

The present work analyzes the developmental pattern of Notch1 immunolabeling in the developing optic tectum (OT) from E2 (neuroepithelium) until E12 (end of basic laminar organization). During the early developmental stages (DS) Notch expression is restricted to progenitor neuroepithelial cells (PNEs) bodies (generative zone). This zone is surrounded by Notch negative, NeuroD-, β III-tubulin- and Syt1-positive postmitotic premigratory neurons. This pattern persists during the neuronogenic phase revealing a role of Notch signaling in maintaining PNE cell population by inhibiting neuronal differentiation. Hes5 is not co-expressed by PNEs suggesting that it does not participate in Notch signaling during these DSs. When the first transitory cell compartment (TCC1) appears, these differentiating neurons express cytoplasmic Notch staining revealing a second phase of Notch activity associated to neuronal differentiation. From this DS onwards neurons co-express Notch and Hes5. As development progresses, nuclear Notch staining is observed in these neurons indicating an increasing nuclear translocation. Simultaneously, the neuropile superficial to TCC2 displays intense neurite staining suggesting a role of Notch signaling in neuritogenesis. Later, scattered differentiating neurons within TCC3 and 4 start to display nuclear Notch staining. By E12, Notch is also expressed by radial and migrating glial revealing a role of Notch signaling during gliogenesis. These findings suggest multiple developmental roles for the Notch signaling pathway.

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Program/Abstract # 483

Role of the homeodomain transcription factor *Dbx1* in patterning the developing diencephalon

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The diencephalon is a forebrain structure that is subdivided into three transverse domains, or prosomeres, which include floor, basal, alar, and roof plates. The alar plate gives rise to prethalamus from p3, thalamus from p2, and pretectum from p1, all of which are critical for brain function. Transcription factors are expressed in differential, position-dependent patterns in the ventricular zone of these three regions. Lineage tracing showed that cells expressing particular transcription factors contribute to neurons that populate specific sets of thalamic nuclei, indicating a link between transcription factor expression in progenitor cells and positional fate of postmitotic neurons. However, the role of transcription factors in specifying postmitotic neural populations of the diencephalon remain unclear. The expression domain of *Dbx1* has two borders: a sharp caudal border at the boundary of the anterior and posterior pretectum and a rostral border that is graded within the caudal domain of the thalamic ventricular zone (pTH-C). Our hypothesis is that *Dbx1* specifies postmitotic fate by establishing or maintaining boundaries within the diencephalon, which is accomplished by repressing genes normally expressed in adjacent regions within its expression domain. Preliminary evidence from *Dbx1-lacZ* knockout mice indicates the posterior pretectum marker *Mash1* is ectopically expressed in anterior pretectum, where *Dbx1* is normally expressed. This suggests that *Dbx1* normally represses posterior pretectal fate and may act to fine-tune the boundary between the anterior and posterior pretectum.

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Program/Abstract # 484

Deciphering the mechanism of engrailed function during mouse cerebellar foliation

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During mammalian development, the cerebellum (Cb) arises from rhombomere 1 of the developing hindbrain. The mammalian homologs of the fly segmentation gene engrailed (*en*), *En1* and *En2*, are required for specification of the cerebellar anlage and have been implicated in late patterning of the developing Cb. However, little is known about the mechanism(s) by which the Engrailed genes regulate later cerebellar patterning. Within the developing cerebellar primordium, two main regions of neurogenesis exist, the ventricular zone and rhombic lip, which give rise to Purkinje and granule cells, respectively. During late embryonic and early postnatal development, the Cb undergoes a drastic increase in tissue size accompanied by folding of the Cb along the A–P axis into lobules. The formation of these lobules occurs through the initiation of fissures and the proliferation of granule cells. Between E17 and 18.5, four primary fissures form and separate the Cb into five lobes. We show that leading up to and during the specification of these fissures, the Engrailed genes are expressed dynamically in cells derived from both the ventricular zone and rhombic lip. Using tissue-specific conditional gene inactivation, we show that *En1* and *En2* are required early for specification and/or production of cells derived from the ventricular zone. Furthermore, we show that loss of *En1* and *En2* in ventricular zone derived cells or in granule cells results in severe A–P patterning defects. We propose that the Engrailed genes pattern cerebellar foliation along the A–P axis by specifying positional and temporal cues, which result in formation of the four primary fissures.

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Program/Abstract # 485

Boundary dispute in the developing forebrain – *DLX2* vs *PAX6*

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Rationale: The ECM molecule Tenascin-C (*Ten-C*) gene promoter contains candidate binding sites for proteins containing homeodomains, paired domains and paired class homeodomains. *DLX2* and *PAX6* may control the expression of *Ten-C* at the cortical-striatal boundary, thereby affecting neuronal migration during forebrain development.

Results: *TEN-C* is reduced and *DLX2* expression extends dorsally in the *Pax6* KO but *TEN-C* is unaffected in the *Dlx1/2* DKO mouse. Both *DLX2* and *PAX6* bind to regions of the *Ten-C* promoter in vitro and in vivo and transactivate expression of a *Ten-C* reporter gene construct in vitro. Using ChIP–reChIP assays, both *DLX2* and *PAX6* bind to a small region of the *Ten-C* promoter in situ. *DLX2* and *PAX6* also form protein–protein complexes in vitro and in situ.

Conclusions: It is unclear why *DLX2* transactivates *Ten-C* expression in vitro yet loss of *Dlx1/Dlx2* function does not affect *Ten-C* expression at the cortical-striatal boundary in vivo. Either *PAX6* or *DLX2* compete for binding to the *Ten-C* promoter or *DLX2* acts as a transcriptional activator in vivo. Further characterization of the interactions between these transcription factors and their target genes will improve our understanding of forebrain development.

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Hox-directed motoneuron subtype development and the role of the *Hoxd11* homeodomain

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