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Transcriptional regulatory mechanisms underlying the GABAergic neuron fate in different diencephalic prosomeres

Sini-Maaria Virolainen[1], Kaia Achim[1], Paula Peltopuro[1], Marjo Salminen[2] and Juha Partanen[1]*

SUMMARY

Diverse mechanisms regulate development of GABAergic neurons in different regions of the central nervous system. We have addressed the roles of a proneural gene, *Ascl1*, and a postmitotic selector gene, *Gata2*, in the differentiation of GABAergic neuron subpopulations in three diencephalic prosomeres: prethalamus (P3), thalamus (P2) and pretectum (P1). Although the different proliferative progenitor populations of GABAergic neurons commonly express *Ascl1*, they have distinct requirements for it in promotion of cell-cycle exit and GABAergic neuron identity. Subsequently, *Gata2* is activated as postmitotic GABAergic precursors are born. In P1, *Gata2* regulates the neurotransmitter identity by promoting GABAergic and inhibiting glutamatergic neuron differentiation. Interestingly, *Gata2* defines instead the subtype of GABAergic neurons in the rostral thalamus (pTh-R), which is a subpopulation of P2. Without *Gata2*, the GABAergic precursors born in the pTh-R fail to activate subtype-specific markers, but start to express genes typical of GABAergic precursors in the neighbouring P3 domain. Thus, our results demonstrate diverse mechanisms regulating differentiation of GABAergic neuron subpopulations and suggest a role for *Gata2* as a selector gene of both GABAergic neuron neurotransmitter and prosomere subtype identities in the developing diencephalon. Our results demonstrate for the first time that neuronal identities between distinct prosomeres can still be transformed in postmitotic neuronal precursors.

KEY WORDS: Diencephalon, Thalamus, Pretectum, Prethalamus, vLGN, IGL, Neurogenesis, GABAergic neuron, Transcription factor, Gata2, Ascl1, Mouse, Cre-recombinase, Proneural gene, Terminal selector gene

INTRODUCTION

The thalamic complex in the diencephalon contains nuclei primarily involved in relay and processing of sensory information. These nuclei are located in three main anatomical regions: prethalamus (former ventral thalamus), thalamus (former dorsal thalamus) and pretectum (Jones, 2007). The nuclei in the prethalamus and pretectum contain abundant GABAergic neurons and project to extracortical targets. By contrast, the thalamic nuclei are mainly glutamatergic and involved in direct information exchange between the sensory systems (auditory, somatosensory, visual) and the cortex. The GABAergic diencephalic nuclei include the ventral lateral geniculate nucleus (vLGN) and the intergeniculate leaflet (IGL), which are located near the prethalamus-thalamus boundary. The vLGN and IGL are closely related structures and have been considered to be an unseparated complex in several studies (Kataoka and Shimogori, 2008; Kitamura et al., 1997). They receive input from the retina and other sources and regulate circadian clock and visuomotor responses (Harrington, 1997). According to a traditional view, vLGN and IGL are prethalamic nuclei because they are GABAergic, do not project to the cortex and are located adjacent to the prethalamus (Jones, 2007). However, recent studies have shown that some of the IGL and vLGN neurons have an origin distinct from other prethalamic nuclei.

The developing diencephalon also consists of three units called prosomeres (P1/pretectum, P2/thalamus and P3/prethalamus) and a narrow zona limitans interthalamica (Zli) domain located between P2 and P3 (Puelles and Rubenstein, 2003; Scholpp and Lumsden, 2010) (Fig. 1A). The identities of the prosomeres are established early during patterning of the neuroepithelium and are manifested by the expression of domain-specific transcription factors, including *Fez* (*Fezf1/2*; in P3), *Irx3* and *Otx2* (in P2). Later, Zli is an important source of the morphogen Shh, which is crucial for further patterning of both the developing prethalamus and thalamus (Hashimoto-Torii et al., 2003; Jeong et al., 2011; Kiecker and Lumsden, 2004; Scholpp et al., 2007; Vieira et al., 2005; Vue et al., 2009). Other local signals regulating thalamic development include fibroblast growth factors and Wnts (Braun et al., 2003; Kataoka and Shimogori, 2008; Martinez-Ferre and Martinez, 2009; Zhou et al., 2004).

Together with the domain-specific transcription factors, Zli-derived signals regulate the activity of proneural genes *Ngn1* (*Neurog1*), *Ngn2* (*Neurog2*) and *Ascl1* in the neural progenitor cells of the ventricular zone (VZ) (Scholpp and Lumsden, 2010; Vue et al., 2007). These transcriptional networks are thought to regulate both cell-cycle exit and neuronal identity at the level of proliferative progenitor cells. *Ngn1* and *Ngn2* are expressed in the progenitors of a large caudal domain of P2/thalamus (pTH-C), which gives rise to the thalamic glutamatergic neurons (Chen et al., 2009; Garcia-Lopez et al., 2004; Vue et al., 2007). In turn, *Ascl1* is expressed in the progenitors of P3/prethalamus, P1/pretectum, and a narrow domain in the most rostral part of P2 (pTh-R; also called Rim, P2ZL) (Kataoka and Shimogori, 2008; Vue et al., 2007). Progenitors in P1 and pTh-R produce postmitotic neuronal precursors expressing genes encoding the GABAergic marker *Gad1* and the transcription factors *Tal1*, *Tal2* and *Sox14* (Hashimoto-Torii et al., 2003; Kataoka and Shimogori, 2008). P1-derived precursors contribute to pretectal nuclei, whereas the pTh-R-derived precursors were recently shown to contribute to the IGL and vLGN (Jeong et al., 2011; Vue et al., 2007). In addition, vLGN

[1]Department of Biosciences and Institute of Biotechnology, Viikki Biocenter, PO Box 56, Viikinkaari 5, FIN00014-University of Helsinki, Helsinki, Finland. [2]Department of Veterinary Biosciences, P.O. Box 66, Agnes Sjobergin katu 2, FIN00014-University of Helsinki, Helsinki, Finland.

*Author for correspondence (juha.m.partanen@helsinki.fi)

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has contributions from Zli and P3 (Delaunay et al., 2009; Kitamura et al., 1997; Suzuki-Hirano et al., 2011). P3 also produces GABAergic neuron precursors but their molecular identity is distinct from pTh-R and P1. The GABAergic precursors born in P3 express genes encoding the transcription factors *Dlx1*, *Dlx2*, *Dlx5* and *Arx*, but are negative for *Tal1*, *Tal2* and *Sox14* (Panganiban and Rubenstein, 2002; Kataoka and Shimogori, 2008; Kitamura et al., 1997). By contrast, the organizer region Zli expresses *Ngn2* and produces a small population of glutamatergic neurons.

Regulation of postmitotic differentiation and mechanisms specifying the different GABAergic neuron subpopulations in the P1/prethalamus, pTh-R and P3/pretectum are still incompletely understood. *Ascl1* is important for GABAergic neurogenesis in the telencephalon (Parras et al., 2002), and also appears to control it in the diencephalon. Development of ectopic GABAergic neurons in P2/thalamus of conditional *Otx2* mutants correlates with upregulation of *Ascl1* expression (Puelles et al., 2006). Also, changes in *ascl1* expression due to modulation of Her6 or Ngn1 activity correlate with changes in GABAergic neurogenesis in zebrafish (Scholpp et al., 2009). Finally, inactivation of *Ascl1* has been reported to lead to loss of GABAergic neurons in the posterior diencephalon (P2 and P1) (Miyoshi et al., 2004). However, *Ascl1* might regulate multiple aspects of GABAergic neurogenesis (Guillemot, 2007; Peltopuro et al., 2010) and its exact roles in distinct regions of diencephalon remain unknown.

Determination of neuronal identities is best understood at the level of proliferative progenitor cells (Dessaud et al., 2008; Guillemot, 2007). However, the definitive markers of neuronal identity are only turned on as progenitors exit the cell cycle and become postmitotic neuronal precursors. Furthermore, there are examples of transcription factors, putative 'terminal selector genes' (Hobert, 2008), which are often activated only in the postmitotic precursors and are important determinants of either GABAergic or glutamatergic identity in the spinal cord, cerebellum and telencephalon (Cheng et al., 2005; Glasgow et al., 2005; Hoshino et al., 2005; Rouaux and Arlotta, 2010). We showed previously that the zinc-finger transcription factor Gata2 is an essential postmitotic selector of the GABAergic neuron identity in the embryonic midbrain (Kala et al., 2009). *Gata2* expression is induced when the midbrain GABAergic neuron precursors leave the cell cycle and differentiate. In tissue-specific *Gata2* mutants, the midbrain neural precursors fail to activate genes characteristic for GABAergic neurons but start to express genes typical for adjacent glutamatergic neurons. Gata2 cooperates with Tal transcription factors during development of the haematopoietic system, V2b spinal interneurons and midbrain GABAergic neurons (Joshi et al., 2009; Osada et al., 1995) (our unpublished results). As *Tal1* and *Tal2* mark some of the diencephalic GABAergic neuron precursors (see above), it is possible that a Gata-Tal complex also regulates their differentiation.

In the present study, we have studied the functions of Ascl1 and Gata2 during GABAergic neuron development in the diencephalon. Our results demonstrate distinct transcriptional regulatory mechanisms controlling differentiation of prethalamic, pTh-R and pretectal GABAergic neurons. Furthermore, we suggest that *Gata2* is a terminal selector gene, which can specify both the neurotransmitter and neuronal subtype identities during postmitotic differentiation.

MATERIALS AND METHODS

Mice

Foxg1^{Cre} (Hebert and McConnell, 2000), *Gata2*^{F} (*Gata2*^{Flox}) (Haugas et al., 2010), *Ascl1*^{KO} (Guillemot et al., 1993) and *Gad1*^{GFP} (*Gad67*^{GFP}) (Tamamaki et al., 2003) alleles have been described previously. For staging, the day of vaginal plug was counted as embryonic day (E) 0.5. Embryos were fixed in 4% paraformaldehyde in PBS, and transferred to polymer wax (Merck). As controls, we used wild-type (WT), *Foxg1*$^{cre/+}$; *Gata2*$^{F/+}$, *Foxg1*$^{+/+}$; *Gata2*$^{F/F}$ or *Ascl1*$^{KO/+}$ littermates, which were phenotypically indistinguishable from each other. All the results were reproduced with at least three embryos per genotype. Samples were sectioned at 5 μm for immunohistochemistry (IHC) and in situ hybridization (ISH) analyses. All the experiments were approved by the committee of experimental animal research of Finland.

BrdU labelling

To label the actively proliferative cells, a single dose of BrdU (3 mg/100 g body weight) was administered intraperitoneally to the pregnant females 1 or 12 hours before dissection of embryos.

In situ mRNA hybridization and immunohistochemistry

mRNA in situ hybridization (ISH) analyses on paraffin sections were performed as described (Wilkinson and Green, 1990) using digoxigenin- or ^{35}S-labelled antisense cRNA probes. The two different detection methods were used owing to variation in sensitivity between the probes. Mouse cDNA probes used for ISH analysis were: *Arx* (Kataoka and Shimogori, 2008), *Ascl1* (previously known as *Mash1*), *Dlx1* (from J. L. R. Rubenstein, University of California San Francisco, USA), cyclin D1 (IMAGE 3155470), *Fev* (also known as *Pet1*), *Gad1* (also known as *Gad67*), *Gata2*, *Gata3* (Lillevali et al., 2004), *Helt* (also known as *Mgn*) (Guimera et al., 2006b), *Mab21l1* (IMAGE 4526962), *Ngn2* (Jukkola et al., 2006), *Nkx2-2* (IMAGE480100), *Npy* (IMAGE 482891), *Penk1* (*Penk*) (IMAGE 6432610), *Pitx2* (Guimera et al., 2006b), *Pou4f1* (from S.-L Ang, NIMR, UK), *Rora* (Vue et al., 2009), *Shh* (from A. McMahon, Harvard University, USA), *Six3* (IMAGE 761326), *Slc17a6* (*Vglut2*) (Guimera et al., 2006b), *Sox14* (IMAGE 6828289), *Tal1* (IMAGE 6826611), *Tal2* (IMAGE 40051579).

Immunohistochemistry (IHC) was performed as described (Kala et al., 2008) with the following antibodies: rabbit anti-Arx [from Kunio Kitamura, 1:500 (Kitamura et al., 2002)], mouse anti-BrdU (GE Healthcare RPN20AB, 1:400), rabbit anti-cyclin D1 (LabVision, Neomarkers RM-9104-SO, 1:400), rabbit anti-Gata2 (Santa Cruz sc-9008, 1:250), mouse anti-Gata3 (Santa Cruz sc-268, 1:200), goat anti-GFP (Abcam ab6673, 1:500), rabbit anti-GFP (Abcam ab290, 1:600), guinea pig anti-Heslike (Helt, 1:500; from R. Kageyama, Kyoto University, Japan), mouse anti-HuC/D (Molecular Probes A21271, 1:800), mouse anti-Mash1 (Ascl1, BD Biosciences 556604, 1:200), mouse anti-Nkx2-2 [Developmental Studies Hybridoma Bank (DSHB) 74.5A5, 1:250], rabbit anti-NPY (Immunostar 22940, 1:800), rabbit anti-p57^{Kip2} (Neomarkers RB-1637-P0, 1:500), mouse anti-Pax7 (DSHB, 1:300), mouse anti-proliferating cell nuclear antigen (PCNA) (Dako Cytomation M-0878, 1:800), mouse anti-phosphohistone 3 (PH3) (Cell Signaling Technology #9706S, 1:250), mouse anti-Sox2 (Millipore AB5603, 1:400) rabbit anti-tyrosine hydroxylase (Millipore MAB318, 1:300) and mouse anti-Slc17a6 (Vglut2; Sigma V2514, 1:1000).

Alexa Fluor 488- and Alexa Fluor 568-conjugated goat anti-rabbit IgG and anti-mouse IgG, and donkey anti-rabbit IgG, anti-mouse IgG and anti-goat IgG (1:400, Invitrogen) were used as secondary antibodies.

For combined ISH and IHC, primary antibodies were added together with the anti-DIG-POD Fab fragments (Roche). The TSA Fluorescence Palette System (PerkinElmer) was used to visualize the ISH signal. Images were processed using Adobe Photoshop or CorelDraw X4 software. Red pseudocolour images were produced by replacing the dark-field images with red and overlaying the resulting image with the respective bright-field image.

RESULTS

Gata2, Gata3 and their partners are expressed in regions of GABAergic neurogenesis in the posterior embryonic diencephalon

As *Gata2* is expressed in the GABAergic precursors in the midbrain (Kala et al., 2009), we postulated that it has similar cell-type

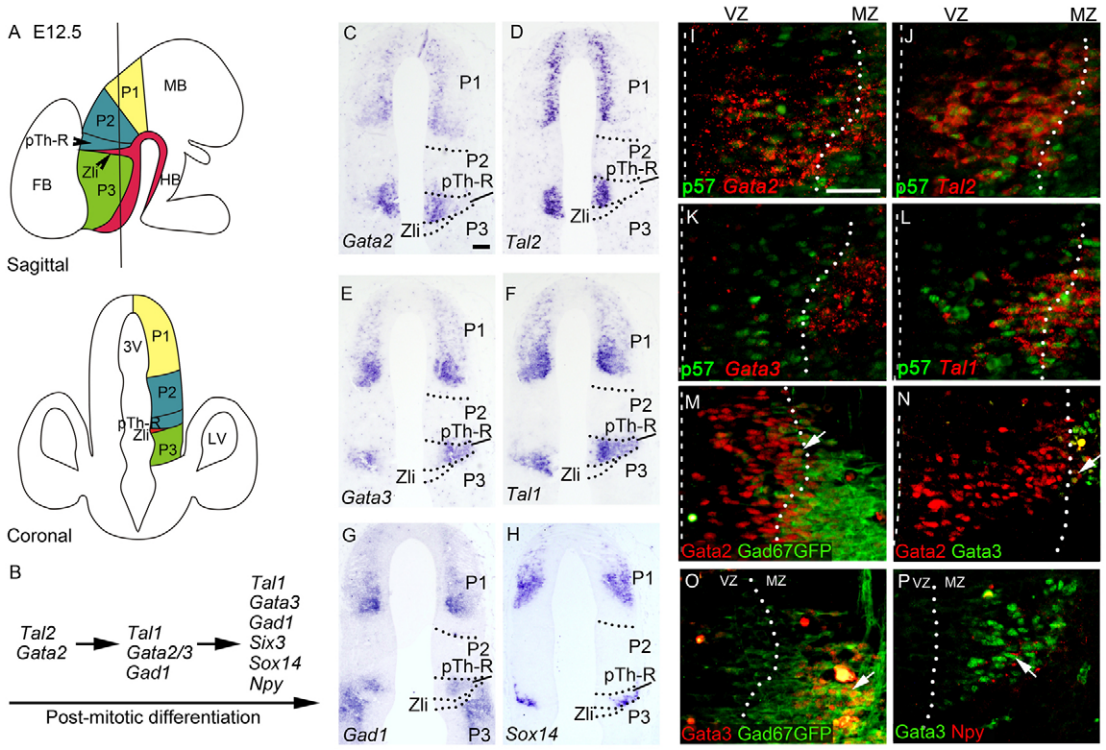

Fig. 1. *Gata2*, *Gata3* and the Gata-associated genes *Tal1* and *Tal2* are co-expressed with GABAergic markers in posterior diencephalon early neuronal precursors. (**A**) Prosomeric model of the mouse diencephalon at E12.5, sagittal and coronal views. The line in the sagittal view indicates the level of the coronal view and the coronal sections analysed in this study. Red indicates the *Shh* expression domain. P1-P3, prosomeres of diencephalon; pTh-R, rostral region of P2; Zli, zona limitans interthalamica; FB, forebrain; MB, midbrain; HB, hindbrain; 3V, 3rd ventricle; LV, lateral ventricle. (**B**) Summary of the expression dynamics of *Gata2/3*, *Tal1/2* and GABAergic markers during GABAergic differentiation in the diencephalon. (**C-H**) ISH analysis of *Gata2*, *Gata3*, *Tal1*, *Tal2*, *Gad1* and *Sox14* mRNA expression on coronal sections of E12.5 WT diencephalon. Dotted lines indicate the prosomere boundaries. The border between P1 and P2 was defined by the expression of *Gbx2* on adjacent sections. (**I-L**) ISH for *Gata2/3* and *Tal1/2* combined with IHC for $p57^{Kip2}$ (p57) in pTh-R. (**M-P**) Co-IHC analysis of Gata2 and Gad67GFP (M), Gata2 and Gata3 (N), Gata3 and Gad67GFP (O) and Gata3 and NPY (P) in the pTh-R region. Analyses were performed on E12.5 WT (N,P) or $Gad67^{GFP/+}$ (M,O) embryos. Arrows indicate double-labelled cells. White dotted lines indicate the ventricular surface and the border between VZ and MZ. Scale bars: in C, 100 μm for C-H; in I, 50 μm for I-P.

specificity in the diencephalon. Therefore, we compared first the expression patterns of *Gata2* and *Gata3* and their putative co-factors, *Tal1* and *Tal2*, with markers of GABAergic progenitors, *Ascl1* and *Helt*, as well as to glutamic acid decarboxylase 1 (*Gad1*) in postmitotic GABAergic neurons. *Gata2* and *Gad1* expression was first detected in the postmitotic zone of P1 at ~E10.5 (supplementary material Fig. S1A-C). At E11.5-12.5, we detected co-expression of *Gata2/3* and *Tal1/2* in P1 and pTh-R, which give rise to $Gad1^{+}$ GABAergic neurons (Fig. 1C-G; supplementary material Fig. S1D-H). Our IHC analyses further demonstrated co-expression of Gata2 protein in Ascl1- and Helt-positive GABAergic progenitors (supplementary material Fig. S2A-D). We also detected Gata2 and Gata3 expression in $Gad1^{+}$ GABAergic neurons by co-IHC for Gata2/3 and GFP in the $Gad1^{GFP/+}$ mouse embryos (Fig. 1M,O). In addition, a number of other factors associated with the GABAergic fate in P1 and pTh-R, including *Six3*, *Sox14* and neuropeptide Y (*Npy*) (Kataoka and Shimogori, 2008; Lim and Golden, 2007; Vue et al., 2009), were also expressed in *Gata2/3* positive regions (Fig. 1H,P; supplementary material Fig. S4M,O,Q, note the typical punctuated *Npy* staining adjacent to *Gata3*-positive nuclei) (Silva et al., 2005). No *Gata2/3* or *Tal1/2* expression was detected in the more anterior brain regions, including P3, where *Ascl1* was expressed and which gives rise to $Gad1^{+}$ GABAergic neurons.

At E12.5, we observed *Gata2* in the ventricular, intermediate and mantle zones (VZ, IZ and MZ, respectively; Fig. 1C,I,M), whereas the expression of Gata3 was restricted to the Gad1-expressing cells in the MZ (Fig. 1E,K,O). Like *Gata2*, *Tal2* was observed mainly in the VZ and IZ (Fig. 1D,J), whereas *Tal1* appeared to be expressed strongly in the IZ and MZ (Fig. 1F,L). Co-labelling with the cyclin-dependent kinase (CDK) inhibitor $p57^{Kip2}$ and p27, markers of early IZ precursors (Gui et al., 2007; Nguyen et al., 2006), showed that *Gata2* and *Tal1/2* are expressed in the early postmitotic cells in VZ and IZ (Fig. 1I,J,L; supplementary material Fig. S2G,H), whereas *Gata3* is confined to the differentiating cells in MZ (Fig. 1K). No co-labelling of Gata2 and BrdU was detected after a short (1 hour) BrdU administration pulse, which should label nuclei of progenitors in the S phase (supplementary material Fig. S2E,F). Upon a longer labelling pulse (12 hours), some BrdU-labelled nuclei started expressing Gata2 and could be traced to the IZ/MZ (supplementary material Fig. S2M,N). Furthermore, Gata2-positive nuclei were mostly devoid of PCNA, a marker of proliferative cells (supplementary material Fig. S2K,L) and never expressed phospho-histone H3, a marker of G2/M phase nuclei (supplementary material Fig. S2O,P). In addition, we observed several cells co-expressing Gata2 and the postmitotic neuron marker HuC/D, indicating that Gata2 is

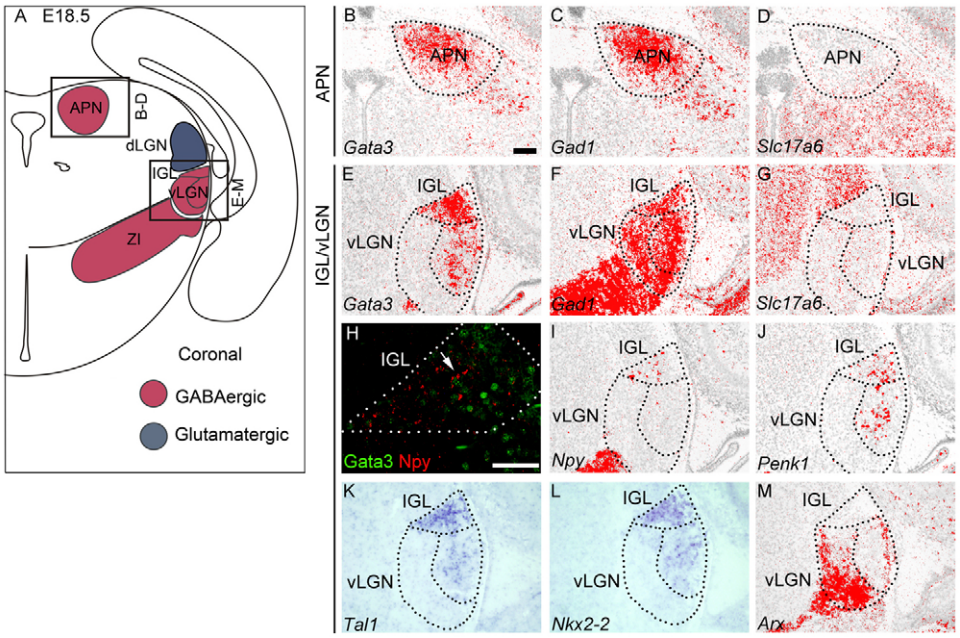

Fig. 2. *Gata2* and *Gata3* expression in the thalamic nuclei of E18.5 mouse brain. (**A**) Schematic of the mature diencephalon showing the main GABAergic (red) and glutamatergic (blue) nuclei. Coronal view. (**B-G**) ISH analysis of *Gata3*, *Gad1* and *Slc17a6* expression in E18.5 APN (B-D) and vLGN/IGL complex (E-G). (**H**) IHC analysis of Gata3 and Npy in the IGL. (**I-M**) ISH with *Npy*, *Penk1*, *Tal1*, *Nkx2-2* and *Arx* mRNA probes on IGL/vLGN. Dotted lines indicate boundaries of the brain nuclei. APN, anterior pretectal nucleus; dLGN and vLGN, dorsal and ventral lateral geniculate nucleus, respectively; IGL, intergeniculate leaflet; ZI, zona incerta. Scale bars: in B, 100 μm for B-G,I-M; in H, 50 μm.

expressed in postmitotic neuronal precursors (supplementary material Fig. S1C and Fig. S2I,J).

In summary, our results suggest that *Gata2* expression is activated in early postmitotic GABAergic precursors in P1 and pTh-R. The expression of *Gata2* was probably initiated very soon after cell-cycle exit when precursor cell nuclei still resided in the VZ and coincided with expression of *Tal2*. By contrast, other markers of the GABAergic lineage, including *Gata3*, *Tal1*, *Sox14*, *Six3*, *Npy* and *Gad1*, are activated after the onset of *Gata2* expression (Fig. 1B).

Gata3 is expressed in the pretectum and IGL/vLGN in the prenatal brain

To study the putative derivatives of *Gata2/3*-expressing P1 and pTh-R GABAergic precursors, we first mapped the expression of Gata and Tal factors in the GABAergic nuclei in caudal diencephalon of E18.5 brain. We detected no expression of *Gata2* or *Tal2* in the diencephalon at E18.5 (data not shown). However, *Gata3* was expressed in the *Gad1*$^{+}$ anterior pretectal nucleus (APN; Fig. 2B,C), IGL and in scattered cells in lateral vLGN (Fig. 2E,F), in agreement with an earlier report (Zhao et al., 2008). Consistent with its expression in more differentiated postmitotic precursors (see above), we also found *Tal1* in the same regions as *Gata3* (Fig. 2K; data not shown). IGL contains two functionally distinct populations of GABAergic neurons expressing either Npy or enkephalin (Penk1) as co-neurotransmitter (Jones, 2007). In IGL, *Gata3/Tal1* expression was seemingly uniform and overlapped with *Nkx2-2* as well as both Npy^{+} and *Penk1*$^{+}$ neurons (Fig. 2E,H-L). Also in the lateral vLGN we found *Gata3* and *Tal1* in regions expressing *Nkx2-2* and *Penk1* (Fig. 2E,K,L), but not *Arx* or *Dlx1*, which are expressed in ventral and medial vLGN (Fig. 2M and Fig. 7J; data not shown). In the lateral vLGN, *Gata3*$^{+}$ neurons appeared to intermingle with *Gata3*-negative cells. In summary, restricted *Gata3/Tal1* expression in the GABAergic neuron populations suggests that *Gata/Tal*-expressing cells in embryonic P1 and pTh-R might specifically contribute to the GABAergic nuclei in the caudal diencephalon, APN, IGL and vLGN.

Analysis of conditional *Gata2* mutants: Gata2 regulates GABAergic neuron differentiation in P1 and pTh-R

To study the function of Gata2 in diencephalic development, we used a conditional *Gata2* mutant strain (*Foxg1*^{cre}*; Gata2*$^{F/F}$) in order to circumvent the early haematopoietic defect seen in the *Gata2*-null mutants (Tsai et al., 1994). The *Foxg1*^{cre} allele has been reported to catalyse recombination primarily in the telencephalon and inner ear (Hebert and McConnell, 2000). However, genetic background was shown to have a major effect on *Foxg1*^{cre}-mediated recombination and, thus, the recombination pattern can be much wider than the detectable *Foxg1* expression. In the *Foxg1*^{cre}*; Gata2*$^{F/F}$ embryos, *Gata2* was widely and efficiently inactivated at an early stage. However, presumably owing to incomplete recombination in the yolk sac, haematopoiesis is rescued and the mutants survive until birth (Haugas et al., 2010). We detected no *Gata2* expression in the brains of *Foxg1*^{cre}*; Gata2*$^{F/F}$ embryos at E12.5 (supplementary material Fig. S3).

We examined first the expression of genes associated with GABAergic fate in *Foxg1*^{cre}*; Gata2*$^{F/F}$ diencephalon. No defects in GABAergic neuron differentiation were observed in P3 (supplementary material Fig. S4B). By contrast, complete loss of *Gad1*, *Gata3*, *Six3*, *Npy*, *Tal1* and *Sox14* expression was detected in the mutant P1 and pTh-R at E12.5 (Fig. 3A-D and Fig. 4A,B,Q,R; supplementary material Fig. S4A,B,G-J,M-R). Despite the failure in GABAergic neuron differentiation, the GABAergic progenitor markers *Ascl1* and *Helt* (Fig. 3G,H; supplementary material Fig. S4E,F,U,V), as well as other regional markers such as *Shh*, *Ngn2* and *Nkx2-2*, were normally expressed in the VZ throughout the P1-P3 (supplementary material Fig. S4S,T,X,Y; data not shown). Interestingly, expression of *Tal2*, a putative Gata2 co-factor, also appeared to be unaffected in the VZ and IZ of the mutants (Fig. 5A,B,I,J; supplementary material Fig. S4K,L). In summary, in the absence of Gata2, postmitotic GABAergic markers were downregulated, but gene expression in GABAergic progenitors remained unaffected in P1 and pTh-R.

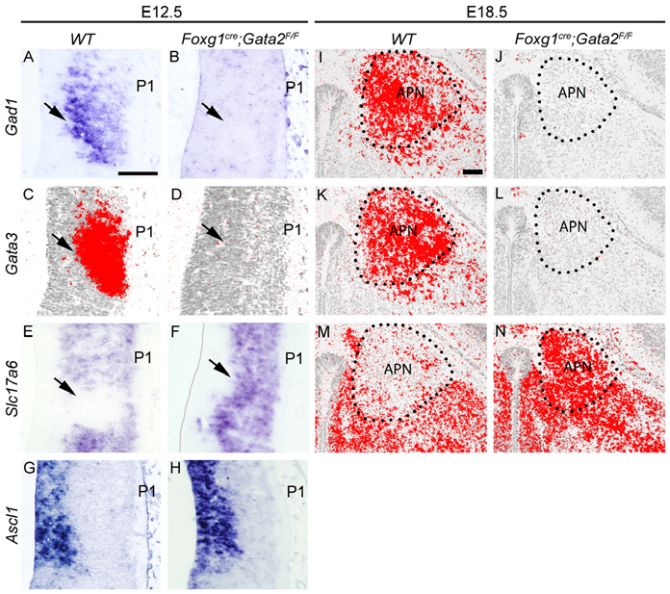

Fig. 3. GABAergic-to-glutamatergic fate transformation in the P1 postmitotic precursors of *Foxg1^{cre}; Gata2$^{F/F}$* mutants. (**A-N**) ISH analysis of *Gad1*, *Slc17a6*, *Gata3* and *Ascl1* expression in WT and *Foxg1^{cre}; Gata2$^{F/F}$* mouse embryos at E12.5 (A-H) and *Gad1*, *Slc17a6* and *Gata3* at E18.5 (I-N). Coronal sections of P1 region (A-H) and pretectum (I-N) are shown. Arrows indicate the region where the fate transformation is most apparent. APN, anterior pretectal nucleus. Scale bars: 100 μm.

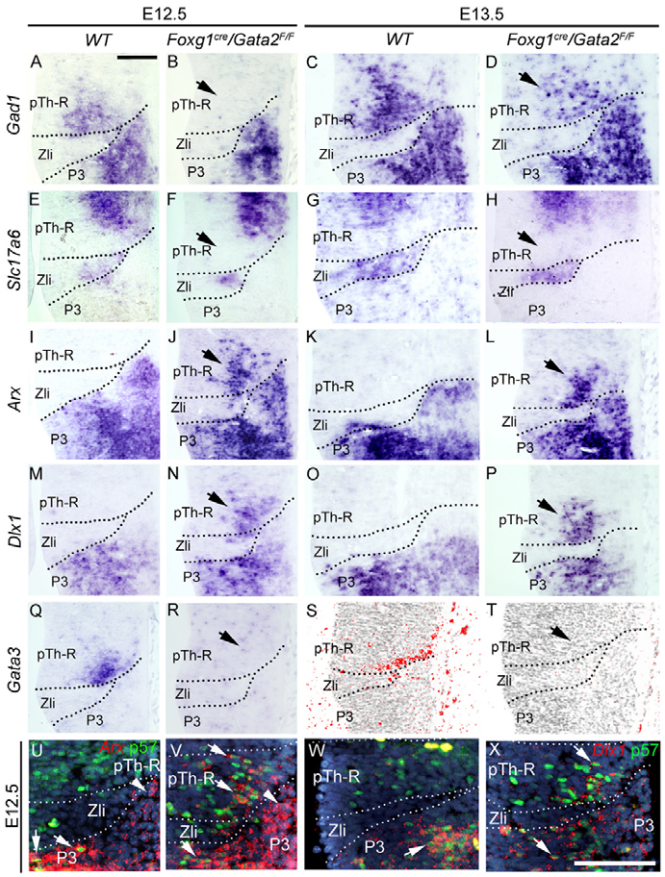

Fig. 4. GABAergic subtype switch in the absence of Gata2 in the pTh-R precursors. (**A-T**) ISH analysis of *Gad1* (A-D), *Slc17a6* (E-H), *Arx* (I-L), *Dlx* (M-P) and *Gata3* (Q-T) expression in WT and *Foxg1^{cre}; Gata2$^{F/F}$* mouse embryos at E12.5 and E13.5. Black arrows indicate the affected pTh-R region. (**U-X**) ISH for *Arx* (U,V) or *Dlx1* (W,X) combined with IHC for the intermediate zone marker p57^{Kip2}. White arrows indicate the p57^{Kip2} and *Arx*/*Dlx1* co-expressing cells; white arrowheads indicate p57^{Kip2}-negative *Arx*-expressing cells in the lateral P3. Dotted lines indicate the prosomere boundaries. Scale bars: 100 μm.

Gata2 selects the GABAergic neurotransmitter identity in P1

As GABAergic-to-glutamatergic fate transformation takes place in midbrain neuronal precursors in the absence of *Gata2* (Kala et al., 2009), we hypothesized that this would happen also in the developing diencephalon. Indeed, we detected ectopic expression of the glutamatergic neuron marker *Slc17a6* in the putative GABAergic domain of P1 in E12.5 *Foxg1^{cre}; Gata2$^{F/F}$* embryos (Fig. 3A,B,E,F; supplementary material Fig. S4A-D, arrows). We detected no apparent changes in tissue morphology, apoptosis or generation of postmitotic precursors in the embryonic diencephalon (data not shown). Therefore, the ectopic *Slc17a6* expression in P1 probably indicates fate transformation in *Foxg1^{cre}; Gata2$^{F/F}$* mutants. Thus, inactivation of *Gata2* appears to result in a switch from GABAergic to glutamatergic neurotransmitter phenotype in the diencephalic P1 precursors.

Gata2 selects the GABAergic neuron subtype in pTh-R

Strikingly, despite the loss of GABAergic neuron markers in pTh-R (Fig. 4A,B, arrow), we detected no upregulation of *Slc17a6* in this area in *Foxg1^{cre}; Gata2$^{F/F}$* mutants at E12.5 (Fig. 4E,F, arrow). To determine whether the fate transformation in pTh-R occurs later in development, we analysed the expression of GABA- and glutamatergic neuron markers a day later, in E13.5 embryos. However, similar to E12.5 pTh-R, we detected no upregulation of *Slc17a6* in the mutant pTh-R at E13.5 either (Fig. 4G,H, arrow; supplementary material Fig. S5C-D′). Instead, we found that *Gad1* expression was now restored in the intermediate cell population of pTh-R (Fig. 4C,D, arrow; supplementary material Fig. S5A-B′). By contrast, we still did not detect expression of other markers typical for pTh-R GABAergic neurons, such as *Gata3*, *Tal1* or *Npy* (Fig. 4S,T; supplementary material Fig. S5E-H′; data not shown). As in P1, we did not detect defects in production of postmitotic precursors or their survival in the mutants (data not shown). This suggested that in *Foxg1^{cre}; Gata2$^{F/F}$* diencephalon, the pTh-R precursors acquire a different GABAergic phenotype in which *Gata2/3*, *Tal1* and *Npy* are not expressed. Such neurons develop in the adjacent P3 region. We therefore analysed expression of markers for P3 GABAergic neurons, *Dlx1* and *Arx*. Indeed, *Dlx1* and *Arx* expression was robustly upregulated in the *Gata2*-deficient pTh-R intermediate zone precursor population already at E12.5 (Fig. 4I-P, arrows). By E13.5, expression of *Dlx1* and *Arx* was even more prominent and coincided with the *Gad1*-expressing region in the pTh-R of *Foxg1^{cre}; Gata2$^{F/F}$* mutants (Fig. 4D,L,P, arrows). These data suggest that in pTh-R, *Gata2*-deficient GABAergic precursors activate a GABAergic differentiation pathway characteristic to P3 progenitors and thus acquire a more rostral GABAergic neuron phenotype.

Alternatively, *Arx*-expressing cells from lateral P3 could move to populate pTh-R in the *Foxg1^{cre}; Gata2$^{F/F}$* mutants. We find this unlikely for the following reasons: first, we always observed a narrow gap in the mantle zone between the *Arx*-expressing cells in

P3 and ectopic *Arx* expression in pTh-R (Fig. 4L); second, *Dlx1* is not expressed in the lateral P3 yet is ectopically induced in the pTh-R in *Foxg1^{cre}; Gata2$^{F/F}$* mutants (Fig. 4O,P); and third, Gad1 is not expressed in ectopic *Arx*- and *Dlx*-positive cells at E12.5 (Fig. 4B,J,N). Furthermore, Zli is a lineage-restricted compartment between pTh-R and P3 (Garcia-Lopez et al., 2004; Kiecker and Lumsden, 2004; Larsen et al., 2001). Nevertheless, we addressed this possibility by analysing whether the *Arx*- and *Dlx1*-expressing cells in pTh-R represent recently born cells in the intermediate zone indicative of local origin in pTh-R. Indeed, the early IZ precursor marker p57^{Kip2} was co-expressed with *Arx* and *Dlx1* in the pTh-R of the *Foxg1^{cre}; Gata2$^{F/F}$* mutants (Fig. 4U-X). By contrast, the differentiating GABAergic neurons in the neighbouring P3 had already lost p57 expression (Fig. 4V, arrowhead). This further supports the conclusion that the local pTh-R GABAergic precursors undergo a transformation from *Gata3/Tal1*- to *Arx/Dlx1*-expressing cell identity in the absence of *Gata2*.

Tal2-expressing precursors switch their fate in *Gata2* mutants

Tal2 is co-expressed together with *Gata2* in precursors giving rise to GABAergic neurons, yet the expression of *Tal2* does not require *Gata2* function (Fig. 5A,B,K,O; supplementary material Fig. S4K,L) (K.A., P.P., J.P., unpublished results). This allowed us to use *Tal2* as a marker of GABAergic neurogenesis and analyse the fate of *Tal2*-expressing precursors in the absence of Gata2 function. For analysis of P1, we performed fluorescence ISH for *Tal2*, *Slc17a6* and *Mab21l1* (a marker of IZ precursors in the glutamatergic precommissural and commissural parts of P1) (Vue et al., 2007) (Fig. 5A-H,L-N) as well as IHC for Slc17a6 and Pax7 (strongly expressed in the commissural part of P1) (Merchán et al., 2011) (Fig. 5C-F,L). Analyses of adjacent sections suggested that the *Tal2*-expressing cells in P1 of the *Foxg1^{cre}; Gata2$^{F/F}$* mutants gave rise to MZ precursors expressing *Slc17a6* (Fig. 5C-F,L,M). Furthermore, *Mab21l1* expression coincided with the domain positive for *Tal2* in the IZ of *Foxg1^{cre}; Gata2$^{F/F}$* mutants (Fig. 5D,F,H,L-N). This was most clearly seen in the IZ of juxtacomissural P1, which in WT embryos uniformly expresses *Tal2* and gives rise only to GABAergic neurons. Thus, in the absence of Gata2, the *Tal2*-positive precursors appear to adopt the glutamatergic identity of precomissural or commissural P1. For analysis of pTh-R, we combined fluorescence ISH for *Tal2* with IHC for Arx. In contrast to WT embryos, we detected Arx-positive nuclei adjacent to and partly overlapping with *Tal2*-expressing cells in *Foxg1^{cre}; Gata2$^{F/F}$* mutants (Fig. 5I,J). This strongly suggests that, without Gata2, the *Tal2*-positive GABAergic precursors in pTh-R adopt a prethalamic phenotype.

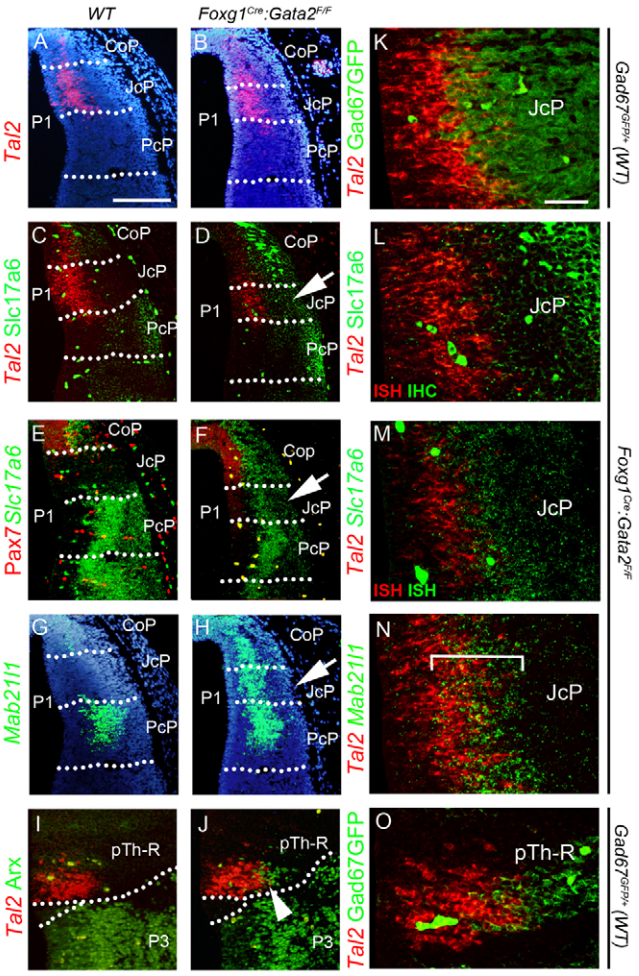

Fig. 5. Identity switch of Tal2-expressing precursors in *Foxg1^{cre}; Gata2$^{F/F}$* mouse embryos. (**A-O**) ISH for *Tal2* (A-D,I-O), *Slc17a6* (E,F,M) and *Mab21l1* (G,H,N) combined with IHC for Pax7 (E,F), Arx (I,J), Slc17a6 (C,D,L) and Gad67GFP (K,O) in WT, *Gad67$^{GFP/+}$* and *Foxg1^{cre}; Gata2$^{F/F}$* embryos at E12.5. ISH signals on adjacent sections analysed for expression of *Tal2* and *Slc17a6* (M), or *Tal2* and *Mab21l1* (N) are overlaid. White arrows and white square bracket indicate the JcP region where the fate transformation is most apparent in P1; white arrowhead indicates the region of identity switch in pTh-R. Dotted lines indicate the prosomere boundaries. CoP, commissural pretectum; JcP, juxtacommissural pretectum; PcP, precommissural pretectum. Scale bars: in A, 100 μm for A-J; in K, 50 μm for K-O.

Distinct requirements for Ascl1 in P1 and pTh-R

Ascl1 is differently required in the dorsal (m1-2) and ventral (m3-5) GABAergic progenitors in the developing midbrain (Peltopuro et al., 2010) and is a potential regulator of various aspects of neuronal progenitor proliferation, cell-cycle exit and migration (Castro et al., 2011; Pacary et al., 2011). To study how *Ascl1* regulates GABAergic neuron development in different diencephalic progenitor populations, we analysed expression of GABAergic and glutamatergic markers in *Ascl1^{KO}* diencephalon at E12.5. We observed complete loss of *Gad1*$^{+}$, *Gata2*$^{+}$ and Gata3^{+} postmitotic cells in *Ascl1*-deficient P1 (Fig. 6A,B,I-L, arrow). Furthermore, we found upregulation of *Ngn2* in the P1 VZ and consequent ectopic *Slc17a6*$^{+}$ glutamatergic neuron differentiation (Fig. 6E-H). By contrast, *Ngn2* was not upregulated in the pTh-R and P3. Instead, we detected upregulation of the Ngn repressor *Helt* (Nakatani et al., 2007) in the VZ (Fig. 6C,D). Consistently, *Gad1*$^{+}$, *Gata2*$^{+}$ and Gata3^{+} cells were produced in the pTh-R of *Ascl1* mutants (Fig. 6A,B,I-L). However, thickening of the Sox2^{+} VZ and reduction in the HuC/D^{+} MZ indicated delayed cell-cycle exit in the mutant P1 and especially in P3 at E12.5 (Fig. 6M,N; data not shown). By contrast, upregulation of HuC/D and p27 in the VZ as well as downregulation of cyclin D1 and mosaic Sox2 expression indicated premature cell-cycle exit and reduced progenitor cycling in the pTh-R (Fig. 6M,N; supplementary material Fig. S6A-H), suggesting that Ascl1 might regulate progenitor proliferation specifically in this region. Changes in cell-cycle exit might also explain the observed increase in the Gata2^{+} Gata3^{+} double-positive cells in the *Ascl1* mutant pTh-R (Fig. 6K,L, insets). However, the

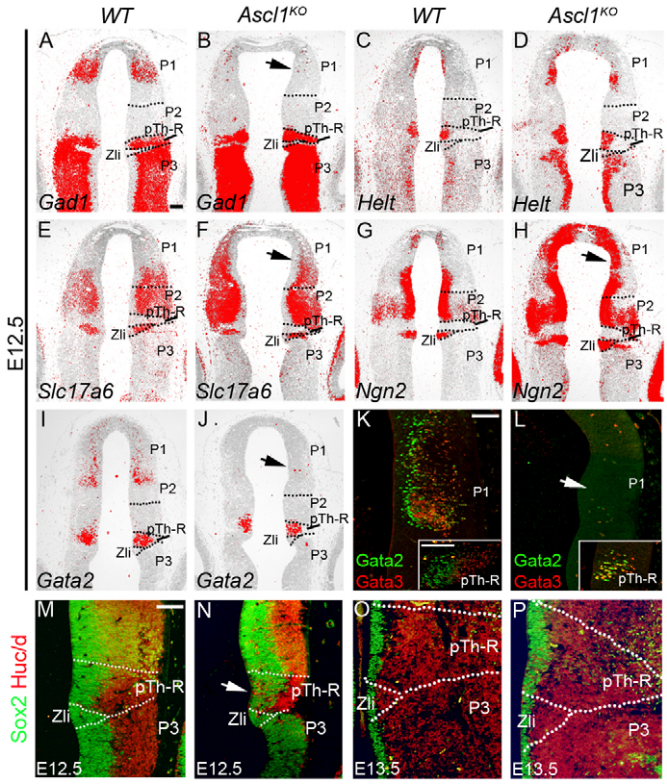

Fig. 6. Fate transformation and delayed neurogenesis in distinct diencephalic domains in the *Ascl1*^{KO} mouse embryos. (**A-J**) ISH with *Gad1*, *Helt*, *Slc17a6*, *Ngn2 and Gata2* probes on WT and *Ascl1*^{KO} diencephalon at E12.5. (**K**,**L**) Gata2 and Gata3 co-IHC on WT and *Ascl1*^{KO} diencephalon at E12.5. (**M-P**) Sox2 and HuC/D co-IHC on WT and *Ascl1*^{KO} diencephalon at E12.5 (M,N) and E13.5 (O,P). Close-up from P2-P3 region is presented in M-P. Arrows in B, F and H indicate the region where the fate transformation is most apparent; in J and L the downregulation of Gata2 and Gata3 in P1; and in N the aberrant upregulation of HuC/D in the pTh-R region. Dotted lines indicate the prosomere boundaries. Scale bars: 100 µm.

defects in neurogenesis appeared to be reversible as by E13.5 the Sox2- and HuC/D-expressing cell layers had acquired a more normal appearance throughout the diencephalon in *Ascl1*^{KO} mutants (Fig. 6O,P). In summary, the proneural gene *Ascl1* appears to play distinct and even opposing roles in the diencephalic neuronal progenitors, promoting cell-cycle exit in the P1 and P3, and supporting progenitor proliferation in the pTh-R. At the same time, *Ascl1*-deficient P1 undergoes a GABAergic-to-glutamatergic fate transformation concomitant with upregulation of proneural gene *Ngn2* expression, whereas in the more rostral diencephalon, pTh-R-P3, the fate selection process is unaltered.

Neuronal derivatives of caudal diencephalon maintain their identity switch in perinatal brain

To elucidate the consequences of observed fate transformations to the prenatal brain, we examined the derivatives of P1 and pTh-R at E16.5-18.5. Consistent with the phenotype in the E12.5 P1, the expression of *Gad1* or *Gata3* was completely absent from the *Foxg1*^{cre}*; Gata2*$^{F/F}$ as well as *Ascl1*^{KO} pretectal area at E18.5 (Fig. 3I-L; data not shown). Instead, the normally GABAergic APN contained abundant *Slc17a6*$^{+}$ glutamatergic neurons in the mutant brains (Fig. 3M,N). Thus, the GABAergic-to-glutamatergic fate transformation in the pretectal area is persistent throughout neuronal differentiation until birth.

The pTh-R-derived neurons contribute to the IGL/vLGN complex in the diencephalon (Jeong et al., 2011). Our analyses showed that vLGN and IGL contain numerous *Gata3*$^{+}$ cells that are probably derivatives of *Gata2*-expressing pTh-R precursors. To analyse the fate of *Gata2*-dependent pTh-R derivatives, we compared the expression of the P2-type GABAergic neuron markers *Npy*, *Penk1*, *Gata3* and *Tal1* with that of the P3-type markers *Arx* and *Dlx1* in the geniculate complex of WT and *Foxg1*^{cre}*; Gata2*$^{F/F}$ prenatal brains. Consistent with our observations at earlier developmental stages, we detected no expression of *Gata3*, *Tal1*, *Npy* or *Penk1* in the E18.5 *Foxg1*^{cre}*; Gata2*$^{F/F}$ brains (Fig. 7D,E,G,H; data not shown). However, we also did not observe any apparent changes in the expression of *Gad1* and *Slc17a6* (Fig. 7A,B; data not shown), or in the size of the vLGN/IGL. Instead, we detected downregulation of *Nkx2-2* (Fig. 7M,N), which could indicate reduction in pTh-R contribution or reflect the transformation to P3-type cell fate as the P3 cells seem to lose Nkx2-2 expression as they move to the MZ (supplementary material Fig. S4S). Indeed, cells expressing the P3 GABAergic neuron markers *Dlx1* and *Arx* were found in the IGL and lateral vLGN area in the *Foxg1*^{cre}*; Gata2*$^{F/F}$ mutants (Fig. 7J,K; data not shown). In conclusion, the vLGN complex maintains a GABAergic neurotransmitter phenotype in the absence of *Gata2*, but the neuronal subtype composition in the complex is altered.

Because we observed a defect in neurogenesis but not in the cell-type identity in the pTh-R of developing *Ascl1*^{KO} embryos, we also analysed the expression of the IGL/vLGN markers *Gad1*, *Gata3*, *Npy*, *Penk1*, *Dlx1* and *Arx* in prenatal *Ascl1*^{KO} brains. In these brains, we could detect the expression of all these markers characteristic to WT vLGN/IGL, although the spatial organization of the cells expressing different markers seemed to be disrupted (Fig. 7C,F,I,L). Overall, unlike in the *Foxg1*^{cre}*; Gata2*$^{F/F}$ brain, the vLGN/IGL seems to display a WT gene expression profile in *Ascl1*^{KO}, indicating unaltered subtype composition. In summary, the cell-fate changes observed in the embryos undergoing neurogenesis are reflected in the prenatal brain structures both in the *Foxg1*^{cre}*; Gata2*$^{F/F}$ and *Ascl1*^{KO} mice.

DISCUSSION

GABAergic neurons are important components of several diencephalic nuclei in prethalamus, thalamus and pretectum. However, relatively little is known about the factors that control their differentiation. Here, we demonstrate that neuronal identity and cell-cycle exit are differently regulated in various GABAergic progenitor subpopulations of the developing diencephalon. In addition, we suggest that terminal selector genes activated upon cell-cycle exit are important for the identities of diencephalic neurons. Our results demonstrate distinct selector functions for Gata2 in different regions of the developing diencephalon (Fig. 8; supplementary material Fig. S7). *Gata2* is not expressed, and is therefore not required for GABAergic neurogenesis, in the anterior diencephalon (P3). By contrast, loss of Gata2 function results in an apparently complete GABAergic-to-glutamatergic fate transformation of postmitotic neuronal precursors in the posterior diencephalon (P1). Yet in the medial diencephalon (pTh-R), Gata2 regulates the type of GABAergic neurons produced. In *Gata2* mutants, the pTh-R GABAergic neurons do not switch neurotransmitter identity, but do start to express markers typical for more anterior GABAergic neurons in the P3. To our knowledge, this is the first demonstration of a neuronal fate transformation to

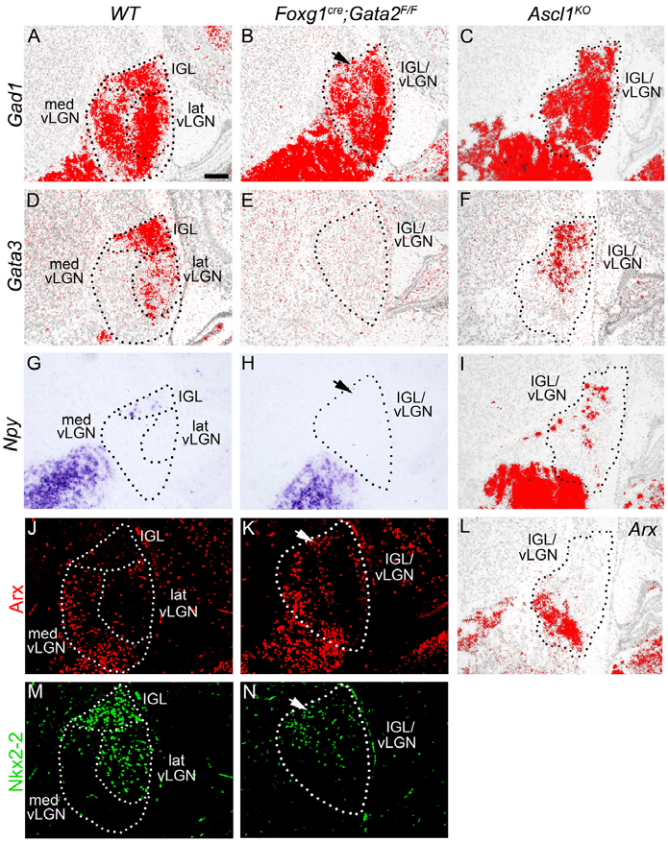

Fig. 7. Distinct alterations in the subcellular composition of the vLGN/IGL complex in *Foxg1^{cre}; Gata2$^{F/F}$* and *Ascl1^{KO}* mouse brains. (**A-I**,**L**) ISH analysis of *Gad1* (A-C), *Gata3* (D-F), *Npy* (G-I) and *Arx* (L) mRNA expression in WT (left), *Foxg1^{cre}; Gata2$^{F/F}$* (middle) and *Ascl1^{KO}* brains (right column). (**J**,**K**,**M**,**N**) IHC analysis of Arx (J,K) and Nx2-2 (M,N) in WT (J,M) and *Foxg1^{cre}; Gata2$^{F/F}$* (K,N) brains. Close-ups from the vLGN/IGL area are presented. Arrows indicate the neuronal subtype switch in the IGL in the *Gata2* mutant. Dotted lines indicate boundaries of the brain nuclei. IGL and lateral (lat) and medial (med) part of vLGN are delineated in the WT. Only the whole vLGN/IGL complex is delineated in the mutants, as the distinct subdivisions cannot be unequivocally distinguished. vLGN, ventral lateral geniculate nucleus; IGL, intergeniculate leaflet. Scale bar: 100 μm.

a different prosomeric identity that is caused by a change in transcription factor activity in postmitotic neuronal precursor cells.

Ascl1 differently regulates GABAergic neuron differentiation in distinct GABAergic neuron subpopulations in the diencephalon

We demonstrate that Ascl1 has distinct functions in the different GABAergic neuron progenitor populations of the diencephalon. In P1, Ascl1 regulates neuronal identity through repression of *Ngn2*. Loss of *Ascl1* resulted in upregulation of *Ngn2* in the VZ progenitors and glutamatergic markers in postmitotic precursors resulting in a complete failure of GABAergic neurogenesis in P1 (supplementary material Fig. S7). These observations are consistent with earlier studies, which showed that the proneural genes *Ascl1* and *Ngn2* cross-repress each other in the forebrain and that *Otx2*-mediated repression of *Ascl1* is required for glutamatergic differentiation in the caudal thalamus (P2, pTh-C) (Parras et al., 2002; Puelles et al., 2006). In contrast to P1, GABAergic neurons

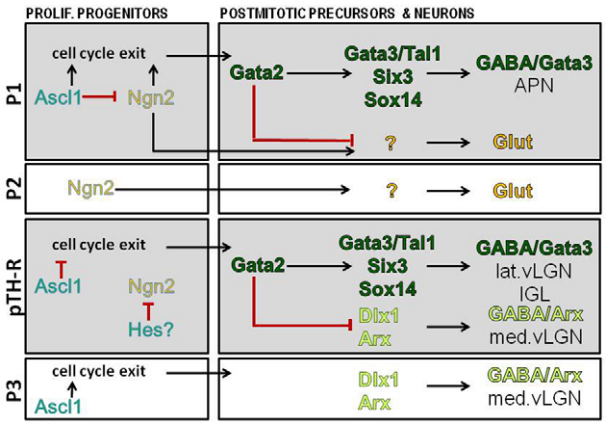

Fig. 8. The functions of Ascl1 and Gata2 in the developing diencephalon. Model for Ascl1- and Gata2-regulated events in proliferative progenitors and postmitotic precursors of diencephalic prosomeres. In neuronal progenitors, Ascl1 promotes cell-cycle exit in P1 and P3, but supports proliferation in the pTh-R region. During postmitotic differentiation, Gata2 controls the expression of downstream transcription factors (Six3, Sox14, Gata3, Tal1) and GABAergic neuron-specific genes (Gad1, Npy) in P1. In pTh-R, Gata2 also counteracts the functions of Dlx1 and Arx that promote the P3 subtype identity of GABAergic cells.

were still produced in the pTh-R and posterior P3 in the *Ascl1^{KO}*. A recent study offers a possible explanation for the different effects of *Ascl1* inactivation in P1 and pTh-R. In zebrafish, the basic helix-loop-helix (bHLH) transcription factor Her6 represses *ngn2* (*neurog3* – Zebrafish Information Network) expression affecting the balance between the proneural genes *ascl1* and *ngn2* and allowing GABAergic neurogenesis (Scholpp et al., 2009). In mouse, as *Her6* is expressed in rostral P2 but not in P1, loss of the *Ngn2* repressor *Ascl1* might lead to *Ngn2* upregulation in P1 but might not be sufficient to induce it in pTh-R. Another bHLH-Orange (bHLH-O) transcription factor Helt, an Ngn repressor in the embryonic midbrain (Nakatani et al., 2007), is also expressed in the diencephalon and is required for normal GABAergic neuron development in the posterior pretectum (Guimera et al., 2006a; Guimera et al., 2006b). Interestingly, loss of *Ascl1* inhibited cell-cycle exit in P3, but appeared to promote it in pTh-R. A recent study identified both cell-cycle repressors and promoters as *Ascl1* targets (Castro et al., 2011). Our results suggest that *Ascl1* might have distinct targets in different neuroepithelic domains. In summary, the proneural gene networks in the proliferative progenitors appear to be wired differently in various GABAergic progenitor populations in the diencephalon (Fig. 8).

Gata2 is required for GABAergic identity in P1and GABAergic subtype identity in pTh-R

Analyses of conditional *Gata2* mutants suggest that in the absence of *Gata2*, neurogenic cell-cycle exit is undisturbed, but the cell-type specification process fails in GABAergic precursors of P1 and pTh-R. However, the P1 and pTh-R neuronal precursors respond differentially to the loss of *Gata2*. Similar to the midbrain (Kala et al., 2009), *Gata2* is an essential postmitotic selector of the GABAergic versus glutamatergic neuron identity in P1. However, the fate transformation in *Gata2* mutant P1 is not restricted to the neurotransmitter identity, as without *Gata2* the *Tal2*-positive precursors also acquire other features of the glutamatergic P1.

By contrast, expression of GABAergic neuron-specific gene expression is not blocked but is delayed by the loss of *Gata2* in pTh-R. Strikingly, our results demonstrate that *Gata2* is a selector of the GABAergic neuron subtype identity in pTh-R. In the absence of *Gata2*, the pTh-R precursors adopt the phenotype of the adjacent GABAergic region in P3, where *Gata2* is normally not expressed. During the normal development of GABAergic neurons in P3, expression of *Arx* and *Dlx1* precedes the expression of *Gad1*. This might also be the reason why *Gad1* activation is delayed in the *Gata2* mutant pTh-R. We hypothesize that only after transformation of the neuronal identity and upregulation of the P3-specific GABAergic neuron regulators can the definitive GABAergic neuron markers be activated in pTh-R in the *Gata2* mutants.

Is *Gata2* a terminal selector gene?

Selection of neuronal identity is thought to depend progressively upon extracellular signal-regulated events in progenitor cells to cell-intrinsic mechanisms at the precursor stage (Edlund and Jessell, 1999). Although cell-cycle exit has been implicated as a crucial period in this transition, the mechanisms are still poorly understood. To date, several postmitotic transcription factors that regulate neurotransmitter identities have been identified. By contrast, the known determinants of regional/prosomeric identities, such as homeodomain transcription factors, operate already at the level of proliferative progenitors. Our results highlight the cell-cycle exit as a culmination point of cell-fate determination and demonstrate that postmitotic loss of a single transcription factor can result in a change of the neuronal identity to the fate of a different prosomere.

Studies of neuronal differentiation in *Caenorhabditis elegans* have led to the concept of 'terminal selector genes', which regulate neuron-type-specific gene batteries (Hobert, 2008). Terminal selector genes were suggested to be expressed throughout the life of a neuron, positively autoregulate themselves, regulate postmitotically expressed terminal differentiation genes, including downstream transcription factors, and be essential for the identity rather than the production of neuronal precursors. Interestingly, *Gata2* appears to fulfil most of these criteria. Although *Gata2* expression is not maintained in mature neurons, this function might be substituted by *Gata3*, which is closely related to *Gata2* and is its transcriptional target. Gata/Tal factors are well known to be involved in positive autoregulatory loops in various cell types (Fujiwara et al., 2009; Lugus et al., 2007) and such autoregulation has also been implicated in the midbrain (Nozawa et al., 2009). *Gata2* is activated upon cell-cycle exit and its targets include several other transcription factors, which might be responsible for more limited aspects of GABAergic neuron identity. As our results suggest that *Gata2* specifies not only the GABAergic neurotransmitter identity, but more generally the identity of the differentiating neuron, *Gata2* is an excellent candidate for a vertebrate 'terminal selector gene'.

Molecular and developmental heterogeneity of the GABAergic neurons in the IGL/vLGN

IGL and vLGN are highly heterogenic nuclei, which consist of several types of (mainly) GABAergic neurons. IGL is a mixture of GABAergic neurons expressing one of the two co-neurotransmitters Npy and Penk1. In this study, *Gata3* was abundantly expressed in both types of IGL neurons. In addition, *Gata3* and *Penk1* were expressed in the lateral part of vLGN in a mosaic pattern. Our results show that *Gata2* is a selector gene for both *Npy*- and *Penk1*-positive GABAergic cells in the IGL and lateral part of the vLGN, whereas other factors control the specification of *Gata3*-negative GABAergic neurons in the vLGN. Our results support the recent conclusions on the origins of the IGL and vLGN neurons (Jeong et al., 2011; Vue et al., 2007) and further suggest that both the *Npy*- and *Penk1*-expressing neurons are derived from the pTh-R. The mechanisms that distinguish between these two neuronal subtypes remain unknown.

Conclusions

The identity of a developing neuron is regulated at multiple levels. Our results demonstrate that, similar to telencephalon, proneural genes are important regulators of neuronal identity in proliferative progenitors in the posterior diencephalon. However, we suggest that the proneural gene networks are different between the diencephalic domains giving rise to distinct GABAergic neuron subpopulations. Another important regulatory step affecting the identity of a developing neuron occurs at the point of cell-cycle exit. We show that the transcription factor *Gata2* acts as a postmitotic cell-fate selector, which can be used differently in different GABAergic precursor populations in the developing diencephalon. During embryonic development, Gata factors have been proposed to act as pioneer transcription factors involved in early marking and opening of genes for expression (Zaret et al., 2008). Consistently, the successful and apparently complete cell-fate transformation upon the loss of *Gata2* suggests that *Gata2* acts relatively early in the transcriptional regulatory cascades leading to differentiation of GABAergic neurons and their specific GABAergic subtypes.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

Supplementary material available online at
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