peregrín S, Tessier-Lavigne M, Garel S, Galcerán J, Lerma J, López-Bendito G (2012) Spontaneous activity regulates Robo1 transcription to mediate a switch in thalamocortical axon growth. *Nat Neurosci* 15:1134–1143. [CrossRef Medline](#)
- Molnár Z, Adams R, Goffinet AM, Blakemore C (1998) The role of the first postmitotic cortical cells in the development of thalamocortical innervation in the reeler mouse. *J Neurosci* 18:5746–5765. [Medline](#)
- Mueller W, Schütz D, Nagel F, Schulz S, Stumm R (2013) Hierarchical organization of multi-site phosphorylation at the CXCR4 C terminus. *PLoS one* 8:e64975. [CrossRef Medline](#)
- Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, Kikutani H, Kishimoto T (1996) Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature* 382:635–638. [CrossRef Medline](#)
- Parnavelas JG (2000) The origin and migration of cortical neurones: new vistas. *Trends Neurosci* 23:126–131. [CrossRef Medline](#)
- Price DJ, Clegg J, Duocastella XO, Willshaw D, Pratt T (2012) The importance of combinatorial gene expression in early mammalian thalamic patterning and thalamocortical axonal guidance. *Front Neurosci* 6:37. [CrossRef Medline](#)
- Pujol F, Kitabgi P, Boudin H (2005) The chemokine SDF-1 differentially

- regulates axonal elongation and branching in hippocampal neurons. *J Cell Sci* 118:1071–1080. [CrossRef Medline](#)
- Rubenstein JL (2011) Annual research review: development of the cerebral cortex: implications for neurodevelopmental disorders. *J Child Psychol Psychiatry* 52:339–355. [CrossRef Medline](#)
- Sánchez-Alcañiz JA, Haeghe S, Mueller W, Pla R, Mackay F, Schulz S, López-Bendito G, Stumm R, Marín O (2011) Cxcr7 controls neuronal migration by regulating chemokine responsiveness. *Neuron* 69:77–90. [CrossRef Medline](#)
- Sessa A, Mao CA, Colasante G, Nini A, Klein WH, Broccoli V (2010) Tbr2-positive intermediate (basal) neuronal progenitors safeguard cerebral cortex expansion by controlling amplification of pallial glutamatergic neurons and attraction of subpallial GABAergic interneurons. *Genes Dev* 24:1816–1826. [CrossRef Medline](#)
- Stumm R, Höllt V (2007) CXCR4 chemokine receptor 4 regulates neuronal migration and axonal pathfinding in the developing nervous system: implications for neuronal regeneration in the adult brain. *J Mol Endocrinol* 38:377–382. [CrossRef Medline](#)
- Stumm RK, Rummel J, Junker V, Culmsee C, Pfeiffer M, Kriegstein J, Höllt V, Schulz S (2002) A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: isoform-selective regulation of SDF-1 expression modulates CXCR4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia. *J Neurosci* 22:5865–5878. [Medline](#)
- Stumm RK, Zhou C, Ara T, Lazarini F, Dubois-Dalcq M, Nagasawa T, Höllt V, Schulz S (2003) CXCR4 regulates interneuron migration in the developing neocortex. *J Neurosci* 23:5123–5130. [Medline](#)
- Stumm R, Kolodziej A, Schulz S, Kohtz JD, Höllt V (2007) Patterns of SDF-1 α and SDF-1 γ mRNAs, migration pathways, and phenotypes of CXCR4-expressing neurons in the developing rat telencephalon. *J Comp Neurol* 502:382–399. [CrossRef Medline](#)
- Suzuki-Hirano A, Ogawa M, Kataoka A, Yoshida AC, Itoh D, Ueno M, Blackshaw S, Shimogori T (2011) Dynamic spatiotemporal gene expression in embryonic mouse thalamus. *J Comp Neurol* 519:528–543. [CrossRef Medline](#)
- Tanaka DH, Yanagida M, Zhu Y, Mikami S, Nagasawa T, Miyazaki J, Yanagawa Y, Obata K, Murakami F (2009) Random walk behavior of migrating cortical interneurons in the marginal zone: time-lapse analysis in flat-mount cortex. *J Neurosci* 29:1300–1311. [CrossRef Medline](#)
- Tiveron MC, Rossel M, Moepps B, Zhang YL, Seidenfaden R, Favor J, König N, Cremer H (2006) Molecular interaction between projection neuron precursors and invading interneurons via stromal-derived factor 1 (CXCL12)/CXCR4 signaling in the cortical subventricular zone/intermediate zone. *J Neurosci* 26:13273–13278. [CrossRef Medline](#)
- Tzeng YS, Li H, Kang YL, Chen WC, Cheng WC, Lai DM (2011) Loss of Cxcl12/Sdf-1 in adult mice decreases the quiescent state of hematopoietic stem/progenitor cells and alters the pattern of hematopoietic regeneration after myelosuppression. *Blood* 117:429–439. [CrossRef Medline](#)
- Vasistha NA, Garcia-Moreno F, Arora S, Cheung AF, Arnold SJ, Robertson EJ, Molnár Z (2014) Cortical and clonal contribution of Tbr2 expressing progenitors in the developing mouse brain. *Cereb Cortex*. Advance online publication. Retrieved June 13, 2014. [CrossRef Medline](#)
- Vue TY, Aaker J, Taniguchi A, Kazemzadeh C, Skidmore JM, Martin DM, Martin JF, Treier M, Nakagawa Y (2007) Characterization of progenitor domains in the developing mouse thalamus. *J Comp Neurol* 505:73–91. [CrossRef Medline](#)
- Wang Y, Li G, Stanco A, Long JE, Crawford D, Potter GB, Pleasure SJ, Behrens T, Rubenstein JL (2011) CXCR4 and CXCR7 have distinct functions in regulating interneuron migration. *Neuron* 69:61–76. [CrossRef Medline](#)
- Wonders CP, Anderson SA (2006) The origin and specification of cortical interneurons. *Nat Rev Neurosci* 7:687–696. [CrossRef Medline](#)