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There was an error in the ePress version of *Development* **138**, 4315-4326 published on 24 August 2011.

In Fig. 7P, the *P*-values are not given in the legend. For region 2, $P=0.02$; for region 3, $P=0.003$.

The final online issue and print copy are correct.

We apologise to authors and readers for this error.

Pallial origin of basal forebrain cholinergic neurons in the nucleus basalis of Meynert and horizontal limb of the diagonal band nucleus

Ana Pombero[1], Carlos Bueno[1], Laura Saglietti[2], Monica Rodenas[1], Jordi Guimera[3], Alexandro Bulfone[4] and Salvador Martinez[1],*

SUMMARY

The majority of the cortical cholinergic innervation implicated in attention and memory originates in the nucleus basalis of Meynert and in the horizontal limb of the diagonal band nucleus of the basal prosencephalon. Functional alterations in this system give rise to neuropsychiatric disorders as well as to the cognitive alterations described in Parkinson and Alzheimer's diseases. Despite the functional importance of these basal forebrain cholinergic neurons very little is known about their origin and development. Previous studies suggest that they originate in the medial ganglionic eminence of the telencephalic subpallium; however, our results identified *Tbr1*-expressing, reelin-positive neurons migrating from the ventral pallium to the subpallium that differentiate into cholinergic neurons in the basal forebrain nuclei projecting to the cortex. Experiments with *Tbr1* knockout mice, which lack ventropallial structures, confirmed the pallial origin of cholinergic neurons in Meynert and horizontal diagonal band nuclei. Also, we demonstrate that Fgf8 signaling in the telencephalic midline attracts these neurons from the pallium to follow a tangential migratory route towards the basal forebrain.

KEY WORDS: Basal prosencephalon, Tangential migration, Pallium, Fgf8, Reelin, Tbr1, Mouse

INTRODUCTION

Cholinergic neurons of the basal forebrain (ChBF) are located in three main areas: anteromedial [medial septal nucleus (MS), or Ch1], intermediate [vertical and horizontal limbs of the diagonal band nucleus (VDB and HDB), or Ch2/Ch3] and caudolateral [the basal nucleus of Meynert (MB), or Ch4] (Mesulam et al., 1984; Mesulam, 1990). Even though ChBF are functionally related to attention and cognition and their degeneration is implicated in Parkinson and Alzheimer's diseases (Bohnen and Albin, 2010), their origin and migration are still unclear. Previously, ChBF were reported to originate in the subpallium (Sussel et al., 1999; Marin et al., 2000; Marin and Rubenstein, 2001; Xu et al., 2008); however, we have observed a different origin and analyzed the cellular mechanisms involved in ChBF development.

The formation of heterogeneous neuronal regions in the brain depends on complex temporospatial patterns of gene expression, regulating the local production of neurons and cellular migrations from distant germinative areas. Thus, the vertebrate cortex from the pallial telencephalon (pallium) derives from the proliferation of local neurons (Molyneaux et al., 2007; Cobos et al., 2001b) and from the interneuron migration of ventral subpallial structures (Wichterle et al., 1999; Cobos et al., 2001a; Tuorto et al., 2003; Wonders and Anderson, 2006). Although clonal experiments in avian embryos have demonstrated a countercurrent migration of cells from the pallium to the subpallium (Striedter et al., 1998; Cobos et al., 2001a), this has yet to be analyzed in detail.

In this work, we analyzed pallio-subpallial neuronal migration in mouse embryos. Neuroepithelial transplants in organotypic cultures of GFP and control mouse brains were performed. We observed a tangential migration of cells positive for calretinin and reelin from the ventral pallium (VP) to the subpallium. Moreover, since these migratory cells also expressed the pallial gene *Tbr1*, we studied ChBF in *Tbr1* mutant (*Tbr1⁻/⁻*) mice, whose brains present a severe disruption of the VP (Bulfone et al., 1998; Hevner et al., 2001; Hevner et al., 2002). In *Tbr1⁻/⁻* mice, no cholinergic cells were detected in the HDB, MB (the corticopetal ChBF) or the subpallial-derived amygdala. Finally, through in vitro assays and analysis of *Fgf8* hypomorphic mice, we substantiate Fgf8 as a candidate attractive signal from the medioventral telencephalon.

Thus, our study describes a new, pallial origin of the corticopetal ChBF. The differences in ChBF ontogenesis shown here might explain their different connection patterns (cortex versus hippocampus) and functions in cognitive processes, as well as their specific responses to pathological stimuli. Conversely, the common ontogenetic origin of various ChBF nuclei and olfactory structures might explain the early co-morbidity in neurodegenerative diseases.

MATERIALS AND METHODS
Animals
All animal experiments were performed in compliance with the Spanish and European Union laws on animal care in experimentation (Council Directive 86/609/EEC), as well as being analyzed and approved by the Animal Experimentation Committee of our university.

Control mice (ICR) and CD1-GFP transgenic mice (Hadjantonakis et al., 2002) were used for the organotypic cultures and transplant experiments at E14.5. *Tbr1⁻/⁻* mice (Bulfone et al., 1998) were analyzed at P0 and P21, using *Tbr1⁺/⁻* mice as controls. *Fgf8* mutant mice (Meyers et al., 1998) were studied at E14.5-16.5; mutant alleles were maintained on a mixed 129/CD1 Swiss genetic background. *Fgf8^{null/+}* and *Fgf8^{neo/+}* mice were crossed to produce *Fgf8^{neo/null}* embryos, and *Fgf8^{neo/+}* and *Fgf8^{neo/+}* to

[1]Instituto de Neurociencias (UMH-CSIC), 03550 San Juan, Alicante, Spain. [2]CNR Institute of Neuroscience and Department of Medical Pharmacology, University of Milano, 20129 Milano, Italy. [3]HMGU-Institute of Developmental Genetics, 85764 Munich, Germany. [4]Bioflag, Polaris Science Park, 09010 Pula, Italy.

*Author for correspondence (smartinez@umh.es)

produce *Fgf8^{neo/neo}* embryos. PCR genotyping was performed as previously described (Hebert and McConnell, 2000). *Fgf8^{neo/+}* embryos were used with wild-type embryos as controls.

Organotypic slice culture and transplants

Organotypic slice cultures of E14.5 and E15.5 mouse brains were prepared as previously described (Anderson et al., 1997). Sections including medial ganglionic eminence (MGE) and VP were incubated on floating polycarbonate membrane filters (8 µm pore size, Nunc). Then, the graft was performed as described by Marin et al. (Marin et al., 2001). Depending on the experiment, either part of or the whole VP was grafted homotopically using GFP-expressing mice as donors and control mice as hosts. Grafted sections were incubated for 24 hours, fixed with 4% PFA and mounted. In some cases, the sections were paraffin embedded, cut and processed immunohistochemically using: anti-GFP polyclonal antibodies (1:1000, Molecular Probes, Leiden, The Netherlands; 1:500, Aves Labs, Tigard, OR, USA), anti-reelin monoclonal antibody (1:500, Chemicon, Temecula, CA, USA), anti-Tbr1 polyclonal antibody (1:25, Abcam, Cambridge, UK), anti-choline acetyltransferase (ChAT) polyclonal antibody (1:100, Chemicon), anti-Nkx2.1 polyclonal antibody (1:1000, anti-TTF from Biopat Immunotechnologies, Italy) or anti-Tuj1 monoclonal antibody (1:1000, anti-neuronal class III beta-tubulin from Covance, Berkeley, CA, USA). For fluorescence microscopy, fluorescent secondary antibodies were used (Alexa series, Molecular Probes).

To quantify the migration distance in slice cultures, the longest distance covered by the pallial cells from the graft-host boundary was measured in each experimental paradigm: whole subpallium, lateral ganglionic eminence (LGE) or MGE grafts. Statistical analysis was performed with the Mann-Whitney sum test using SigmaStat software.

Co-cultures in Matrigel

Co-cultures were performed as described by Marin et al. (Marin et al., 2003). E14.5 mice were processed as described above to obtain brain slices. Small pieces of MGE and VP were placed on top of the Matrigel drops (BD Biosciences, Franklin Lakes, NJ, USA) and covered with Matrigel diluted 1:1 in Neurobasal culture medium (Gibco Life Technologies). Co-cultures of MGE and VP were separated by 400 µm and incubated for 24 to 48 hours.

Explant incubation with Fgf8- and SU5402-soaked beads

Heparin on acrylic beads (Sigma) or Affi-Gel Blue Gel beads (Bio-Rad) were preincubated overnight with recombinant mouse Fgf8b (1 µg/µl; R&D Systems) or with the Fgf8 inhibitor SU5402 (40 µM; Calbiochem, La Jolla, CA, USA), respectively. Controls employed beads with BSA (1 µg/µl; Sigma). The soaked beads were co-cultured for 48 hours with VP explants in Matrigel cultures as described above.

To quantify cell migration in cultures, the VP piece was subdivided into four sectors depending on the bead position, and then cells migrating from the sectors nearest to each bead were counted, as well as from the sectors on the opposite side, which were used as controls (see Fig. 7O). Statistical analysis was performed by Student's *t*-test using SigmaPlot software.

Fgf8-expressing cells
Cell culture
HEK293T cells were cultured in 10% fetal bovine serum (FBS)-supplemented Dulbecco´s modified Eagle´s medium (DMEM; Sigma), 100 units/ml penicillin-streptomycin (Sigma) and 2 mM L-glutamine (Sigma).

Mammalian expression vectors
pCMV-eGFP-IRES1hyg was generated by subcloning eGFP cDNA as a *Bam*HI-*Not*I fragment from pEGFP-N1 (Clontech, Cambridge, UK) into *Bam*HI and *Not*I digested pIRES1hyg (Clontech, Cambridge, UK).

The mouse Fgf8b coding region without stop codon was subcloned into the *Eco*RI site of a pEGFP-N1 vector to obtain pCMV-Fgf8b-eGFP-an.

The mRFP1 coding region without stop codon and the cDNA fragment encoding the leader sequence and GPI signal of the human decay-accelerating factor (CD55 molecule, NM_000547) were subcloned into *Bam*HI and *Not*I digested pIRES1hyg vector to generate pCMV-mRFP1-GPI-IRES1hyg.

Transfection methods
One day before transfection (with Lipofectamine 2000, Invitrogen), the cells were seeded at a density of 0.5×10^5 cells/cm^2 in multi-well (12- or 24-well) plates. The cells were incubated with DNA-lipid complexes for 4 hours (following the supplier's instructions), after which the lipofection mix was removed and replaced with fresh medium. Drug selection of stable transfectants was performed with 50-100 µg/ml hygromycin B (hyg; Calbiochem) and G418 disulfate salt solution (Sigma).

Mutant analysis
Tbr1 mutant brain paraffin sections were immunostained following the protocol described above. The antibodies used were: anti-Pax6 monoclonal (1:5, Developmental Studies Hybridoma Bank, Iowa City, IA, USA), anti-ChAT polyclonal (1:100, Chemicon), anti-reelin monoclonal (1:500, Chemicon) and anti-Nkx2.1 polyclonal (1:1000, Biopat Immunotechnologies). To quantify differences between wild type and *Tbr1^{-/-}* in the basal forebrain (BF), cells expressing Pax6, ChAT and reelin were counted at P0 and P21 (*n*=3 for each stage). Cell counts were performed using a parallel series of sections stained by these specific markers in three different control embryos and three mutant embryos, counting four consecutive sections of the BF in each series. Statistical analysis was performed by Student's *t*-test using SigmaPlot software.

Fgf8 mutant mice were analyzed at E14.5-16.5. Paraffin coronal sections were immunostained with anti-ChAT polyclonal antibody (1:100, Chemicon) and stained with Cresyl Violet.

RESULTS
Tangential migration from the ventral pallium to the subpallium

In order to detect pallial-subpallial migration, we prepared sagittal explants from E14.5 control mice (ICR), where VP from GFP mice (GFP-VP) was homotopically grafted after the excision of equivalent host territories. In some experiments, GFP-VP was transplanted in place of the host anterior olfactory nucleus (AO) and, specifically, the olfactory cortex close to the pallio-subpallial boundary (Fig. 1A). After 24 hours in culture, a large number of GFP$^+$ cells were detected throughout the superficial subpallium (*n*=7; Fig. 1A-C,I), whereas fewer GFP$^+$ cells were detected scattered throughout the striatum (ST) (*n*=6; Fig. 1A,B,D). Immunohistochemical analysis of the explants confirmed that the GFP$^+$ migrating cells in the olfactory tubercle (Tu) and superficial subpallium were positive for reelin (Fig. 1E-H), Tbr1 (Fig. 1I-L) and calretinin (CR; also known as calbindin 2 – Mouse Genome Informatics) (data not shown), but negative for Nkx2.1 (Fig. 1M-P). Moreover, to confirm that these migrating cells were young postmitotic neurons we demonstrated that the GFP$^+$ migrating cells expressed class III beta-tubulin (Tubb3) in their trailing process, a neuron-specific protein that is recognized by Tuj1 monoclonal antibody (Fig. 1Q-S).

At E14.5, the presence of fusiform CR-positive neurons in the superficial subpallium suggests a tangential migration through the subpial regions of Tu and preoptic area (POA) (Fig. 1T). Moreover, these CR$^+$ cells appeared oriented in opposite directions, away and towards the VP, sharing a common migratory substrate (Fig. 1U), and were also reelin$^+$ (Fig. 1V). Pax6 and ChAT double-positive basal forebrain (BF) neurons were detected (Fig. 1W), indicating the maintenance of this pallial marker (Carney et al., 2009) in ChBF. Interestingly, *Tbr1* is expressed in postmitotic cells of the developing cortex from E10 to adulthood (Bulfone et al., 1995). Also, *Tbr1* can be detected in the olfactory bulb (OB) and AO, as well as in the POA (Bulfone et al., 1995; Bulfone et al., 1998). In the E14.5
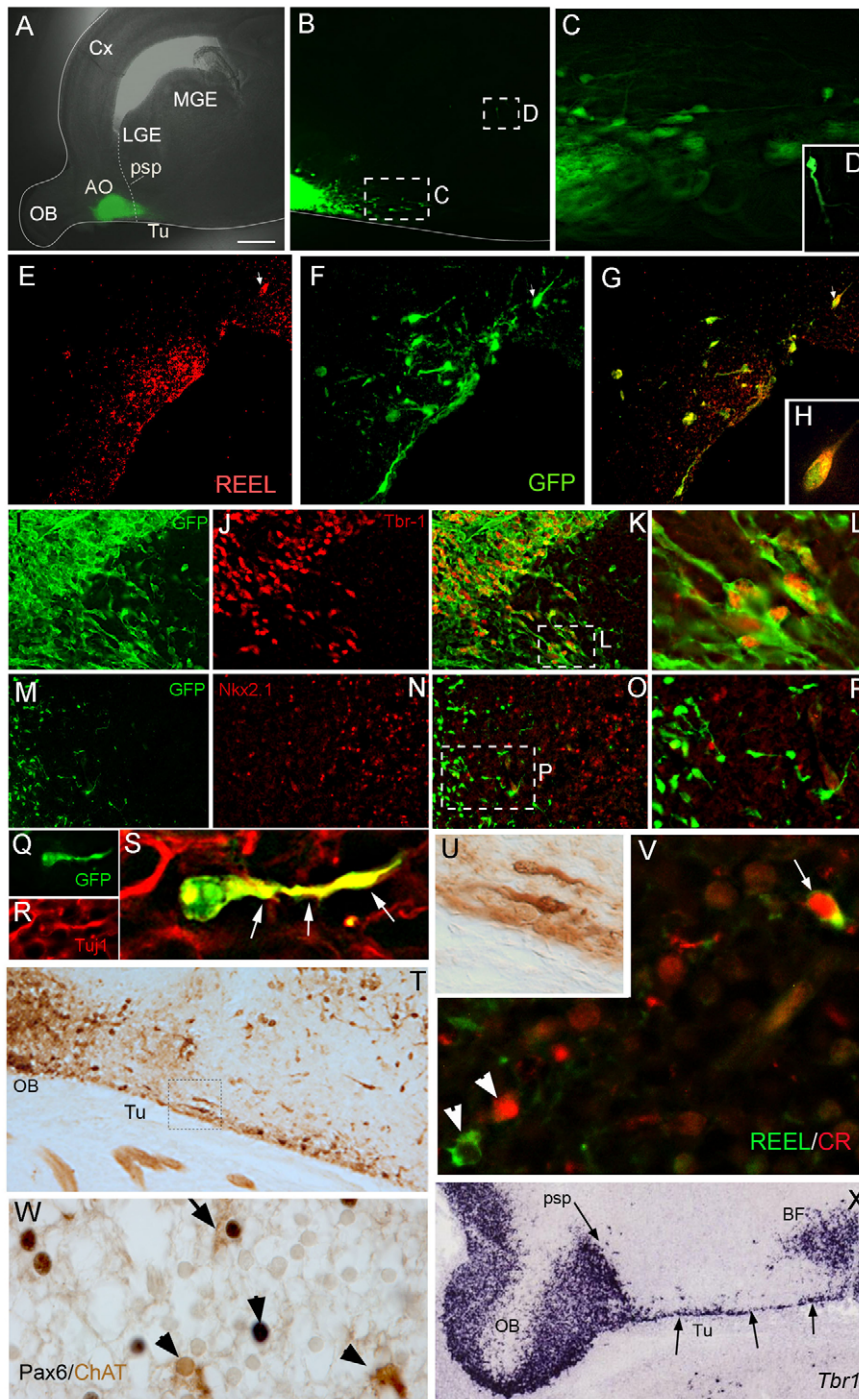
Fig. 1. Pallio-subpallial migration in the mouse embryo. (**A-D**) A 250 μm brain slice (A) with a VP-GFP graft (rostral is always to the left). After 24 hours, GFP⁺ cells were detected in the subpial region (B,C) and in the mantle layer (B,D) of the subpallium. C and D are higher magnification images of B. (**E-H**) Reelin (E) and GFP (F) immunostaining in a 7 μm sagittal section. (G) Merged image of E and F. (H) Higher magnification image of the labeled cell in E and F (arrow). (**I-P**) GFP brain slices (250 μm) with a wild-type (WT) subpallial graft. (I-L) After 24 hours, GFP⁺ and Tbr1⁺ cells migrated from the host VP to the grafted subpallium (I,K); L is a higher magnification image of the boxed area in K, illustrating the presence of Tbr1 protein in most of the nuclei of migrating cells. (M-P) GFP-VP migrating cells on the experimental slides do not show Nkx2.1 immunoreactivity. (**Q-S**) The neuronal character of GFP-VP migrating cells (Q) was confirmed by the presence of Tuj1 immunoreactivity (R) in their training process (arrows in S). (**T,U**) Sagittal sections immunostained with CR antibody at E14.5. Cells migrating both rostrally and caudally were detected in the Tu. U is a magnification of the boxed area in T. (**V**) Reelin and CR co-expression in the Tu (arrow); arrowheads indicate reelin (green) and CR (red) single-labeled neurons. (**W**) Double immunostaining with anti-Pax6 (black nuclei) and anti-ChAT (brown cytoplasm) in the Tu. Arrows indicate double-stained cells and arrowheads label single-labeled cells. (**X**) *Tbr1* is detected in the Tu and BF of an E14.5 mouse embryo (from Eurexpress data). AO, anterior olfactory nucleus; BF, basal forebrain; Cx, cortex; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; OB, olfactory bulb; psp, pallio-subpallial boundary; Tu, olfactory tubercle; VP, ventral pallium. Scale bar: in A, 500 μm for A, 170 μm for B,X, 40 μm for C-G,I-K,M-O,T, 10 μm for H,L,P-R,U-W, and 5 μm for S.

mouse embryo, *Tbr1*-expressing cells were mapped within the superficial subpallium as a cellular stream connecting the VP to the BF region [Fig. 1X; from Eurexpress (Diez-Roux et al., 2011)], confirming that *Tbr1* expression is a suitable marker for pallio-subpallial migration in mouse.

At E15.5 and P14, many cells at the surface of the Tu expressed *Tbr1* (Fig. 2A-D), some of which could be cholinergic neurons (ChAT⁺) (Fig. 2D-G). In the BF, 40% of cholinergic neurons were shown to co-express ChAT and Tbr1 or Nkx2.1 (Fig. 2H,L). However, in the MB there were more pallial Tbr1⁺/ChAT⁺ neurons (Fig. 2I-K) than subpallial Nkx2.1⁺/ChAT⁺ neurons (Fig. 2L-O) (60% and 20%, respectively).

The pallial origin of ChBF was explored by examining ChAT expression in experimental VP migrating cells. To this end, we performed immunohistochemistry on grafted slices (*n*=20) and observed that many VP migrating neurons expressed ChAT when they reached the BF (30-50% of GFP⁺ cells in MB and HDB expressed ChAT; Fig. 3A-F).

Absence of pallio-subpallial ChBF migration in *Tbr1* mutant mice

In Tbr1-deficient mice, the primary olfactory cortex and claustrum (lateral pallium and VP), OB, AO and pallial amygdala (which are VP-derived areas) are disrupted (Bulfone et al., 1998; Hevner et al.,
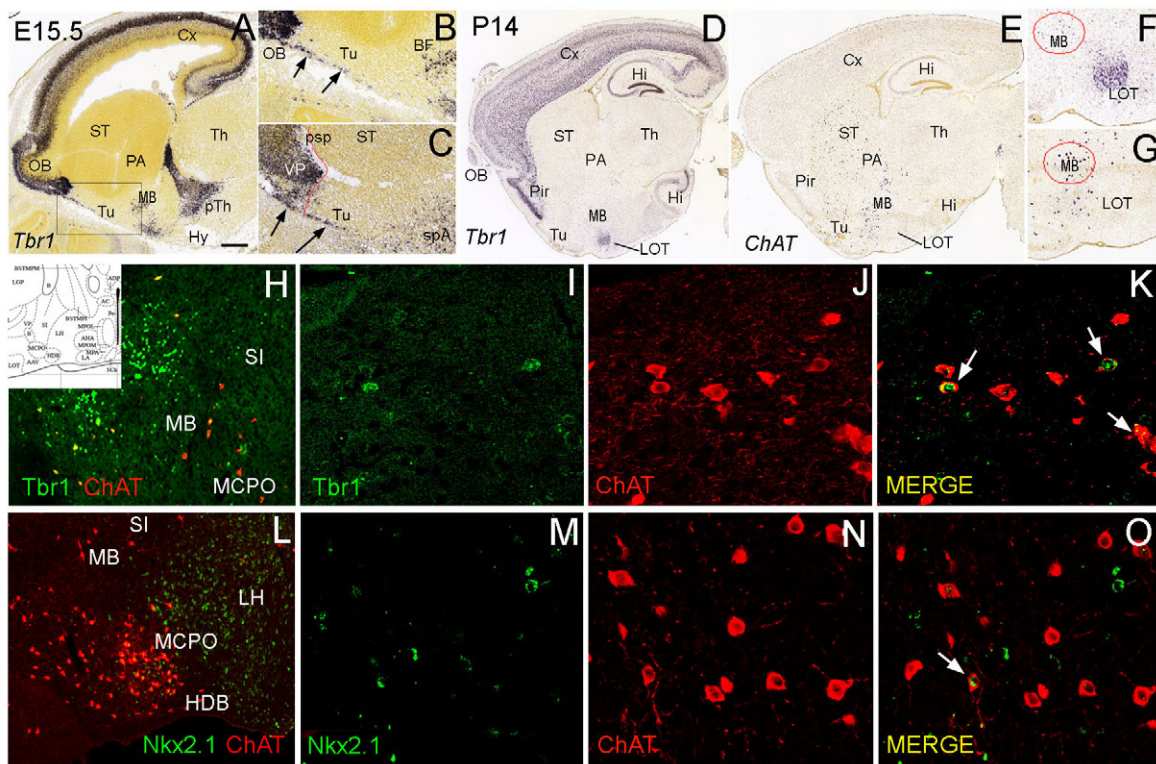
Fig. 2. Tbr1 in ChAT cells of the basal forebrain. (**A-G**) *Tbr1* expression in E15.5 (A-C) and P14 (D) mouse sagittal sections (rostral is always to the left). Arrows in B and C indicate superficial migrating *Tbr1*^{+} cells in the Tu. ChAT-expressing cells at P14 (E,G) colocalize with *Tbr1*^{+} cells (D,F) mainly in the MB nucleus (red circle in F,G) (from Allen Institute, Developing-Mouse-Brain). (**H-O**) *Tbr1* (H) and *Nkx2.1* (L) were detected in ChAT[+] regions in consecutive transverse sections of the adult mouse brain. (I-K) Cellular colocalization of *Tbr1* and ChAT in neurons of the MB nucleus. Few neurons of the MB colocalize both Nkx2.1 and ChAT (M-O). Arrows in K and O indicate double-labeled neurons. AAV, anterior amygdaloid area, ventral part; AC, anterior commissural nucleus; ADP, anterodorsal preoptic nucleus; AHA, anterior hypothalamic area, anterior part; B, basal nucleus (Meynert); BSTMPI, bed nucleus of the stria terminalis, medial division, posterointermediate part; BSTMPM, bed nucleus of the stria terminalis, medial division, posteromedial part; HDB, nucleus of the horizontal limb of the diagonal band; Hi, hippocampus; Hy, hypothalamus; LA, lateroanterior hypothalamic nucleus; LH, lateral hypothalamic area; LGP, lateral globus pallidus; LOT, nucleus of the lateral olfactory tract; MB, Meynert basal nucleus; MCPO, magnocellular preoptic nucleus; MPA, medial preoptic area; MPOL, medial preoptic nucleus, lateral part; MPOM, medial preoptic nucleus, medial part; PA, pallidum; Pe, periventricular hypothalamic nucleus; Pir, piriform cortex; pTh, prethalamus; SCh, suprachiasmatic nucleus; SI, substantia innominata; ST, striatum; Th, thalamus (for other abbreviations see Fig. 1). Scale bar: in A, 500 µm for A,D,E, 170 µm for B,C,F,G,H,L, and 15 µm for I-K,M-O.

2001). Since VP is the source of pallio-subpallial migratory cells, we examined whether this migration is affected in *Tbr1* mutant mice. It has been reported that the majority of the striatal and Tu interneurons derive from the medial ganglionic eminence (MGE) (Marin et al., 2000). We analyzed *Nkx2.1* expression in *Tbr1* mutant mice. *Nkx2.1* is expressed in part of the septum (Se), pallidum (PA), substantia innominata (SI) and the bed nucleus of the stria terminalis (Puelles et al., 1999; Sussel et al., 1999; Shimamura et al., 1995). *Nkx2.1* expression was normal in these nuclei in *Tbr1*^{−/−} brains (Fig. 4A-F), suggesting that the subpallium was not affected.

Tbr1^{−/−} mice were analyzed at P0 (*n*=3; Fig. 5A-H) and P21 (*n*=4; Fig. 5I-Q) for the presence of migrating pallial neurons in the subpallium. According to previous data, the targets for the migrating cells are the Tu, HDB and MB. Thus, three markers were used to identify VP migrating cells: reelin (D'Arcangelo et al., 1997; de Bergeyck et al., 1998), Pax6 (Stoykova et al., 1997; Chapouton et al., 1999; Toresson et al., 2000; Yun et al., 2001) and choline acetyltransferase (ChAT). Immunohistochemical analysis of paraffin-embedded sagittal and coronal sections was performed (Fig. 5) using *Tbr1*^{+/−} mice as controls. At P0, the

controls presented an abundance of reelin^{+} cells in the Tu [22±1 cells/section in four consecutive sections (cs); *n*=3; Fig. 5A,B,D,R], whereas the mutant did not express this marker (*n*=3; Fig. 5C,E,R). Pax6 was detected in deeper strata of subpallial areas than reelin in the control mice (54.5±5 cells/section in four cs; *n*=3; Fig. 5A,B). In *Tbr1*^{−/−} embryos there were significantly fewer Pax6^{+} cells (31.7±1.7 cells/section in four cs; *n*=3; Fig. 5C,R), and these gradually decreased towards the subpallium, resulting in very few dispersed cells in the HDB. Lastly, ChAT^{+} cells were detected in the BF of control mice at P0, as well as in the ventral pallidum (PV) and ST (47±3 cells/section in four cs; *n*=3; Fig. 5F,G,R), whereas very few ChAT^{+} cells were observed in BF of *Tbr1*^{−/−} brains (22±1 cell/section in four cs; *n*=3; Fig. 5H,R).

At P21, the differences between control and mutant mice were even more evident (Fig. 5I-Q,S). *Tbr1* mutants either lacked or expressed very low levels of reelin (Fig. 5K,S), Pax6 (Fig. 5S) and ChAT (44±3 versus 11±1 cells/section in four cs of WT versus *Tbr1*^{−/−}, respectively; Fig. 5Q,S) in MB and HDB. Analysis of this region by Cresyl Violet staining further confirmed BF structural anomalies (Fig. 5L-N,P).
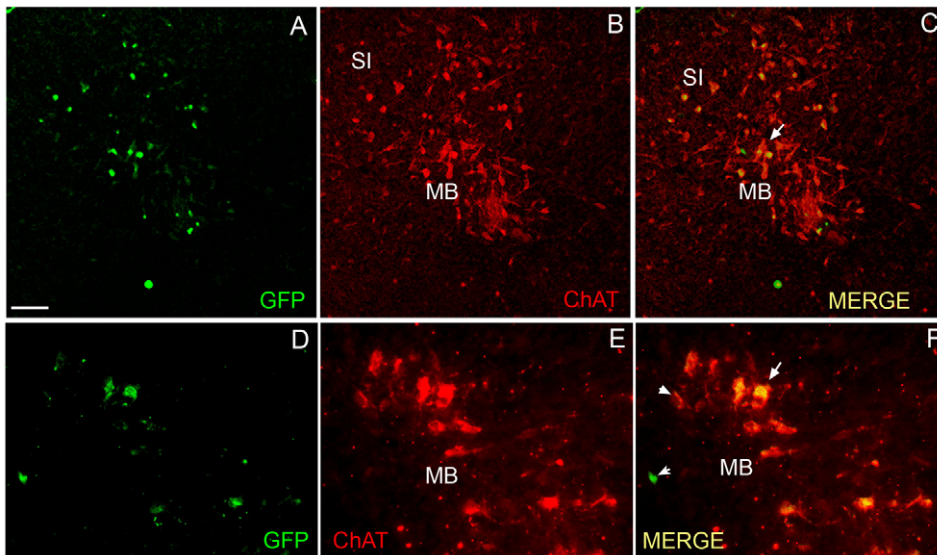
Fig. 3. ChAT is expressed in cells migrating from the ventral pallium. (A-C) Sagittal paraffin sections of VP-GFP grafted into the VP of a control mouse brain explant. GFP+ cells from the graft were observed in the BF, near the MB nucleus (A). (D-F) Higher magnifications of sections from another experimental explant. In B and E, cells expressing ChAT in the MB are shown (red). C and F are merged images of A and B and of D and E, respectively. GFP/ChAT (green and red, respectively) double-positive cells were detected (arrows); arrowheads indicate single-stained cells. For abbreviations, see previous figure legends. Scale bar: in A, 40 μm for A-C, 15 μm for D-F.

Migration mechanisms: possible chemo-attraction from the medial subpallium

A subpallial origin of the cholinergic neurons in the ST and BF, including Tu, has been proposed previously (Sussel et al., 1999; Marin et al., 2000; Marin and Rubenstein, 2001), as *Nkx2.1* mutants lack cholinergic neurons in the subpallium, including the striatal interneurons and BF projecting neurons. These defects have also been associated with a loss of *Lhx8* expression in the developing ventral telencephalon (Zhao et al., 1999; Zhao et al., 2003). Alternatively, subpallium disruption might also eliminate an attractive signal for the pallial migrating cells, resulting in a lack of both subpallial and pallial ChBF.

To analyze whether the medial subpallium (MGE and Se) contains attractive signals for pallio-subpallial migration, explants were prepared from E14.5 GFP mice. Different subpallial areas from control embryos were transplanted into GFP slices. First, we homotopically transplanted the whole subpallium from control to GFP explants (n=16). After 24 hours, a large number of GFP+ cells migrated ventrally from the VP (GFP+ Tbr1+) to the grafted subpallium (Fig. 6A-F). There were numerous GFP+ cells in the mantle layer of the lateral ganglionic eminence (LGE) that migrated further along the superficial areas of the grafted subpallium (Fig. 6B,C,M,N). When only the LGE was transplanted (n=16; Fig. 6G-I), there was a substantial reduction in the number and migratory distance of GFP+ cells migrating into the grafted LGE (Fig. 6H,I,M,N). In these cases, GFP+ pallial-derived cells were detected clustered near the border of the graft. These results are in accordance with the existence of medial attractive signals that act upon pallial-derived neurons. When the medial area of the subpallium was grafted without the LGE, the number of GFP+ cells migrating into the MGE was also reduced (n=15; Fig. 6J-N). In this case, only a few pallial-derived cells were detected invading the grafted MGE (Fig. 6K-N). This observation might indicate that the MGE represents a non-permissive substrate for the pallial cells. This would fit with the superficial localization of the migratory cells in the subpallium, with little invasion into deep LGE domains, and no migration into MGE derivates.

Thus, our results confirm that, in E14.5 mouse embryos, VP neurons migrate tangentially through superficial regions of the subpallium and populate the BF. Also, we show that the medial subpallium has an attractive role for VP cells.

In order to further confirm the chemo-attractive effect of the medial telencephalon, small portions of wild-type (WT) MGE or LGE and GFP-VP were co-cultured in a Matrigel matrix (n=26; Fig. 6O,P). After 36 hours, MGE cells were detected migrating towards the neocortex, as previously seen (Marin et al., 2003), and in over half of the explants (14 of 26) GFP+ pallial cells were observed moving towards the MGE explant (Fig. 6O). Parallel experiments using LGE never showed that VP-derived cells migrate preferentially towards LGE explants (n=12; Fig. 6P). These results corroborate the existence of diffusible signals deriving from the MGE that stimulate the migration of VP cells.

Fgf8 activates and attracts the migration of ventral pallial cells

Fgf8 is produced by the anterior neural ridge (ANR) and is involved in cell survival and telencephalic patterning (Crossley and Martin, 1995; Shimamura and Rubenstein, 1997; Crossley et al., 2001; Kawauchi et al., 2005; Storm et al., 2003; Storm et al., 2006; Bailey et al., 2006). Recently, it has been shown that Fgf8 is required for rostral pallium specification and for the generation of CR+ Cajal-Retzius cells (Zimmer et al., 2010). Moreover, its early expression in the rostral tip of the neural tube persists at later stages in the commissural plate of the telencephalon midline (Fig. 7A) (Crossley et al., 2001), suggesting that Fgf8 might also play a role in pallio-subpallial tangential migration.

Although a strong telencephalic phenotype in *Fgf8* hypomorphic mutants has previously been reported (Storm et al., 2006), the BF was not carefully analyzed. First, we confirmed the progressive reduction of *Fgf8* expression in the anterior telencephalon in *Fgf8^neo/neo* and *Fgf8^neo/null* mice (Fig. 7A-C). Then, we studied the distribution of ChBF in these progressive *Fgf8* hypomorphic mice. Coronal sections of E16.5 brains were immunostained with anti-ChAT antibody and counterstained with Cresyl Violet. Whereas ChAT was detected in MB and HDB cells in WT brains (Fig. 7D,G), this was strongly reduced in *Fgf ^neo/neo* (Fig. 7E,H) and disappeared altogether in the *Fgf8^neo/null* mouse (Fig. 7F,I). Cresyl Violet showed that the BF region and medial subpallium were structurally preserved in the mutants (Fig. 7E,H). These results suggested that the lack of ChAT expression might be a consequence of migratory alterations resulting from the reduction of Fgf8 signaling. In
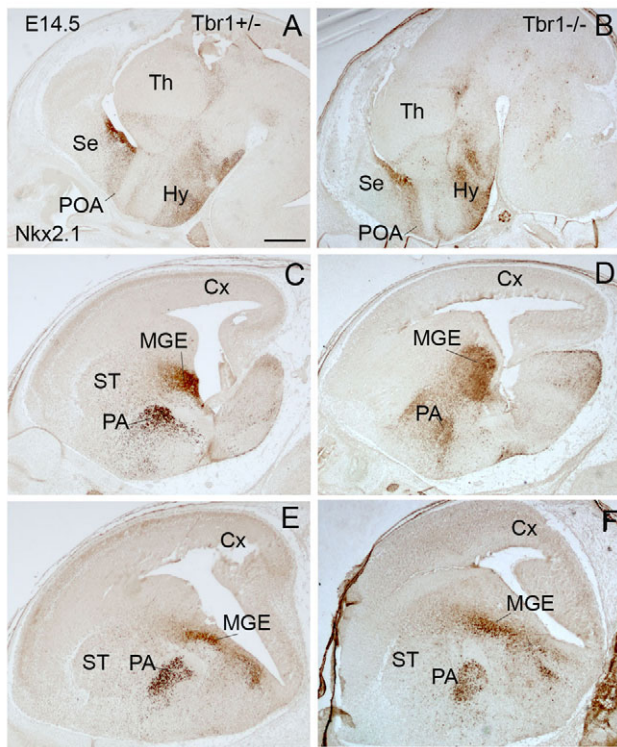
Fig. 4. Nkx2.1 expression in *Tbr1* mutant mice. (A-F) Nkx2.1 immunostaining in sagittal sections (rostral is to the left) from a control mouse (A,C,E) and a *Tbr1* mutant mouse (B,D,F) at E14.5. Nkx2.1 is strongly expressed in the medial septum (A,B), hypothalamus (A,B), MGE (C-F) and pallidum (C-F). No significant differences were detected in the subpallium of *Tbr1*−/− versus control mice. POA, preoptic area; Se, septum (for other abbreviations, see previous figure legends). Scale bar: 500 μm.

order to experimentally demonstrate the attractive role of Fgf8, we grafted *Fgf8*-expressing cells to cultured slides where medial pallium was excised, and this recovered the pallio-subpallial cellular migration (*n*=9/12; Fig. 7J-M). Experiments with control cells did not show any increase in cell movements (*n*=5/5; Fig. 7N). Using VP explants in Matrigel cultures we demonstrated the diffusion of Fgf8 signal in the matrix and the negative effects of factors that block Fgf8 activity (SU5402) in pallial migrating cells (Fig. 7O,P). Altogether, these results implicate Fgf8 in the guidance of cells migrating from the VP towards the superficial area of the subpallium.

DISCUSSION
Pallio-subpallial neuronal migration
Tangential migration is defined as the free movement of cells unrelated to radial glia cells (Rakic, 1972). In the vertebrate telencephalon, tangential migration generally consists of cell movements from the subpallium to the pallium of GABAergic interneurons (De Carlos et al., 1996; Anderson et al., 1997; Lavdas et al., 1999; Marin and Rubenstein, 2003; Marin and Rubenstein, 2001; Cobos et al., 2001a; Olivier et al., 2001; Tuorto et al., 2003; Metin et al., 2007). Here, we describe a countercurrent pallio-subpallial tangential migration in the developing mouse telencephalon. Although this migratory pathway was previously observed in chick and mouse embryos (Fishell et al., 1993; Chapouton et al., 1999; Puelles et al., 2000),

no detailed analysis was performed. Recently, it has been shown that *Emx1*-positive progenitors migrate into the dorsal ST and amygdala to generate GABAergic medium spiny and excitatory neurons, respectively (Cocas et al., 2009). Garcia-Moreno et al. reported that cells from the rostromedial telencephalon move ventrally, following a ventromedial route, and populate Broca's diagonal nuclei and Tu (Garcia-Moreno et al., 2007). These ventromedial migrating cells express reelin and possibly represent medial components of our pallio-subpallial migration. However, the cholinergic differentiation of migrating neurons from VP-derived amygdala (basal and lateral amygdaline complex) in the subpallial amygdala (central complex) (Medina et al., 2004) has not been reported previously. The functional importance of these cholinergic amygdaline cells is clear because stress reactions are regulated by changes in microRNAs acting through the cholinergic function in the central amygdala (Meerson et al., 2010), as well as by nicotine dependence mechanisms (for a review, see Markou, 2008).

Cells expressing *Tbr1* and *Pax6* were previously shown to migrate from the VP to superficial areas of the subpallium (Puelles et al., 2000; Carney et al., 2009). Also, we have demonstrated that Pax6+ cells migrate from the VP into the superficial layers of the BF in mouse embryos. These cells also express reelin, a key protein for neuronal migration and cortical layering (D'Arcangelo et al., 1995; Ogawa et al., 1995; Pearlman et al., 1998). However, reelin is widely expressed in chick embryos, suggesting that its function is not exclusively to control laminar development (Bernier et al., 2000). For example, reelin-activated cell adhesion mechanisms (Hoffarth et al., 1995) could mediate cellular interactions along the superficial migratory stream in the subpallium (Fig. 3I,J). In agreement with these data, a significant alteration in the ChBF has been reported in heterozygous reeler mice (Sigala et al., 2007).

Previous results have identified the existence of a lateral migratory stream through the pallio-subpallial boundary towards the olfactory cortex in mouse (De Carlos et al., 1996; Medina et al., 2004; Carney et al., 2009). Although VP migrating cells may partially follow this stream on their way to the BF, the pallio-subpallial migration must include more dorsal pallial regions because in most of our subpallial-grafted explants (Figs 6 and 7) we have excised the boundary territory from the donor to expose the VP to the grafted subpallium (abundant GFP-Tbr1+ cells and cortical histology at the host edge confirmed the VP origin of migrating cells).

Taken together, our results demonstrate that, in addition to the ventrodorsal tangential migration of interneurons and oligodendrocytes in the developing telencephalon of sauropsids, a dorsoventral migration of cholinergic neurons contributes to the cellular complexity of the basal prosencephalon (Fig. 8A).

### Cholinergic cell deficiency in *Tbr1*−/− mutant mice
The transcription factor *Tbr1* is expressed in cortical glutamatergic neurons of the preplate and cortical layer 6 (Bulfone et al., 1995; Hevner et al., 2001). In this work, we analyzed the pallio-subpallial migration pathway in *Tbr1*−/− mice. Reelin, Pax6 and ChAT expression were absent or strongly decreased in the mutant subpallium. Previous studies have reported that ChAT-positive cells in the subpallium originate from the MGE (Marin et al., 2000; Xu et al., 2008). As such, ChAT expression in the BF should not be affected in *Tbr1*−/− mice as both the MGE and *Nkx2.1* expression in the subpallium were normal. Furthermore, the fate map analysis of *Nkx2.1* expression reported by Xu et al. demonstrated that
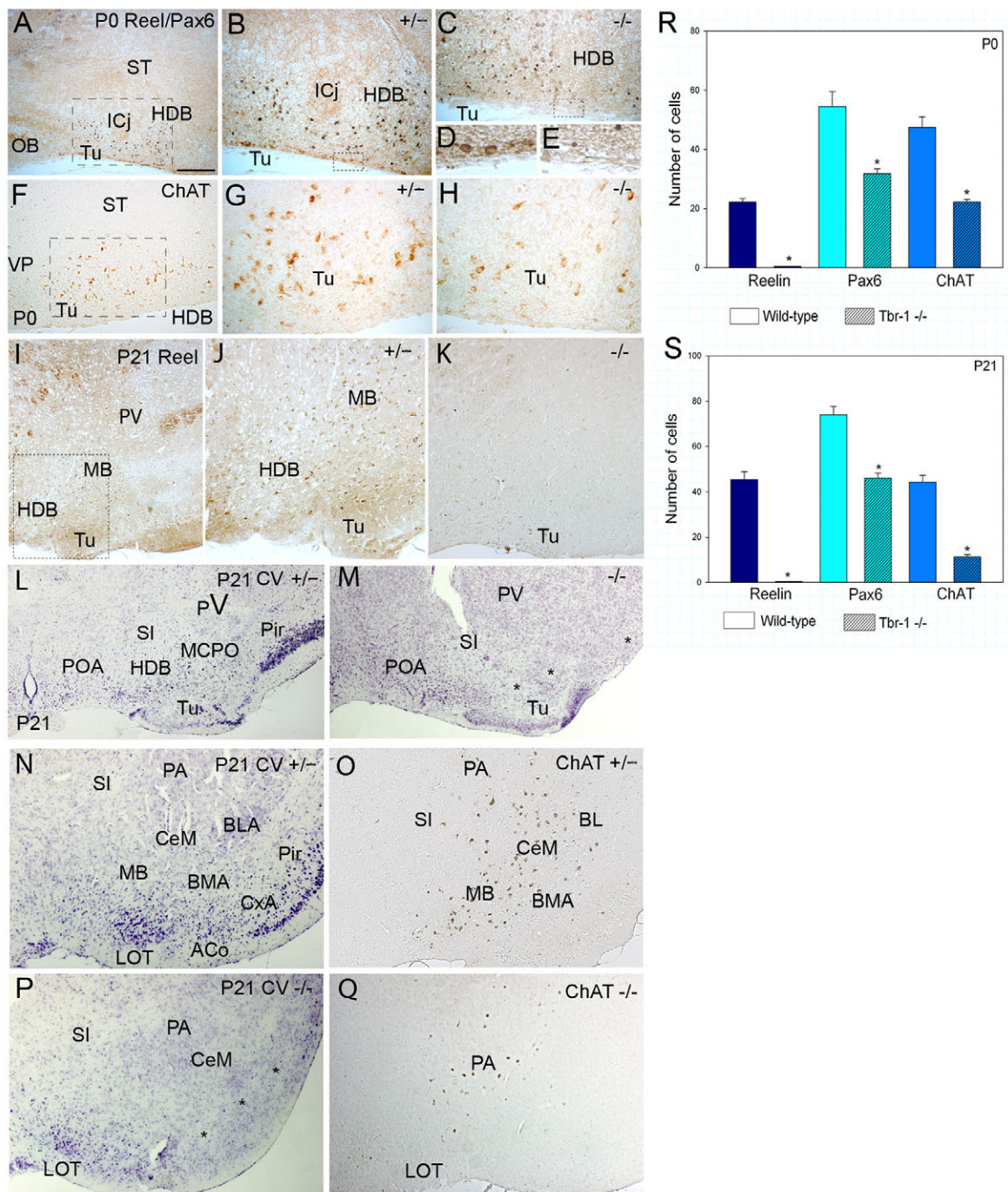
Fig. 5. Analysis of *Tbr1* mutant mice. (**A-E**) Pax6 (black nuclei) and reelin (brown cytoplasm) immunostaining in sagittal sections of P0 control (A,B,D) and *Tbr1*^{-/-} (C,E) mice. B is a magnification of A; D and E are magnifications of B and C, respectively. Reelin^{+} cells were detected at the surface of the Tu in control but not *Tbr1*^{-/-} brains. Pax6^{+} cells were detected in the mantle layer of the Tu and HDB in control (A,B), whereas the number was reduced in mutant mice (C). (**F-H**) Low- and high-magnification micrographs of ChAT immunostaining at P0 in control (F,G) and *Tbr1*^{-/-} (H) mice. ChAT^{+} cells were abundant in control BF (Tu and HDB; F,G and see R), whereas in *Tbr1*^{-/-} few ChAT^{+} cells were detected (H). (**I-K**) Reelin immunostaining in coronal sections of P21 control (I,J) and *Tbr1* mutant (K) mice. J is a magnification of I. (K) Almost no reelin expression was detected in the BF of mutant mice at P21. (**L,M**) Cresyl Violet staining in P21 control (L) and *Tbr1* mutant (M) coronal sections. In *Tbr1*^{-/-}, the Pir and major nuclei of the BF have practically disappeared; only a vestigial Tu could be detected (M). (**N-Q**) Parallel series of coronal sections from control (N,O) and *Tbr1* mutant (P,Q) mice stained with Cresyl Violet (N,P) and anti-ChAT antibody (O,Q). *Tbr1* mutant mice lacked the piriform cortex and cortical amygdala derivatives. Also, the MB and CeM were severely affected. (Q) ChAT immunostaining in a mutant brain, where no cholinergic cells were detected in the BF and very few in the PA (Q). (**R,S**) Bar charts indicating the average ± s.e.m. of cells expressing reelin, Pax6 and ChAT in WT and *Tbr1*^{-/-} brain sections at P0 and P21. ACo, anterior amygdaloid nucleus; BL, basolateral amygdaloid nucleus; BLA, basolateral amygdaloid nucleus, anterior part; BMA, basomedial amygdaloid nucleus, anterior part; CeM, central amygdaloid nucleus, medial division; CxA, cortex-amygdala transition zone; ICj, islands of Calleja; PV, ventral pallidum (for other abbreviations, see previous figure legends). Scale bar: in A, 500 μm for A,F,I,L-Q, 250 μm for B,C,G,H,J,K, and 125 μm for D,E.

DEVELOPMENT
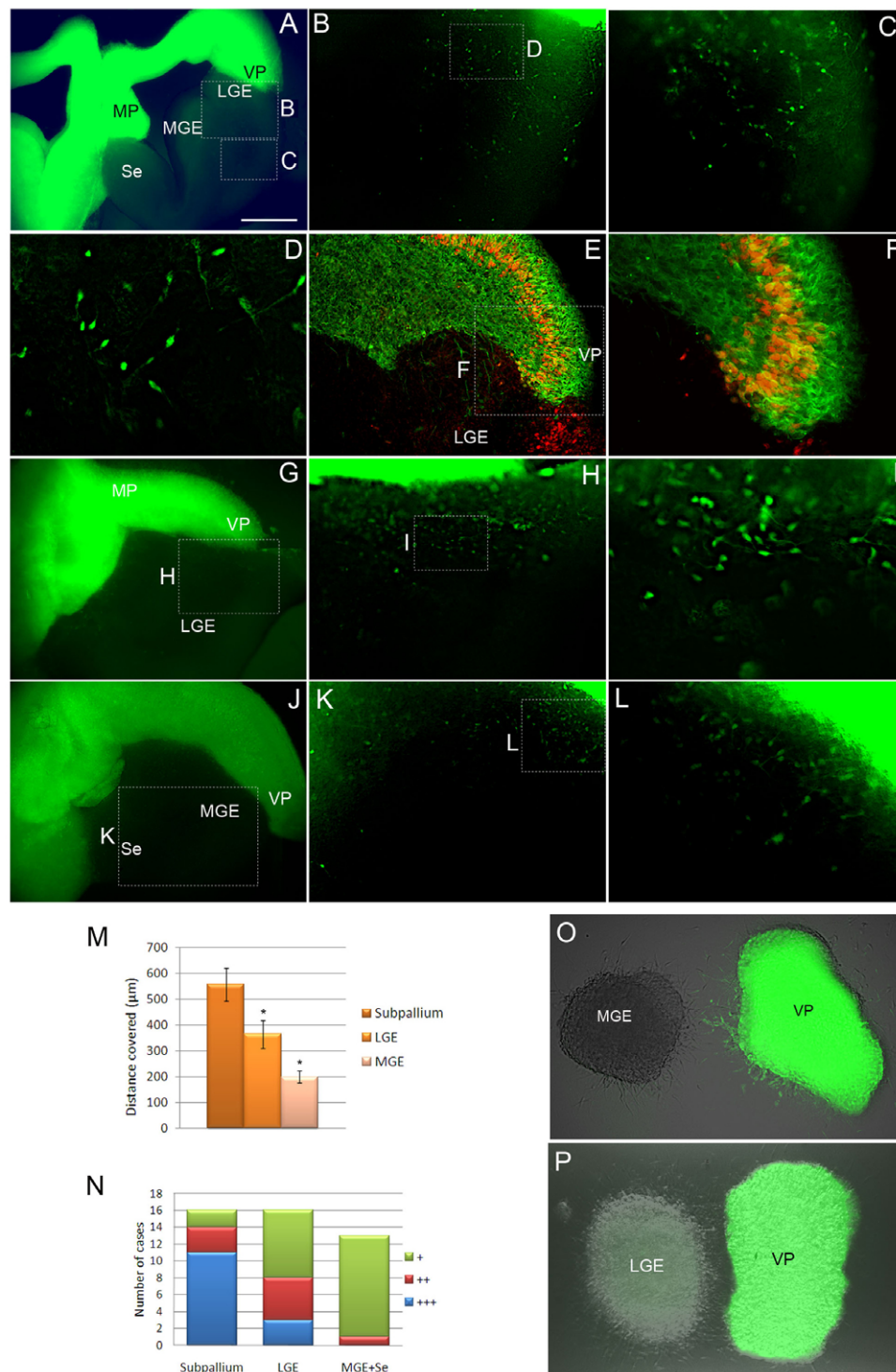
Fig. 6. Medial subpallium induces pallio-subpallial cell migration. (**A-L**) Co-culture of GFP+ pallium and WT subpallium. (A-D) VP-GFP cells invade deep (B) and superficial (C) subpallium. (E,F) Tbr1 immunostaining (red) in a paraffin section of a transplanted slice. (F) enlargement of E, showing the host-graft limit and some Tbr1+ cells migrating into the control subpallium surface. (G-I) Fewer GFP+ migrating cells were detected when the medial portion of the subpallium was not included in the graft, as compared with whole subpallial grafts. I is a magnification of H. (J-L) MGE and Se grafted without LGE. Very few cells were observed near the border of the graft (K,L). L is a magnification of K. (**M,N**) Analysis of the distance covered by the pallial cells (M; average ± s.e.m.) and the number of migrating cells in each experimental case (N). (M) The migrating cells covered greater distances when the whole subpallium was grafted as compared with the grafts with only LGE (*, *P*=0.029) or MGE (*, *P*<0.001); the Mann-Whitney sum test was used for statistical analysis. (N) Color codes represent the number of cases showing migrations that were qualitatively estimated as: few (<5, +), moderate (5-20, ++) and abundant (>20) cells, in each type of graft (i.e. subpallium, LGE or MGE+Se. (**O,P**) Co-cultures of VP-GFP and WT MGE (O) and LGE (P) explants, demonstrating the chemo-attractive effect of the MGE on the VP cells, whereas this effect was not observed in LGE co-cultures. Image taken after 48 hours. MP, medial pallium (for other abbreviations, see previous figure legends). Scale bar: in A, 500 μm for A,G,J,O,P, 170 μm for B,C,E,H,K, and 50 μm for D,F,I,L.
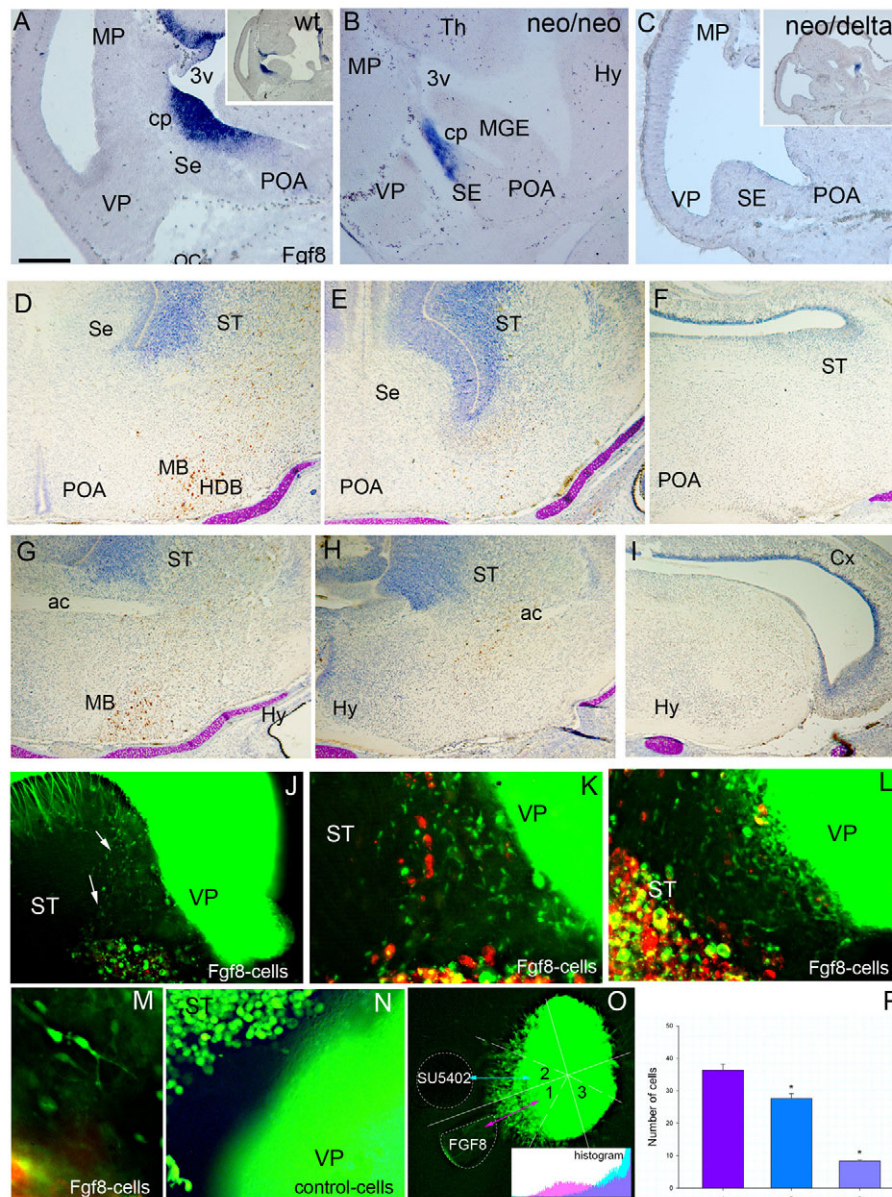
**Fig. 7. Fgf8 activity on pallio-subpallial tangential migration.** (**A**,**D**,**G**) Images of control mouse embryos at E12.5 (A; sagittal plane) and E16.5 (D and G, coronal plane; D is more rostral than G). Fgf8 expression was observed in the Se and cp (A and insert). MB was detected anatomically by Cresyl Violet (CV) staining and by ChAT immunostaining at E16.5 (D,G). (**B**,**E**,**H**) Sections at equivalent brain levels show that in *Fgf8neo/neo* mutant mice, Fgf8 expression is reduced in Se and cp (B). Also, there were fewer ChAT neurons in the MB (E,H). (**C**,**F**,**I**) *Fgf8neo/null* analysis showed that Fgf8 expression is not detected in the telencephalon, as opposed to the hypothalamus (C and insert). MB could not be detected by CV staining or ChAT immunostaining. (**J-P**) Organotypic cultures of sagittal sections obtained from E14.5 GFP mice with a control LGE graft. Fgf8-expressing cells (J,L) or control cells were placed on the LGE and incubated for 24 hours. (J-L) Grafted Fgf8-expressing cells. (J,K) GFP^{+} cells from the VP were detected migrating towards Fgf8-expressing cells. Arrows indicate GFP^{+} cells migrating from the dorsal part of the VP to the striatum. K is a magnification of J. (L) Cells migrating from the ventral area of the VP to the Fgf8-expressing cells placed in the striatum. M is an enlargement of migrating cells. In control experiments in which non-Fgf8-expressing cells were implanted into the graft (N), no cell migration was observed. (O) A VP explant cultured in Matrigel matrix near Fgf8-soaked and SU5402-soaked beads. Cells were observed migrating preferentially towards the Fgf8-soaked bead. Insert in O shows a semi-quantitative analysis (performed using the Histogram tool of Photoshop) through the linear narrow field, as identified by double-headed arrows and color coding: the pink field represents the luminance of migratory cells from VP towards the Fgf8 bead and the light-blue field represents the luminance of migratory cells from VP towards the U5402 bead. (P) Quantification (mean ± s.e.m.) of migrating cells in regions 1, 2 and 3 of VP explants co-cultured with embedded beads in Matrigel matrix (*n*=4). *P*=0.02 (2); *P*=0.003 (3). 3v, third ventricle; cp, commissural plate (for other abbreviations, see previous figure legends). Scale bar: in A, 500 μm for A-I, 170 μm for J-L,N-O, and 25 μm for M.

Nkx2.1 lineage-related cells mainly form MS and VDB nuclei, whereas the HDB nuclei are practically devoid of this transcription factor [see figure 9D in Xu et al. (Xu et al., 2008)]. This observation, in accordance with our expression analysis, suggests a dual origin of ChBF: corticopetal cholinergic neurons of the HDB and MB from the VP (Tbr1^{+}), and hippocampus projecting cholinergic neurons in VDB and MS nuclei from the medial subpallium (Nkx2.1[+]).
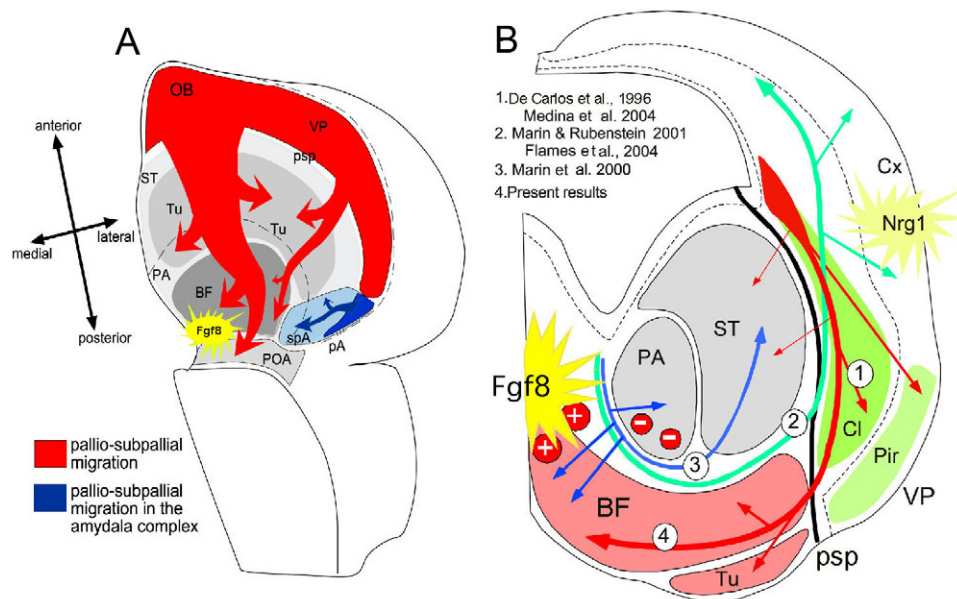
Fig. 8. Migratory routes in the telencephalon. (**A**) Ventral view of the telencephalon depicting the location of BF structures. Fgf8 stimulates pallial cells from the VP (red domain) and pallial amygdala (dark-blue area) to migrate ventrally, crossing the pallio-subpallial boundary, and to populate superficial structures of the subpallium (gray areas) and subpallial amygdala (light blue). (**B**) Coronal section depicting the major cellular migration routes (color-coded arrows and numbers). The chemo-attractive signals are represented by red circles with a plus symbol, and non-permissive signals are represented by red circles with a minus symbol. Neurons from the VP migrate radially to form the claustrum and olfactory cortex. Also, they migrate tangentially towards the ventral prosencephalon, populating the BF, where they differentiate into corticopetal cholinergic cells. Cl, claustrum; pA, pallial amygdala; spA, subpallial amygdala (for other abbreviations, see previous figure legends).

Finally, the LIM-homeobox gene *Lhx8* is required for the development of cholinergic neurons in the mouse BF (Marin et al., 2000; Asbreuk et al., 2002; Zhao et al., 2003). *Lhx8* mutant mice present alterations of the cholinergic system in the dorsal ST; however, neurons in the ventral ST and Tu are unaffected, possibly owing to their pallial origin (Zhao et al., 2003) (this work). Elshatory and Gan (Elshatory and Gan, 2008) reported that most of the cholinergic neurons in the MB express Islet1 and disappear when Islet1 is conditionally inactivated with Six3-cre, suggesting a subpallial origin of these cells. The results from Elshatory and Gan's study do not rule out the possibility of a VP origin of ChBF. First, as they did not perform independent clonal experiments, one cannot eliminate the possibility of alternative interpretations, such as the activation of Six3 in VP-derived cells or the involvement of compensatory mechanisms. Second, they report that some cholinergic neurons remained in the transgenic MB, which is in agreement with our results.

Trophic factor-induced activation of pallio-subpallial neuronal migration
Our results indicate that pallial cells migrate due to signals released from the medial subpallium (MGE and Se). It was previously shown that the tangential ventrodorsal migration of interneurons is regulated by the simultaneous expression of chemo-repulsive and chemo-attractive signals in the POA and cortex, respectively (Marin et al., 2003; Wichterle et al., 2003). We believe that a similar mechanism could control pallio-subpallial migration, and our experiments indicate that Fgf8 signaling from the telencephalic midline is a key signal for this process. The morphogenetic activity of Fgf8 during neural tube development, both in the isthmic and anterior prosencephalic organizers (Crossley et al., 1996; Martinez et al., 1999; Storm et al., 2006), suggests a long-distance effect and

supports an attractive role for this signal. Since the transcriptional regulation of *Fgf8* expression in these signaling centers is relatively uncharacterized, further analysis of the possible control of *Fgf8* and Fgf receptor (*Fgfr*) genes by transcription factors, such as Tbr1, in the BF needs to be undertaken. The reduction of cell migration by SU5402 in Matrigel explants proved the involvement of Fgfrs. Fgfrs are required for the normal morphogenetic activity of Fgf8 signaling during dorsal prosencephalic development (Paek et al., 2009; Stevens et al., 2010). Some cells in the developing BF express Fgfr, but because they have been related to the oligodendroglial lineage (Bansal et al., 2003; Furusho et al., 2011) a more precise analysis is required to explore their co-existence with pallio-subpallial migrating neurons.

Once they reach their destination in the BF, the VP cells seem to differentiate into cholinergic neurons. As the neurons in the claustrum, ChBF establish cortical connections and are involved in complex brain functions, such as cognition (reviewed by Sarter et al., 2005), as well as in the physiopathology of dementia in neurodegenerative diseases (Alzheimer's and Parkinson) and schizophrenia (reviewed by Bohnen and Albin, 2010). Amygdaline cholinergic cells are functionally involved in addiction and the stress response (Markou, 2008; Meerson et al., 2010).

The cholinergic hypothesis of geriatric memory dysfunction (Bartus et al., 1982) indicates a link between ChBF degeneration and cognitive disorders. Furthermore, the degeneration of ChBF is considered an early symptom in neurodegenerative diseases (for a review, see Hampel et al., 2008). Similarly, olfactory alterations have been identified as a risk factor and an early marker of neurodegenerative diseases (Tabert et al., 2005; Arnold et al., 2010). The early degeneration of granular neurons in the OB (Wesson et al., 2010) and of cholinergic neurons of the MB (Whitehouse et al., 1982) occur as two independent processes. Our

present results demonstrate that these neurons have a common origin in ventral pallial progenitors and that they undergo an extensive migration from their origin to their final destination. Investigating the role of the amyloid precursor protein (APP) in this neuronal migration (Young-Pearse et al., 2010) and the specific trophic dependencies related to these common ontogenetic processes in the ChBF (Stewart and Appel, 1988) could represent new directions to further our knowledge of neurodegenerative-related cognitive disorder pathophysiology.

References
Anderson, S. A., Eisenstat, D. D., Shi, L. and Rubenstein, J. L. (1997). Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* **278**, 474-476.

Arnold, S. E., Lee, E. B., Moberg, P. J., Stutzbach, L., Kazi, H., Han, L. Y., Lee, V. M. and Trojanowski, J. Q. (2010). Olfactory epithelium amyloid-beta and paired helical filament-tau pathology in Alzheimer disease. *Ann. Neurol.* **67**, 462-469.

Asbreuk, C. H., van Schaick, H. S., Cox, J. J., Kromkamp, M., Smidt, M. P. and Burbach, J. P. (2002). The homeobox genes Lhx7 and Gbx1 are expressed in the basal forebrain cholinergic system. *Neuroscience* **109**, 287-298.

Bailey, A. P., Bhattacharyya, S., Bronner-Fraser, M. and Streit, A. (2006). Lens specification is the ground state of all sensory placodes, from which FGF promotes olfactory identity. *Dev. Cell* **11**, 505-517.

Bansal, R., Lakhina, V., Remedios, R. and Tole, S. (2003). Expression of FGF receptors **1**, 2, 3 in the embryonic and postnatal mouse brain compared with Pdgfralpha, Olig2 and Plp/dm20: implications for oligodendrocyte development. *Dev. Neurosci.* **25**, 83-95.

Bartus, R. T., Dean, R. L., 3rd, Beer, B. and Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408-414.

Bernier, B., Bar, I., D'Arcangelo, G., Curran, T. and Goffinet, A. M. (2000). Reelin mRNA expression during embryonic brain development in the chick. *J. Comp. Neurol.* **422**, 448-463.

Bohnen, N. I. and Albin, R. L. (2010). The cholinergic system and Parkinson disease. *Behav. Brain Res.* **221**, 564-573.

Bulfone, A., Smiga, S. M., Shimamura, K., Peterson, A., Puelles, L. and Rubenstein, J. L. (1995). T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. *Neuron* **15**, 63-78.

Bulfone, A., Wang, F., Hevner, R., Anderson, S., Cutforth, T., Chen, S., Meneses, J., Pedersen, R., Axel, R. and Rubenstein, J. L. (1998). An olfactory sensory map develops in the absence of normal projection neurons or GABAergic interneurons. *Neuron* **21**, 1273-1282.

Carney, R. S., Cocas, L. A., Hirata, T., Mansfield, K. and Corbin, J. G. (2009). Differential regulation of telencephalic pallial-subpallial boundary patterning by Pax6 and Gsh2. *Cereb. Cortex* **19**, 745-759.

Chapouton, P., Gartner, A. and Gotz, M. (1999). The role of Pax6 in restricting cell migration between developing cortex and basal ganglia. *Development* **126**, 5569-5579.

Cobos, I., Puelles, L. and Martinez, S. (2001a). The avian telencephalic subpallium originates inhibitory neurons that invade tangentially the pallium (dorsal ventricular ridge and cortical areas). *Dev. Biol.* **239**, 30-45.

Cobos, I., Shimamura, K., Rubenstein, J. L., Martinez, S. and Puelles, L. (2001b). Fate map of the avian anterior forebrain at the four-somite stage, based on the analysis of quail-chick chimeras. *Dev. Biol.* **239**, 46-67.

Cocas, L. A., Miyoshi, G., Carney, R. S., Sousa, V. H., Hirata, T., Jones, K. R., Fishell, G., Huntsman, M. M. and Corbin, J. G. (2009). Emx1-lineage progenitors differentially contribute to neural diversity in the striatum and amygdala. *J. Neurosci.* **29**, 15933-15946.

Crossley, P. H. and Martin, G. R. (1995). The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. *Development* **121**, 439-451.

Crossley, P. H., Martinez, S. and Martin, G. R. (1996). Midbrain development induced by FGF8 in the chick embryo. *Nature* **380**, 66-68.

Crossley, P. H., Martinez, S., Ohkubo, Y. and Rubenstein, J. L. (2001). Coordinate expression of Fgf8, Otx2, Bmp4, and Shh in the rostral prosencephalon during development of the telencephalic and optic vesicles. *Neuroscience* **108**, 183-206.

D'Arcangelo, G., Miao, G. G., Chen, S. C., Soares, H. D., Morgan, J. I. and Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* **374**, 719-723.

D'Arcangelo, G., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K. and Curran, T. (1997). Reelin is a secreted glycoprotein recognized by the CR-50 monoclonal antibody. *J. Neurosci.* **17**, 23-31.

de Bergeyck, V., Naerhuyzen, B., Goffinet, A. M. and Lambert de Rouvroit, C. (1998). A panel of monoclonal antibodies against reelin, the extracellular matrix protein defective in reeler mutant mice. *J. Neurosci. Methods* **82**, 17-24.

de Carlos, J. A., Lopez-Mascaraque, L. and Valverde, F. (1996). Dynamics of cell migration from the lateral ganglionic eminence in the rat. *J. Neurosci.* **16**, 6146-6156.

Diez-Roux, G., Banfi, S., Sultan, M., Geffers, L., Anand, S., Rozado, D., Magen, A., Canidio, E., Pagani, M., Peluso, I. et al. (2011). A high-resolution anatomical atlas of the transcriptome in the mouse embryo. *PLoS Biol.* **9**, e1000582.

Elshatory, Y. and Gan, L. (2008). The LIM-homeobox gene Islet-1 is required for the development of restricted forebrain cholinergic neurons. *J. Neurosci.* **28**, 3291-3297.

Fishell, G., Mason, C. A. and Hatten, M. E. (1993). Dispersion of neural progenitors within the germinal zones of the forebrain. *Nature* **362**, 636-638.

Flames, N., Long, J. E., Garratt, A. N., Fischer, T. M., Gassmann, M., Birchmeier, C., Lai, C., Rubenstein, J. L. and Marín, O. (2004) Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. *Neuron* **44**, 251-261.

Furusho, M., Kaga, Y., Ishii, A., Hebert, J. M. and Bansal, R. (2011). Fibroblast growth factor signaling is required for the generation of oligodendrocyte progenitors from the embryonic forebrain. *J. Neurosci.* **31**, 5055-5066.

Garcia-Moreno, F., Lopez-Mascaraque, L. and de Carlos, J. A. (2007). Origins and migratory routes of murine Cajal-Retzius cells. *J. Comp. Neurol.* **500**, 419-432.

Hadjantonakis, A. K., Macmaster, S. and Nagy, A. (2002). Embryonic stem cells and mice expressing different GFP variants for multiple non-invasive reporter usage within a single animal. *BMC Biotechnol.* **2**, 11.

Hampel, H., Burger, K., Teipel, S. J., Bokde, A. L., Zetterberg, H. and Blennow, K. (2008). Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. *Alzheimers Dement.* **4**, 38-48.

Hebert, J. M. and McConnell, S. K. (2000). Targeting of cre to the Foxg1 (BF-1) locus mediates loxP recombination in the telencephalon and other developing head structures. *Dev. Biol.* **222**, 296-306.

Hevner, R. F., Shi, L., Justice, N., Hsueh, Y., Sheng, M., Smiga, S., Bulfone, A., Goffinet, A. M., Campagnoni, A. T. and Rubenstein, J. L. (2001). Tbr1 regulates differentiation of the preplate and layer 6. *Neuron* **29**, 353-366.

Hevner, R. F., Miyashita-Lin, E. and Rubenstein, J. L. (2002). Cortical and thalamic axon pathfinding defects in Tbr1, Gbx2, and Pax6 mutant mice: evidence that cortical and thalamic axons interact and guide each other. *J. Comp. Neurol.* **447**, 8-17.

Hoffarth, R. M., Johnston, J. G., Krushel, L. A. and van der Kooy, D. (1995). The mouse mutation reeler causes increased adhesion within a subpopulation of early postmitotic cortical neurons. *J. Neurosci.* **15**, 4838-4850.

Kawauchi, S., Shou, J., Santos, R., Hebert, J. M., McConnell, S. K., Mason, I. and Calof, A. L. (2005). Fgf8 expression defines a morphogenetic center required for olfactory neurogenesis and nasal cavity development in the mouse. *Development* **132**, 5211-5223.

Lavdas, A. A., Grigoriou, M., Pachnis, V. and Parnavelas, J. G. (1999). The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. *J. Neurosci.* **19**, 7881-7888.

Marin, O. and Rubenstein, J. L. (2001). A long, remarkable journey: tangential migration in the telencephalon. *Nat. Rev. Neurosci.* **2**, 780-790.

Marin, O. and Rubenstein, J. L. (2003). Cell migration in the forebrain. *Annu. Rev. Neurosci.* **26**, 441-483.

Marin, O., Anderson, S. A. and Rubenstein, J. L. (2000). Origin and molecular specification of striatal interneurons. *J. Neurosci.* **20**, 6063-6076.

Marin, O., Yaron, A., Bagri, A., Tessier-Lavigne, M. and Rubenstein, J. L. (2001). Sorting of striatal and cortical interneurons regulated by semaphorin-neuropilin interactions. *Science* **293**, 872-875.

Marin, O., Plump, A. S., Flames, N., Sanchez-Camacho, C., Tessier-Lavigne, M. and Rubenstein, J. L. (2003). Directional guidance of interneuron migration to the cerebral cortex relies on subcortical Slit1/2-independent repulsion and cortical attraction. *Development* **130**, 1889-1901.

Markou, A. (2008). Review. Neurobiology of nicotine dependence. *Philos. Trans. R. Soc. Lond. B* **363**, 3159-3168.

Martinez, S., Crossley, P. H., Cobos, I., Rubenstein, J. L. and Martin, G. R. (1999). FGF8 induces formation of an ectopic isthmic organizer and isthmocerebellar development via a repressive effect on Otx2 expression. *Development* **126**, 1189-1200.

Medina, L., Legaz, I., Gonzalez, G., de Castro, F., Rubenstein, J. L. and Puelles, L. (2004). Expression of Dbx1, Neurogenin 2, Semaphorin 5A, Cadherin 8, and Emx1 distinguish ventral and lateral pallial histogenetic divisions in the developing mouse claustroamygdaloid complex. *J. Comp. Neurol.* **474**, 504-523.

Meerson, A., Cacheaux, L., Goosens, K. A., Sapolsky, R. M., Soreq, H. and Kaufer, D. (2010). Changes in brain MicroRNAs contribute to cholinergic stress reactions. *J. Mol. Neurosci.* **40**, 47-55.

Mesulam, M. M. (1990). Human brain cholinergic pathways. *Prog. Brain Res.* **84**, 231-241.

Mesulam, M. M., Mufson, E. J., Levey, A. I. and Wainer, B. H. (1984). Atlas of cholinergic neurons in the forebrain and upper brainstem of the macaque based on monoclonal choline acetyltransferase immunohistochemistry and acetylcholinesterase histochemistry. *Neuroscience* **12**, 669-686.

Metin, C., Alvarez, C., Moudoux, D., Vitalis, T., Pieau, C. and Molnar, Z. (2007). Conserved pattern of tangential neuronal migration during forebrain development. *Development* **134**, 2815-2827.

Meyers, E. N., Lewandoski, M. and Martin, G. R. (1998). An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination. *Nat. Genet.* **18**, 136-141.

Molyneaux, B. J., Arlotta, P., Menezes, J. R. and Macklis, J. D. (2007). Neuronal subtype specification in the cerebral cortex. *Nat. Rev. Neurosci.* **8**, 427-437.

Ogawa, M., Miyata, T., Nakajima, K., Yagyu, K., Seike, M., Ikenaka, K., Yamamoto, H. and Mikoshiba, K. (1995). The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron* **14**, 899-912.

Olivier, C., Cobos, I., Perez Villegas, E. M., Spassky, N., Zalc, B., Martinez, S. and Thomas, J. L. (2001). Monofocal origin of telencephalic oligodendrocytes in the anterior entopeduncular area of the chick embryo. *Development* **128**, 1757-1769.

Paek, H., Gutin, G. and Hebert, J. M. (2009). FGF signaling is strictly required to maintain early telencephalic precursor cell survival. *Development* **136**, 2457-2465.

Pearlman, A. L., Faust, P. L., Hatten, M. E. and Brunstrom, J. E. (1998). New directions for neuronal migration. *Curr. Opin. Neurobiol.* **8**, 45-54.

Puelles, L., Kuwana, E., Puelles, E. and Rubenstein, J. L. (1999). Comparison of the mammalian and avian telencephalon from the perspective of gene expression data. *Eur. J. Morphol.* **37**, 139-150.

Puelles, L., Kuwana, E., Puelles, E., Bulfone, A., Shimamura, K., Keleher, J., Smiga, S. and Rubenstein, J. L. (2000). Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. *J. Comp. Neurol.* **424**, 409-438.

Rakic, P. (1972). Mode of cell migration to the superficial layers of fetal monkey neocortex. *J. Comp. Neurol.* **145**, 61-83.

Sarter, M., Hasselmo, M. E., Bruno, J. P. and Givens, B. (2005). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res. Brain Res. Rev.* **48**, 98-111.

Shimamura, K. and Rubenstein, J. L. (1997). Inductive interactions direct early regionalization of the mouse forebrain. *Development* **124**, 2709-2718.

Shimamura, K., Hartigan, D. J., Martinez, S., Puelles, L. and Rubenstein, J. L. (1995). Longitudinal organization of the anterior neural plate and neural tube. *Development* **121**, 3923-3933.

Sigala, S., Zoli, M., Palazzolo, F., Faccoli, S., Zanardi, A., Mercuri, N. B. and Spano, P. (2007). Selective disarrangement of the rostral telencephalic cholinergic system in heterozygous reeler mice. *Neuroscience* **144**, 834-844.

Stevens, H. E., Smith, K. M., Maragnoli, M. E., Fagel, D., Borok, E., Shanabrough, M., Horvath, T. L. and Vaccarino, F. M. (2010). Fgfr2 is

required for the development of the medial prefrontal cortex and its connections with limbic circuits. *J. Neurosci.* **30**, 5590-5602.

Stewart, S. S. and Appel, S. H. (1988). Trophic factors in neurologic disease. *Annu. Rev. Med.* **39**, 193-201.

Storm, E. E., Rubenstein, J. L. and Martin, G. R. (2003). Dosage of Fgf8 determines whether cell survival is positively or negatively regulated in the developing forebrain. *Proc. Natl. Acad. Sci. USA* **100**, 1757-1762.

Storm, E. E., Garel, S., Borello, U., Hebert, J. M., Martinez, S., McConnell, S. K., Martin, G. R. and Rubenstein, J. L. (2006). Dose-dependent functions of Fgf8 in regulating telencephalic patterning centers. *Development* **133**, 1831-1844.

Stoykova, A., Gotz, M., Gruss, P. and Price, J. (1997). Pax6-dependent regulation of adhesive patterning, R-cadherin expression and boundary formation in developing forebrain. *Development* **124**, 3765-3777.

Striedter, G. F., Marchant, T. A. and Beydler, S. (1998). The "neostriatum" develops as part of the lateral pallium in birds. *J. Neurosci.* **18**, 5839-5849.

Sussel, L., Marin, O., Kimura, S. and Rubenstein, J. L. (1999). Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development* **126**, 3359-3370.

Tabert, M. H., Liu, X., Doty, R. L., Serby, M., Zamora, D., Pelton, G. H., Marder, K., Albers, M. W., Stern, Y. and Devanand, D. P. (2005). A 10-item smell identification scale related to risk for Alzheimer's disease. *Ann. Neurol.* **58**, 155-160.

Toresson, H., Potter, S. S. and Campbell, K. (2000). Genetic control of dorsal-ventral identity in the telencephalon: opposing roles for Pax6 and Gsh2. *Development* **127**, 4361-4371.

Tuorto, F., Alifragis, P., Failla, V., Parnavelas, J. G. and Gulisano, M. (2003). Tangential migration of cells from the basal to the dorsal telencephalic regions in the chick. *Eur. J. Neurosci.* **18**, 3388-3393.

Wesson, D. W., Levy, E., Nixon, R. A. and Wilson, D. A. (2010). Olfactory dysfunction correlates with amyloid-beta burden in an Alzheimer's disease mouse model. *J. Neurosci.* **30**, 505-514.

Whitehouse, P. J., Price, D. L., Struble, R. G., Clark, A. W., Coyle, J. T. and Delon, M. R. (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**, 1237-1239.

Wichterle, H., Garcia-Verdugo, J. M., Herrera, D. G. and varez-Buylla, A. (1999). Young neurons from medial ganglionic eminence disperse in adult and embryonic brain. *Nat. Neurosci.* **2**, 461-466.

Wichterle, H., varez-Dolado, M., Erskine, L. and varez-Buylla, A. (2003). Permissive corridor and diffusible gradients direct medial ganglionic eminence cell migration to the neocortex. *Proc. Natl. Acad. Sci. USA* **100**, 727-732.

Wonders, C. P. and Anderson, S. A. (2006). The origin and specification of cortical interneurons. *Nat. Rev. Neurosci.* **7**, 687-696.

Xu, Q., Tam, M. and Anderson, S. A. (2008). Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. *J. Comp. Neurol.* **506**, 16-29.

Young-Pearse, T. L., Suth, S., Luth, E. S., Sawa, A. and Selkoe, D. J. (2010). Biochemical and functional interaction of disrupted-in-schizophrenia 1 and amyloid precursor protein regulates neuronal migration during mammalian cortical development. *J. Neurosci.* **30**, 10431-10440.

Yun, K., Potter, S. and Rubenstein, J. L. (2001). Gsh2 and Pax6 play complementary roles in dorsoventral patterning of the mammalian telencephalon. *Development* **128**, 193-205.

Zhao, Y., Guo, Y. J., Tomac, A. C., Taylor, N. R., Grinberg, A., Lee, E. J., Huang, S. and Westphal, H. (1999). Isolated cleft palate in mice with a targeted mutation of the LIM homeobox gene lhx8. *Proc. Natl. Acad. Sci. USA* **96**, 15002-15006.

Zhao, Y., Marin, O., Hermesz, E., Powell, A., Flames, N., Palkovits, M., Rubenstein, J. L. and Westphal, H. (2003). The LIM-homeobox gene Lhx8 is required for the development of many cholinergic neurons in the mouse forebrain. *Proc. Natl. Acad. Sci. USA* **100**, 9005-9010.

Zimmer, C., Lee, J., Griveau, A., Arber, S., Pierani, A., Garel, S. and Guillemot, F. (2010). Role of Fgf8 signalling in the specification of rostral Cajal-Retzius cells. *Development* **137**, 293-302.