

Transcription factor LIM homeobox 7 (Lhx7) maintains subtype identity of cholinergic interneurons in the mammalian striatum

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The generation and maintenance of a plethora of neuronal subtypes is essential for normal brain function. Nevertheless, little is known about the molecular mechanisms that maintain the defining characteristics of neurons following their initial postmitotic specification. Using conditional gene ablation in mice, we demonstrate here that the homeodomain protein LIM homeobox (Lhx7) is essential for maintaining the morphological and molecular characteristics of cholinergic interneurons of the striatum. Lhx7-depleted cholinergic interneurons extinguish expression of several subtype-specific markers, including choline acetyl transferase and *Isl1*, and are respecified into Lhx6-expressing mature GABAergic interneurons. Additional expression studies support a model where Lhx7 controls the choice between cholinergic or GABAergic identity by gating a cross inhibitory regulation between *Isl1* and Lhx6. By demonstrating that the switch between alternative striatal interneuron fates depends on persistent activity of a single transcription factor, we provide evidence that the intrinsic plasticity of mammalian forebrain neuronal subtypes is maintained after the initial specification and lineage commitment and possibly throughout life.

neuronal subtype specification | medial ganglionic eminence-derived interneurons

The information-processing ability of the nervous system depends on the formation of functional neuronal circuits composed of a highly heterogeneous population of neurons. Recent progress has identified molecular cascades that control the generation of distinct neuronal subtypes during development (1–3) but the mechanisms that maintain the identity of neurons following lineage commitment and differentiation are currently unclear. Identifying such mechanisms is critical for understanding phenotypic plasticity in the nervous system and, ultimately, influencing its ability to adjust during normal development, disease, or injury.

The striatum is a subcortical structure that integrates multiple inputs from the cortex, thalamus, and midbrain and relays information to the output domains of the basal ganglia via its principal population of projection neurons (4, 5). Balanced striatal output, which is critical for motor and cognitive activity, depends on local circuits controlled by distinct subpopulations of GABAergic and cholinergic interneurons (5). Both GABAergic and cholinergic striatal interneurons (GSIs and CSIs, respectively) are derived from Nkx2.1-expressing progenitors of the medial ganglionic eminence (MGE) (6, 7), which, upon exit from the cell cycle, express the LIM homeodomain (LIM HD) transcription factors LIM homeobox 6 (Lhx6) and LIM homeobox 7 (Lhx7) (8). A subset of these precursors maintains expression of *Lhx6* and differentiate into mature GABAergic interneurons expressing the neuropeptide somatostatin (Sst) or the calcium-binding protein parvalbumin (Pv) (9, 10). The remaining precursors induce expression of the LIM HD protein *Isl1*, down-regulate *Lhx6*, and differentiate into CSIs (8). In adult animals, all GSIs are *Lhx6*⁺ and a fraction of these also coexpresses *Lhx7*. In contrast, *Lhx6* is undetectable in CSIs, which invariably coexpress *Isl1* and *Lhx7*. Genetic studies have established the requirement of *Lhx7* and *Isl1* for the specification of forebrain cholinergic neurons (11–14), but the potential role of these factors in maintaining the

identity of CSIs following their specification and differentiation is currently unknown.

By combining *in vivo* fate mapping and phenotypic analysis of CSIs in which *Lhx7* was conditionally ablated, we demonstrate here that *Lhx7* is required subsequently to cholinergic subtype specification to actively maintain the phenotypic characteristics of these interneurons and prevent them from adopting GABAergic identity. Gain-of-function studies also suggest that *Lhx7* is a molecular node that resolves the lineage choices of striatal interneuron precursors by modulating antagonistic expression of *Isl1* and *Lhx6*. Our experiments reveal a remarkable degree of plasticity of postmitotic neurons and raise the possibility that cell differentiation choices can be readjusted at all developmental stages by manipulating the levels of key transcriptional regulators.

Results

Deletion of *Lhx7* from Committed Postmitotic Cholinergic Neurons of the Forebrain.

To examine the role of *Lhx7* in maintaining the subtype identity of CSIs following their initial specification, we generated a conditional allele of the locus (*Lhx7*^{fl}), which allows cell type- and stage-specific deletion of the gene (Fig. S1 and *Materials and Methods*). The fate of *Lhx7*-depleted cells was followed by combining *Lhx7*^{fl} with *Lhx7*^{LacZ}, a null allele generated previously by inserting the *LacZ* reporter into the *Lhx7* locus (12). To confirm that *Lhx7*^{fl} is converted into a null allele upon Cre-mediated recombination, we analyzed brain sections from *Lhx7*^{+/LacZ}, *Lhx7*^{fl/LacZ}, and β -actin::Cre;*Lhx7*^{fl/LacZ} animals (15). Consistent with *Lhx7* deletion in β -actin::Cre;*Lhx7*^{fl/LacZ} mice, *Lhx7*-positive cells were absent (Fig. S1D) and CSIs were dramatically reduced in the striatum (Fig. S1E–G) (8, 12–14). Interestingly, reduction of CSIs was associated with increased number of *Lhx6*-expressing striatal interneurons (Fig. S1F), a characteristic feature of *Lhx7*-null mice (8, 12–14). Therefore, upon Cre-mediated recombination, *Lhx7*^{fl} is converted into a null allele.

Because choline acetyltransferase (ChAT) mRNA is first detected in committed, immature cholinergic neurons of the striatum and basal forebrain at embryonic stages (Fig. 1C), we reasoned that deletion of *Lhx7* by a transgene expressing Cre under the control of ChAT regulatory sequences (*ChAT*::Cre) would bypass the early requirement of the gene for cholinergic specification and allow us to address its role in maintaining the identity of the cholinergic sublineage. To validate our strategy, we fate-mapped cholinergic precursors *in vivo* by combining a *ChAT*::Cre transgene (16) with the Cre-dependent R26ReYFP lineage reporter (17) and the *Lhx7*^{LacZ} allele (to mark *Lhx7*-expressing cells with β -gal). Animals were analyzed during embryogenesis for expression of Cre and YFP in β -gal⁺ cells of the

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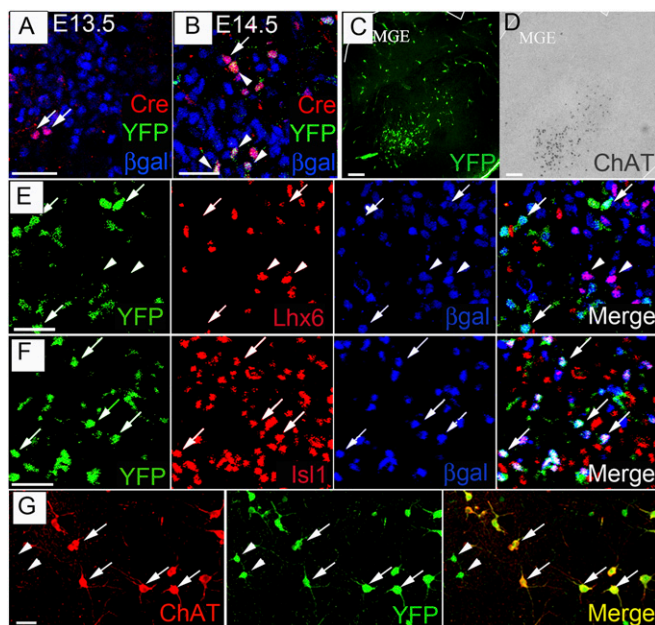


Fig. 1. The *ChAT::Cre* transgene is specifically expressed by *Lhx7*⁺ forebrain cholinergic neurons. (A–D) Ventral forebrain sections from E13.5 (A) or E14.5 (B–D) *ChAT::Cre;Lhx7^{+/LacZ};R26ReYFP* embryos immunostained for Cre, YFP, and β -gal (A and B) or YFP (C) or processed for ISH using a ChAT-specific riboprobe (D). Arrows in A and B indicate Cre⁺YFP⁺ cells, and arrowheads in B indicate Cre⁺YFP⁺ cells. All Cre⁺ cells express β -gal. In the serial sections of ventral forebrain shown in C and D, the distribution of YFP signal is similar to that of ChAT mRNA. (E and F) Sections from the MGE-LGE boundary sections of E14.5 *ChAT::Cre;Lhx7^{+/LacZ};R26ReYFP* embryos triple-immunostained for YFP, Lhx6, and β -gal (E) or YFP, Isl1, and β -gal (F). Lhx6-expressing cells (arrowheads) are negative for YFP, and YFP⁺ cells (arrows) do not express Lhx6 (E). In contrast, YFP is detected in β -gal⁺ neurons expressing *Isl1* (F). (G) Adult striatum sections immunostained for ChAT and YFP. YFP is expressed in virtually all cholinergic (ChAT⁺) neurons (arrows); some noncholinergic cells also express YFP (arrowheads). [Scale bars: 50 μ m (A, B, E, and F); 100 μ m (C and D).]

MGE (where striatal interneurons are born) and LGE (the striatal anlage). At embryonic day (E)13.5, some β -gal⁺ cells expressed Cre (but not YFP) (Fig. 1A), and at E14.5, many β -gal⁺ cells coexpressed Cre and YFP (Fig. 1B). β -gal⁺YFP⁺ cells did not express Ki67, indicating that they were postmitotic (Fig. S2) (8). Induction of Cre and YFP in the striatal anlage followed a caudal to rostral temporal order (similar to the wave of birth and maturation of cholinergic neurons in the basal forebrain) (18), and the overall pattern of expression of YFP in the ventral forebrain was comparable to that of ChAT RNA (compare Fig. 1C and D). Importantly, the vast majority of YFP⁺ cells in the basal forebrain of *ChAT::Cre;Lhx7^{+/LacZ};R26ReYFP* animals coexpressed β -gal, and many expressed the cholinergic interneuron marker *Isl1* (Fig. 1F), but all were negative for the GABAergic marker *Lhx6* (Fig. 1E). Furthermore, in the striatum of adult *ChAT::Cre;Lhx7^{+/LacZ};R26ReYFP* mice, virtually all β -gal⁺YFP⁺ neurons coexpressed ChAT (Fig. 1G, arrows). Although, at this stage, we observed a small number of YFP⁺ChAT[−] cells (Fig. 1G, arrowheads), these cells did not express β -gal and, therefore, did not interfere with our subsequent analysis. Taken together, these experiments demonstrate that the *ChAT::Cre* transgene is activated during embryogenesis in postmitotic CSIs after the divergence of the GABAergic and cholinergic lineages of the striatum.

Lhx7 Is Required in Committed CSIs for Terminal Differentiation. To examine whether *Lhx7* is required in the mammalian forebrain to maintain the unique identity of CSIs, we compared the expression of the cholinergic markers ChAT, vesicular acetylcholine transporter (VAcHT), and TrkA (19, 20) in the striatum of adult

ChAT::Cre;Lhx7^{+/LacZ} (control) and *ChAT::Cre;Lhx7^{fl/LacZ}* (conditional mutant) mice. As expected, CSIs coexpressing *ChAT*, *VAcHT*, and *TrkA* were readily detected in control animals (Fig. 2A, arrows). However, in the striatum of conditional mutants, the number of cells expressing these markers was dramatically reduced (compare Fig. 2A and B). Most residual CSIs in these animals expressed all three markers, but, occasionally, we observed cells that expressed ChAT but were negative for TrkA and VAcHT (Fig. 2B, arrowhead). The forebrain of *ChAT::Cre;Lhx7^{fl/LacZ}* animals was also analyzed for expression of *Isl1*, a gene expressed in mature CSIs and required for their specification (8, 11). Consistent with the loss of CSIs, the number of cells expressing *Isl1* was severely reduced (compare Fig. 2C and D), with expression maintained only in residual cholinergic interneurons (Fig. 2D). Together, these findings suggest that deletion of *Lhx7* from committed cholinergic precursors results in depletion of cholinergic neurons in the adult striatum.

To examine whether the decrease of CSIs observed in *ChAT::Cre;Lhx7^{fl/LacZ}* mice results from cell death, we compared the total number of β -gal⁺ cells in control and conditional mutant striatum. We observed that in conditional mutants, the absolute number of β -gal⁺ cells was reduced (Fig. 2G), suggesting a requirement of *Lhx7* activity for the survival of a subset of these cells. However, twice as many cholinergic neurons were lost in the mutant striatum compared with β -gal⁺ cells (Fig. 2G), suggesting that upon deletion of *Lhx7*, a significant fraction of β -gal⁺ cholinergic neurons survive but extinguish expression of cholinergic markers. In support of this view, we recorded an increased number of β -gal⁺ChAT[−] cells in the striatum of *ChAT::Cre;Lhx7^{fl/LacZ}* animals (Fig. 2F and G). Together, our experiments indicate that *Lhx7* is required to maintain the cholinergic identity of immature CSIs.

Upon Deletion of *Lhx7* CSIs Switch Identity and Acquire Molecular and Morphological Features of GSIs.

Because GSIs and CSIs share common *Lhx6*-expressing precursors, we investigated the possibility that deletion of *Lhx7* from committed cholinergic interneurons is associated with reactivation of *Lhx6* and acquisition of GABAergic characteristics. To explore this idea, we double immunostained forebrain sections from control and conditional mutant animals with antibodies for β -gal (to identify all striatal interneurons derived from *Lhx7*-expressing lineages) and *Lhx6*. Relative to controls, the total number of β -gal⁺ cells coexpressing *Lhx6* (β -gal⁺*Lhx6*⁺) in the striatum of *ChAT::Cre;Lhx7^{fl/LacZ}* animals increased by approx. 46% (Fig. 3A–C), with *Lhx6* never detected in residual ChAT⁺ neurons. Moreover, the number of Sst⁺ and Pv⁺ interneurons coexpressing *LacZ* increased in *ChAT::Cre;Lhx7^{fl/LacZ}* animals relative to controls [16.7% increase of Sst⁺ β -gal⁺ interneurons and 27% increase of Pv⁺ β -gal⁺ interneurons (Fig. 3D and E)]. These findings, together with the reduced representation of cholinergic neurons in the total β -gal⁺ subpopulation of the striatum, suggest that *Lhx7*-depleted CSIs switch identity and acquire molecular characteristic of GABAergic interneurons. Interestingly, β -gal⁺ cells were not detected in the cortex of conditional mutants indicating that *Lhx7*-depleted precursors do not adopt a cortical fate.

CSIs can be distinguished from GSIs because of their distinct cellular morphology and relatively large nuclear size (21, 22). Consistent with this, striatal interneurons in *ChAT::Cre;Lhx7^{+/LacZ}* mice, which express the nuclear localized reporter β -gal, include neurons with large and intensely labeled nuclei, as well as neurons with relatively small and weakly labeled nuclei (Fig. 3F, black and white arrowheads, respectively). Interestingly, in *ChAT::Cre;Lhx7^{fl/LacZ}* animals, the number of large and intensely stained nuclei was considerably smaller, whereas the number of small nuclei had increased (Fig. 3G). Triple immunostaining for β -gal, ChAT, and *Lhx6* confirmed that large nuclei corresponded to CSIs, whereas the cells with small nuclei were GSIs (Fig. 3J).

To quantify the effects of *Lhx7* deletion on nuclear morphology, the nuclear surface area and marker expression of striatal interneurons was compared in forebrain sections from control and conditional mutants triple immunostained for β -gal, ChAT, and *Lhx6*. In control sections, β -gal⁺ neurons segregated into two

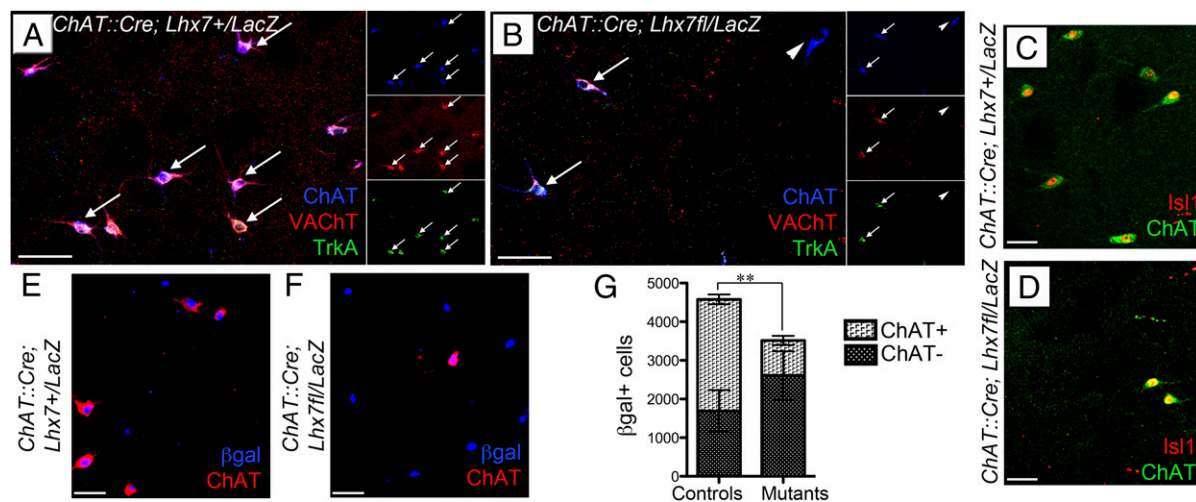


Fig. 2. Lhx7 activity is necessary for maintaining the cholinergic identity of striatal interneurons. (A–F) Sections from the striatum of adult *ChaT::Cre;Lhx7^{+/LacZ}* (A, C, and E) and *ChaT::Cre;Lhx7^{fl/LacZ}* (B, D, and F) animals immunostained for ChAT, VAcHT, and TrkA (A and B), ChAT and Isl1 (C and D), or ChAT and β -gal (E and F). The number of cholinergic cells in conditional mutants is reduced and some ChAT⁺ neurons are negative for VAcHT or TrkA (arrowhead in B). (G) Quantification of the cholinergic and noncholinergic subpopulations of β -gal⁺ cells in control and conditional mutant animals. The dramatic loss of cholinergic neurons in the striatum of *ChaT::Cre;Lhx7^{fl/LacZ}* animals was associated with an increase in the number of noncholinergic β -gal⁺ cells (E–G). Bars represent means \pm SD. [Scale bars: 100 μ m (A and B); 50 μ m (C–F).]

largely nonoverlapping groups; the first was characterized by relatively small nuclear size (peak around 120 μ m²) and expression of *Lhx6* (Fig. 3H, black), and the second by significantly larger nuclear size (peak around 230 μ m²) and expression of ChAT (Fig. 3H, red). Similar analysis from *ChaT::Cre;Lhx7^{fl/LacZ}* animals showed a dramatic reduction in the fraction of β -gal⁺ neurons with large nuclei, a concomitant decrease in the representation of cholinergic neurons and an increase in β -gal⁺ neurons belonging to the *Lhx6*⁺ group (Fig. 3I). The average nuclear size of the *Lhx6*⁺ population was indistinguishable in control and conditional mutant animals, consistent with the idea that cholinergic precursor-derived *Lhx6*⁺ interneurons acquire the nuclear size of GABAergic interneurons (Fig. 3K). Interestingly, we observed a relatively small but statistically significant reduction in the nuclear size of ChAT⁺ neurons in the striatum of *Lhx7^{fl/LacZ}; ChaT::Cre* mice (Fig. 3K), indicating that the residual CSIs of these animals may have subtle phenotypic alterations. We conclude that deletion of *Lhx7* from committed cholinergic interneurons results in molecular and morphological reprogramming of these cells toward the GABAergic identity.

Reprogramming of CSIs Occurs Shortly After *Lhx7* Deletion and Does Not Require Cell Division. To define the stage at which *Lhx7*-depleted CSIs acquire GABAergic characteristics, we analyzed expression of ChAT mRNA in the basal forebrain of *ChaT::Cre;Lhx7^{+/LacZ}* and *ChaT::Cre;Lhx7^{fl/LacZ}* embryos. Reduction in the number of ChAT-expressing cells was evident already at E14.5 (compare Fig. 4 A and B) and was more pronounced by E16.5 (compare Fig. 4 C and D). Therefore, *Lhx7* is required throughout the period of maturation of committed cholinergic precursors to actively maintain cholinergic identity, and deletion of the locus is followed shortly afterward by acquisition of GABAergic character.

To examine whether acquisition of GABAergic characteristics by *Lhx7*-depleted cholinergic interneurons requires reentry into the cell cycle, we immunostained forebrain sections of E13.5, E14.5, E15.5, and E16.5 *ChaT::Cre;Lhx7^{fl/LacZ}* embryos for β -gal and Ki67 (Fig. 4 E and F). At no stage did we observe colocalization of the two markers, indicating that the *Lhx7*-expressing lineage remains postmitotic even after deletion of the gene and that the switch from a cholinergic to a GABAergic fate does not require cell division.

Lhx7 Maintains Cholinergic Identity by Regulating a Switch in Expression of *Lhx6* and *Isl1*. Given the critical roles of *Lhx6* and *Isl1* for GABAergic and cholinergic differentiation, respectively

(10, 11), we posited that cross-repressive interactions between the two genes may control the segregation of the two striatal interneuron sublineages and that *Lhx7* could maintain the cholinergic phenotype by sustaining the expression of *Isl1* and, consequently, the down-regulation of *Lhx6*. To provide support for this hypothesis, we constitutively expressed *Isl1* in the MGE of embryonic brain slices from wild-type embryos and analyzed its effect on *Lhx6* expression. Vectors expressing either GFP (control) or *Isl1*-GFP were electroporated into the ventral region of forebrain slices from E12.5 (Fig. 5B) and E14.5 (Fig. 5C) *Lhx7^{+/LacZ}* embryos. Forty-eight hours later, we quantified the percentage of GFP⁺ cells coexpressing *Lhx6* (Fig. 5A and Materials and Methods). In control E12.5 slices, ~20% of GFP⁺ cells coexpressed *Lhx6*. In contrast, expression of *Isl1* led to a 70% reduction of the fraction of *Lhx6*⁺GFP⁺ cells (6.1%) (Fig. 5B). A similar effect was observed upon electroporation of *Isl1* in E14.5 slices (reduction from 8.7% in GFP-electroporated to 2.6% in *Isl1*-electroporated slices; Fig. 5C). These studies suggest that *Isl1* is capable of downregulating *Lhx6* in the ventral forebrain of mouse embryos.

To further explore the role of *Lhx7* in the regulation of *Lhx6* expression by *Isl1*, we electroporated E14.5 forebrain slices from *Lhx7*-null (*Lhx7^{LacZ/LacZ}*) animals with *Isl1*-GFP or GFP-only constructs (Fig. 5D). We observed that ~10.5% of the GFP-electroporated cells in the MGE of *Lhx7*-null slices coexpressed *Lhx6*. In contrast, only 4% of the *Isl1* electroporated cells coexpressed *Lhx6*, indicating that the ability of *Isl1* to inhibit *Lhx6* expression is largely independent of *Lhx7*. These findings provide support to the idea that cross repressive interactions between *Isl1* and *Lhx6* control sublineage segregation of striatal interneurons.

Discussion

All neuronal and glial subtypes in the CNS are derived from multipotential neuroepithelial progenitors that, under the influence of extracellular signals and intracellular factors, restrict their developmental potential and choose between alternative cell identities (2). “Terminal selector genes” encode transcription factors that coordinately regulate the expression of diverse molecular, morphological and functional characteristics that constitute the terminal phenotype of neurons (23). *Lhx7* and *Lhx6* belong to this class of genes because, respectively, they regulate multiple aspects of cholinergic and GABAergic interneuron differentiation in the mammalian forebrain (8, 10, 12–14). In vertebrates terminal selector genes have been studied mostly in the context of lineage restriction and forward neuronal

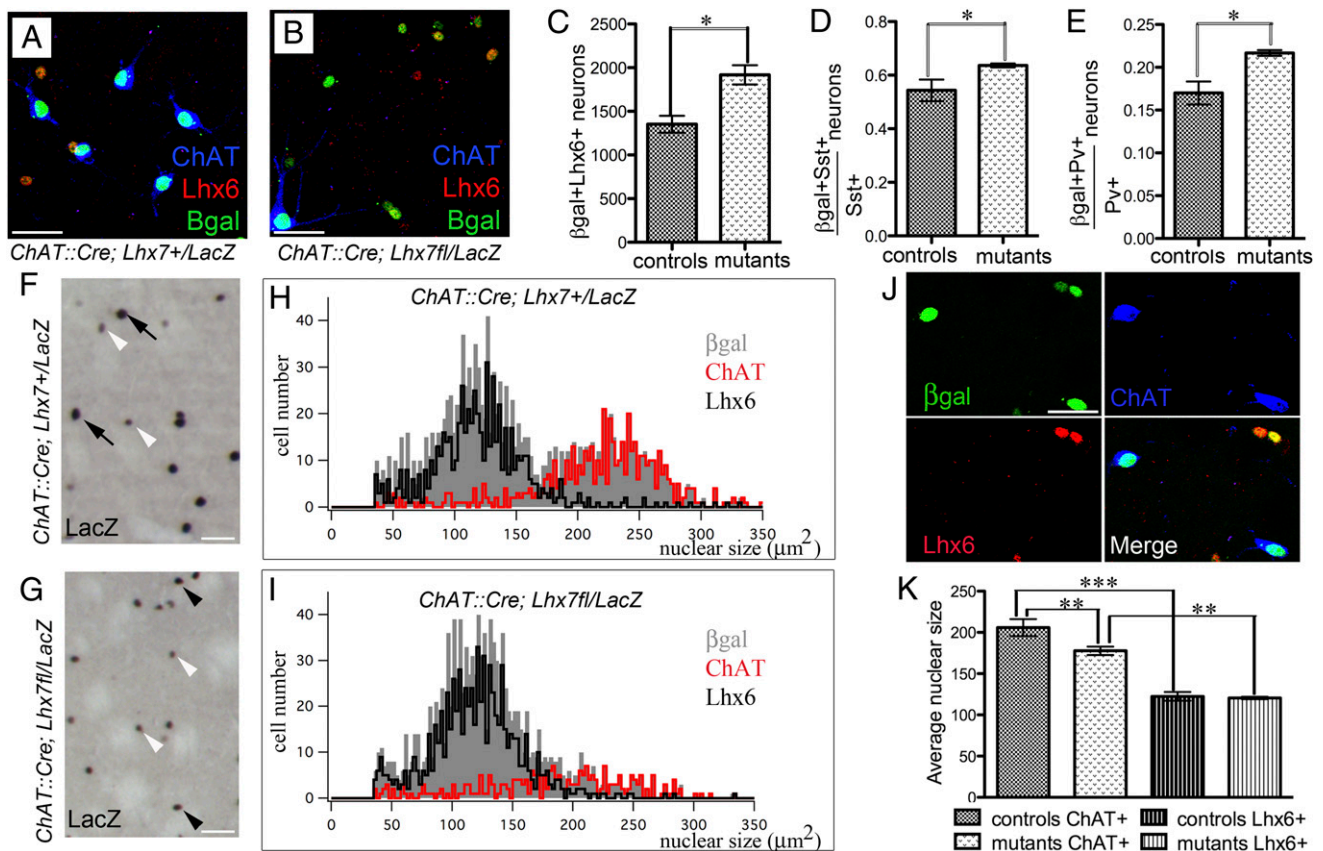


Fig. 3. *Lhx7*-depleted CSI precursors acquire molecular and morphological characteristics of GSIs. (A and B) Sections from the striatum of *ChAT::Cre;Lhx7^{+/LacZ}* (A) and *ChAT::Cre;Lhx7^{fl/LacZ}* (B) animals triple-immunostained for ChAT, Lhx6, and β -gal. (C) Quantification of β -gal⁺Lhx6⁺ cells in the striatum of adult animals from the two genotypes. Note the increased representation of Lhx6⁺ cells among β -gal⁺ neurons in conditional mutants (A–C). (D and E) Quantification of the β -gal⁺ fraction of the Sst⁺ (D) or Pv⁺ (E) GSIs of adult conditional mutants and controls. (F and G) Sections from the striatum of *ChAT::Cre;Lhx7^{+/LacZ}* (F) and *ChAT::Cre;Lhx7^{fl/LacZ}* (G) adult animals immunostained for β -gal. (H and I) Distribution of nuclear size of all β -gal-expressing cells (gray), β -gal⁺Lhx6⁺ (black), and β -gal⁺ChAT⁺ (red) subpopulations in the striatum of *ChAT::Cre;Lhx7^{+/LacZ}* (H) and *ChAT::Cre;Lhx7^{fl/LacZ}* (I) mice. (J) Example of triple-immunostaining (β -gal, Lhx6, ChAT) used for the quantification shown in H and I. (K) Quantification of average nuclear size in the cholinergic (ChAT⁺) and GABAergic (Lhx6⁺) subpopulations in the striatum of *ChAT::Cre;Lhx7^{+/LacZ}* and *ChAT::Cre;Lhx7^{fl/LacZ}* animals. [Scale bars: 50 μ m (A, B, F, G, and J).]

differentiation, but their subsequent role in maintaining neuronal subtype identity is largely unknown. For example, constitutive germ-line deletion of *Tlx1/3*, *Helt*, *Lbx1*, and *Sox6* resulted in the acquisition by the relevant cell lineages of alternative default cell fates (24–28). However, the potential plasticity of the neuronal identities promoted by these transcription factors has not been addressed. Using a conditional gene ablation strategy that deletes *Lhx7* from committed forebrain cholinergic neurons, we demonstrate here that, in addition to its role in commitment and differentiation, *Lhx7* activity is also critical for maintaining the lineage identity of CSIs. Several lines of evidence suggest that our experimental strategy bypasses the requirement of *Lhx7* for the initial specification of striatal cholinergic precursors and allows us to explore its role in lineage-committed cholinergic neurons. First, Cre recombinase and its reporter YFP were induced exclusively in the nonproliferative mantle zone of the MGE, where the late cholinergic marker ChAT is expressed. Second, during embryogenesis, the Cre-dependent YFP reporter was specifically localized in cholinergic precursors marked by β -gal and *Isl1* and was excluded from cells expressing *Lhx6*, which marks unspecified common precursors of striatal interneurons or specified GABAergic interneurons. Third, fate-mapping analysis of *ChAT::Cre*-expressing cells shows that in the adult striatum YFP was specifically expressed by β -gal⁺ cholinergic interneurons.

Striatal cholinergic interneurons are derived from *Lhx6*-expressing postmitotic precursors, which upon cholinergic commitment, induce *Isl1*, down-regulate *Lhx6*, and progressively acquire mature cholinergic markers and characteristic mag-

nocellular morphology (8, 18, 29). In contrast, the lineally related GSIs maintain expression of *Lhx6* and induce subtype-appropriate markers, such as Sst and Pv (Fig. 6). We demonstrate here that deletion of *Lhx7* from committed cholinergic interneurons results not only in up-regulation of *Lhx6* but also in expression of mature GABAergic-subtype markers and adoption of morphological features that are characteristic of GABAergic interneurons. Therefore, *Lhx7*-depleted cholinergic interneurons do not simply revert to an Lhx6⁺ common precursor stage but are capable of differentiating as Pv⁺ or Sst⁺ interneurons. Together, our studies argue that interneurons of the mammalian forebrain maintain remarkable phenotypic plasticity beyond cell cycle exit and commitment to terminal identity. Future studies could address the exciting possibility that GABAergic neurons originating from the *Lhx7*-depleted cholinergic lineage integrate into functional neuronal circuits of the basal forebrain.

Our findings raise the possibility that *Lhx7* activity is necessary to maintain the identity of fully differentiated CSIs during postnatal and adult stages. Interestingly, experiments involving withdrawal of trophic support in adult animals suggest that the cholinergic phenotype must be actively maintained throughout life. Thus, transection of septal cholinergic neuron projections to the hippocampus prevents target-derived nerve growth factor (NGF) signaling and leads to neuronal atrophy and loss of characteristic markers of cholinergic phenotype without cell death (30, 31). On the other hand, NGF infusion in the striatum of adult animals can protect or stimulate the cholinergic phenotype of interneurons (32–34). However, these studies have not determined

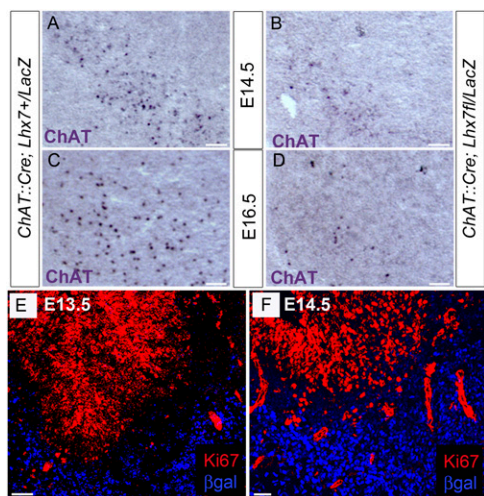


Fig. 4. Loss of cholinergic identity of *Lhx7*-depleted CSIs is not associated with reentry into the cell cycle. (A–D) ISH with a ChAT-specific riboprobe of ventral forebrain sections from E14.5 (A and B) and E16.5 (C and D) *ChAT::Cre; Lhx7^{+/LacZ}* (A and C) and *ChAT::Cre; Lhx7^{fl/LacZ}* (B and D) animals. (E and F) Ventral forebrain sections from E13.5 (E) and E14.5 (F) *ChAT::Cre; Lhx7^{fl/LacZ}* embryos immunostained for β -gal and Ki67. No double-positive cells were detected at any of the stages analyzed. (Scale bars: 50 μ m.)

whether upon withdrawal of neurotrophin signaling cholinergic neurons adopt an alternative neuronal identity. It would be interesting to investigate whether the effect of neurotrophins on cholinergic neurons is mediated by *Lhx7* and *Isl1* and determine whether variations in trophic signaling could trigger physiological changes in cholinergic identity in the mammalian forebrain.

What are the molecular mechanisms that ultimately control the choice and maintain the identity of striatal interneuron subtypes? The mutually exclusive expression of *Isl1* and *Lhx6* at all stages of striatal interneuron differentiation and the ability of *Lhx6* to inhibit *Lhx7*/*Isl1*-induced cholinergic differentiation in chicken embryo

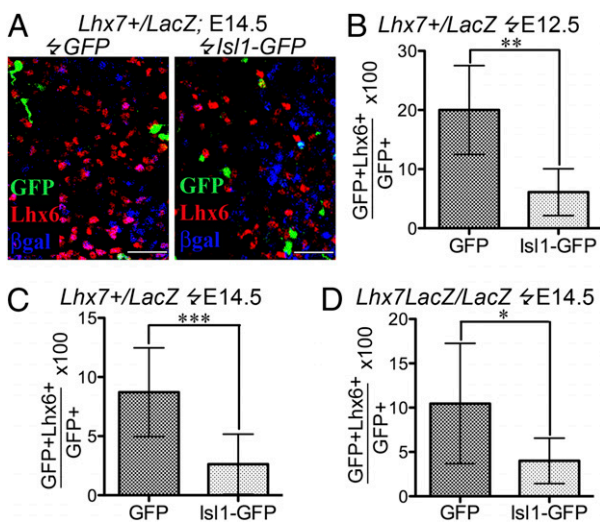


Fig. 5. *Isl1* down-regulates *Lhx6* in the ventral forebrain. (A) Sections from ventral forebrain slices of E14.5 *Lhx7^{+/LacZ}* embryos electroporated with GFP-only vector [left hemisphere (Left)] and *Isl1*-GFP vector [right hemisphere (Right)] were immunostained for GFP, *Lhx6*, and β -gal. (B and C) Quantification of the fraction of *Lhx6*-expressing GFP⁺ cells in ventral forebrain slices from E12.5 (B) or E14.5 (C) *Lhx7^{+/LacZ}* embryos following electroporation of GFP-only or *Isl1*-GFP-expressing vectors. (D) Similar quantification following electroporation of E14.5 *Lhx7^{fl/LacZ}* slices.

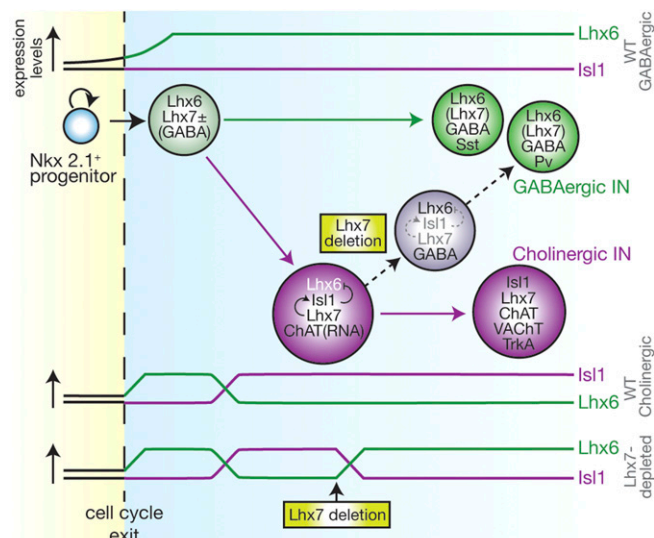


Fig. 6. Model for *Lhx7*-dependent segregation and maintenance of alternative striatal interneuron subtypes. CSIs and GSIs are specified from common *Nkx2.1⁺* MGE-derived progenitors, which, upon exit from the cell cycle, express *Lhx6*, *GABA*, and (at least some of them) *Lhx7*. A subset of these postmitotic precursors induce *Isl1*, which results in down-regulation of *Lhx6*, and differentiate into magnocellular cholinergic neurons expressing *ChAT*, *VACht*, and *TrkA* (Cholinergic IN). Cells that do not induce *Isl1* continue to express high levels of *Lhx6* and differentiate into *Pv⁺* or *Sst⁺* GABAergic interneurons (GABAergic IN). Constitutive deletion of *Lhx7* results in transient induction of *Isl1* and the default differentiation of postmitotic precursors into mature GABAergic interneurons (8), suggesting that *Lhx7* is required for maintaining *Isl1* expression. The ability to adopt a GABAergic identity is not lost after cholinergic specification, because *Lhx7* deletion in committed cholinergic neurons results in loss of *Isl1*, up-regulation of *Lhx6*, and acquisition of molecular and morphological features of GABAergic neurons. The changes in the relative levels of *Lhx6* and *Isl1* during normal differentiation of GSIs and CSIs are shown in the graphs labeled “WT GABAergic” and “WT Cholinergic,” respectively. The changes in the relative levels of *Lhx6* and *Isl1* as a result of *Lhx7* deletion are depicted in the graph labeled “*Lhx7*-depleted.”

spinal cord (8) suggest an antagonistic role of these transcription factors in striatal interneuron specification. Our present findings are consistent with this idea and further suggest that the antagonistic interaction between *Isl1* and *Lhx6* is also required to maintain the identity of CSIs beyond their initial specification (model in Fig. 6). By demonstrating here that overexpression of *Isl1* in the ventral forebrain is sufficient to down-regulate *Lhx6*, we provide direct evidence for an inhibitory cross-regulatory interaction between these factors. However, what is the role of *Lhx7* in such cross regulatory loop between *Lhx6* and *Isl1*? We propose that *Lhx7* activity is required for the sustained expression of *Isl1* and the consequent down-regulation of *Lhx6*. This idea is consistent with the observation that in *Lhx7*-deficient embryos, *Isl1* is only transiently induced in cholinergic interneurons precursors (8) and is supported by our present studies indicating that ablation of *Lhx7* in cholinergic neurons results in down-regulation of *Isl1*, re-expression of *Lhx6* and acquisition of a GABAergic phenotype. Based on these findings, we suggest that *Lhx7* controls the generation of GABAergic and cholinergic interneuron lineages in the striatum by gating the cross inhibitory interaction between *Lhx6* and *Isl1* (model in Fig. 6). It should be noted that although the down-regulation of *Lhx6* is crucial for cholinergic differentiation (8, 10, 12–14), it is not sufficient for instructing cholinergic identity in interneuron precursors because *Lhx6*-null animals do not show an increase in the number of cholinergic interneurons. Accordingly, our observation in this report that the residual cholinergic neurons in *Lhx7* mutants retain *Isl1* but show subtle changes in morphology and expression of mature cholinergic markers suggests that *Lhx7* (perhaps in combination with other factors such as *Isl1*) has additional roles in cholinergic differentiation. Future studies could address in detail

the molecular mechanisms by which *Lhx7* contributes to the commitment and maintenance of the cholinergic lineage during maturation of the mammalian striatum and perhaps in adult animals.

The notion of a stable cellular identity has been challenged in different systems by studies using gene ablation or overexpression of defined sets of transcription factors to reprogram mature cellular phenotypes (35). Thus, deletion of *Pax5* from B cells respecifies them into T cells, a process that requires cell division and the formation of an undifferentiated intermediate (36). In contrast, *in vivo* conversion of pancreatic exocrine cells into islet β cells (37) by the expression of key developmental factors does not involve reversal to a pluripotent stage or reentry into cell cycle. Similarly, the cholinergic to GABAergic switch we have observed in the *Lhx7* conditional mutants is unlikely to require cell division as we found no reexpression of the cell cycle marker *Ki67*. *Lhx7*-depleted cholinergic neurons adopt a neuronal subtype identity (GABAergic) that represents an alternative postmitotic fate of the common precursor during physiological specification. This is consistent with the idea that in the forebrain epigenetic possibilities are determined at the last cell division and subsequent changes in expression profile of postmitotic precursors (such as the GABAergic vs. cholinergic choice by striatal interneuron precursors) are reversible without the need for extensive chromatin remodelling. It has been suggested that cross-antagonism between transcription factors that generate two cell fates could be a regulatory mechanism that allows physiological transitions between these fates (35). Maintaining the flexibility to switch between two types of striatal interneurons by *Lhx7*-controlled expression of *Isl1* and *Lhx6* could fine tune the necessary balance of striatal interneuron subtypes during development. By retaining phenotypic plasticity beyond the initial specification, forebrain neurons could contribute to the ability of the CNS to cope with dynamic changes imposed by developmental requirements, learning, or disease.

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Materials and Methods

Animals. Generation of the *Lhx7fl* allele is described in *SI Materials and Methods*. All animal studies were carried out under a United Kingdom Home Office Project License in a Home Office-designated facility.

In Situ Hybridization Histochemistry and Immunohistochemistry. In situ hybridization (ISH) on sections from embryonic and adult brain were carried out as described in *SI Materials and Methods* (38). The ChAT riboprobe was generated from a 2.2-kb rat cDNA (gift from C. Goidis, Institut de Biologie de l'Ecole normale supérieure, Paris, France).

Quantifications. For all quantifications, the result are expressed as means \pm SD, and statistical analysis of significance was performed using a two-tailed Student *t* test (Prism 5; GraphPad). The level of significance is indicated by **P* < 0.5, ***P* < 0.05, or ****P* < 0.005 in the figures.

Measurement of Nuclear Size and Fluorescence Intensity. To evaluate the nuclear size of β -gal⁺ cells and quantify the expression of β -gal, ChAT, and *Lhx6* in these cells a series of confocal images were analyzed with ImageJ with user-designed macros to establish a signal/noise threshold, select particles, and create areas of local background for each particle. The size of the particles was measured on the β -gal channel, and intensity of fluorescence, subtracted by the local background, was calculated for each particle on the three channels corresponding to β -gal, ChAT, and *Lhx6*. The resulting data were analyzed using Igor Pro (Wavemetrics).

In Vitro Focal Electroporation and Organotypic Slice Culture. Organotypic slice culture and focal electroporation were performed as previously described (39). For details, see *SI Materials and Methods*.

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