Recruitment of FoxP2-Expressing Neurons to Area X Varies During Song Development

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ABSTRACT: **In adult songbirds, neural progenitors proliferate along the lateral ventricles. After migration, many of the subsequently formed neuroblasts integrate into the song nuclei HVC and Area X that participate in auditory-guided vocal motor learning and singing. Recruitment of postembryonically generated neurons into HVC, rodent hippocampus, and olfactory bulb has been linked to learning and memory. The cellular identity and the role of postembryonically generated neurons in Area X are unknown. Here we describe that the majority of new neurons in postembryonic Area X of male zebra finches expressed DARPP32 but not choline acetyltransferase or parvalbumin. This suggests that they are spiny neurons. Retrogradely labeled neurons projecting to thalamic nucleus DLM were not renewed. The spiny neurons in Area X were recently shown to express FoxP2, a transcription factor critical for normal speech and language development in humans. Since increased *FoxP2* mRNA expression was previously observed during periods of vocal plasticity we investigated whether this increase might be associated with neuronal recruitment. Consistent with their spiny phenotype, new neurons in Area X did express FoxP2 and recruitment increased transiently during the juvenile song learning period. Moreover we found that FoxP2 was expressed in the ventricular zone of adult songbirds but was absent from the germinal zones in adult mouse brains, the hippocampus, and the subventricular zone. Together these results raise the possibility that neuronal recruitment and FoxP2 expression in Area X are associated with vocal learning.** © 2007 Wiley Periodicals, Inc. Develop Neurobiol 67: 809–817, 2007

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INTRODUCTION

Correlative data from a variety of invertebrate and vertebrate species implicate adult neurogenesis in behavioral plasticity (Wilbrecht and Nottebohm, 2003; Doetsch and Hen, 2005). In vertebrates, new neurons are generated from stem cells residing in a germinal layer lining the lateral ventricle (Garcia Verdugo et al., 2002). In adult zebra finches (*Taeniopygia guttata*) neuroblasts migrate away from their birthplace and many of them integrate into HVC and Area X (Goldman and Nottebohm, 1983; Nordeen and Nordeen, 1988; Alvarez-Buylla et al., 1994), two regions of the song control system. In contrast to HVC, less information is available about neurogenesis in Area X (Nordeen and Nordeen, 1988; Sohrabji et al., 1993; Alvarez-Buylla et al., 1994; Lipkind et al., 2002). For instance, the neuronal phenotype into which neuroblasts differentiate is unknown. Area X is the first relay of the anterior forebrain pathway, which mediates juvenile song learning (Bottjer et al., 1984; Sohrabji et al., 1990; Scharff and Nottebohm, 1991) and monitors adult song (Williams and Mehta, 1999; Brainard and Doupe, 2000; Kao et al., 2005). Electrophysiologically and neuroanatomically most neuron types in Area X strikingly resemble mammalian striatal neurons (Bottjer and Johnson, 1997; Farries et al., 2005). At least three different populations

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of interneurons exist in Area X (Carrillo and Doupe, 2004; Reiner et al., 2004a): (1) GABAergic cells expressing the Ca^+ binding molecule parvalbumin (Parv), (2) cells coexpressing Parv and the neurotensin-related hexapeptide LANT6, and (3) cholinergic cells (Fig. 3a). Most neurons in Area X that synapse onto the neurons that project to the thalamic nucleus DLM are equivalent to the mammalian medium spiny neurons (Farries and Perkel, 2002). As in mammals, avian spiny neurons express the D1-receptor associated signaling protein dopamine-and-cAMP-regulated phosphoprotein of 32 kDa (DARPP32), the neuropeptide substance P and the transcription factor FoxP2 (Reiner et al., 1998; Carrillo and Doupe, 2004; Haesler et al., 2004; Reiner et al., 2004a). Recent work shows that in Area X expression of *FoxP2*, a transcription factor involved in human speech and language (Lai et al., 2001), might be linked to the function of vocal control circuitry in songbirds (Haesler et al., 2004; Teramitsu and White, 2006). Since Area X recruits considerable numbers of new neurons in young and adult songbirds (Nordeen and Nordeen, 1988; Alvarez-Buylla et al., 1994) we investigated the developmental time course of neuronal recruitment during the song learning phase and its relationship to FoxP2 expression in zebra finches. Here we show that during posthatch development and in adulthood the majority of neurons recruited into Area X differentiate into $DARPP32^+$ spiny neurons and express FoxP2. No new neurons were found among any of the interneuron populations, or among the neurons projecting to DLM. We also show that FoxP2 is highly expressed in the avian, but not the mammalian, ventricular zone (VZ). Finally we document that recruitment of neurons expressing FoxP2 increases transiently during the song learning phase. Together these results raise the possibility that FoxP2 function is linked to striatal neurogenesis in songbirds and may be related to vocal plasticity.

METHODS

Subjects

Male zebra finches (*Taeniopygia guttata*) and male C57BL/6 mice were obtained from breeding colonies at the Max-Planck Institute for Molecular Genetics, Berlin.

BrdU Injections

Dividing cells were labeled with the DNA synthesis marker, 5-bromo-2′-deoxyuridine (BrdU). BrdU was administered intramuscularly (50 mg/kg of body weight, dissolved in 0.4 *N* NaOH with 0.9% NaCl). To identify the phenotype of newborn neurons in Area X, adult zebra finches (4-months old) received 12 BrdU injections (3 per day during 4 consecutive days) and labeled profiles were detected 4 weeks later. To determine if the percentage of new cells expressing neuronal markers increases with longer maturation time, labeled profiles were analyzed 6 weeks after 5 injections of BrdU. To quantify the rate of recruitment of newborn neurons during song learning, five groups of male juveniles zebra finches received five BrdU injections (one every 2 h) at 4, 14, 29, 54, and 79 days after hatching and were sacrificed at 25, 35, 50, 75, and 100 days after hatching respectively.

Immunohistochemistry

For all histological analyses requiring tissue fixation by perfusion, birds and mice were given an overdose of anesthesia and perfused transcardially with saline (NaCl 0.9%) and subsequently with 4% paraformaldehyde (PFA) in 0.1 *M* phosphate buffer (pH 7.3). Brains were excised and kept in PFA overnight. Coronal sections were serially cut using a vibratome (VT1000S, Leica) and collected in PBS (0.1 *M*; pH 7.3).

Phenotype of Newborn Neurons in Area X

Newborn cells ($BrdU^+$) were phenotyped by double immunofluorescence staining with the following primary antibodies: rat anti BrdU (1:200, ImmunologicalsDirect), mouse anti-HuC/D (1:200, Chemicon), rabbit anti-cAMP-regulated phosphoprotein of molecular weight 32,000 (DARPP32, kindly provided by H.C. Hemmings, Jr., The New York Hospital-Cornell Medical Center, NY; 1:20 000), mouse anti-parvalbumin (1:1000; Swant), goat anti-choline acetyltransferase (1:100, Chemicon), rabbit anti-FoxP2 (1:1000, kindly provided by E.E. Morrisey, University of Pennsylvania, Philadelphia). For all experiments, the anti-BrdU antibody was applied on free-floating 30-μm sections that were permeabilized with 0.2% triton and DNA was denatured with HCl (2 *N*, 30 min at 37°C). Double labeling was carried out sequentially, to minimize cross reactivity. Briefly, the slices were first incubated with anti-BrdU overnight. After BrdU detection with Alexa 568-labeled goat anti-rat IgG (1:500, Molecular Probes), slices were fixed in PFA for 10 min and then exposed to one of the primary antibodies cited above. Primary antibodies were then revealed with Alexa 488-labeled secondary antibody (1:200; Molecular Probes) raised against the species in which the primary antibodies were made.

Expression of FoxP2 in Germinal Zones

Adult zebra finches (>90 days) were sacrificed 4 h after a single injection of BrdU. Adult mice (2-months old) were sacrificed either 4 h after a single BrdU injection, or 3 weeks after 5 injections of BrdU. Coronal sections (40 μm) were cut along the bird lateral ventricle, the mouse olfactory bulb, subventricular zone, or the dentate gyrus of the hippocampus. Single labeling of FoxP2 or colabeling of BrdU and FoxP2 with corresponding antibodies was applied on free-floating sections as described above.

Stereotactic Injections

To label DLM-projecting neurons in Area X, we stereotaxically injected rhodamine conjugated beads (Lumafluor) into DLM, 28 days after BrdU injection. Stereotactic injections were performed using a stereotaxic apparatus (MyNeurolab) at the coordinates (relative to the O-point at the bifurcation of the midsagittal sinus): anterio-posterior = +1.2 mm; medio-lateral = 1.3 mm; dorso-ventral = −4.5 mm. All animals were perfused 5 days later. $BrdU^+$ cells were revealed with an Alexa 488-labeled secondary antibody.

Image Analysis and Quantification

Immunofluorescent sections were analyzed with a 40× oil objective, using a Zeiss confocal microscope (LSM510) equipped with lasers Ar 488 and HeNe1 543, with LSM-510 software package for image acquisition and data analysis. Pinholes were optimally set at 94 μm for channel 1 and 106 μm for channel 2. Lateral and z axis resolutions were 0.45 and 1 μm respectively. Area X stood out from the rest of the striatum by the background and/or specific patterns of the different fluorescent immunostainings. $BrdU^+$ cells within Area X were phenotyped by analyzing in three dimensions reconstructed $BrdU^+$ nuclei in the x-z and y-z orthogonal projections for the presence or absence of the neuronal markers Hu and FoxP2. The density of BrdU/Hu and BrdU/FoxP2 neurons was calculated on every third section (90 μm apart) throughout Area X. The total size of Area X was derived by tracing its outline with a 5× objective on adjacent Nissl stained sections, using a computer-interfaced Leica DMIRE2 microscope equipped with Simple PCI software (Compix). The resulting areas were then multiplied by the sampling interval (90 μm) and summed to yield the volumes of Area X. The density of BrdU positive profiles was related to the entire volume of Area X, for each individual bird, to evaluate the total number of BrdU/FoxP2 neurons it contains.

Statistics

We used one-way ANOVAs followed by a least significant difference (LSD) post hoc test. Levels of significance were set at $p < 0.05$.

RESULTS

Newly Generated Neurons in Adult Area X Express FoxP2 and Have Characteristics of Striatal Spiny Neurons

Four weeks after BrdU injections, numerous $BrdU^+$ cells were found within Area X and of those, (66.0% ± 4.6%) expressed the transcription factor FoxP2 [$n = 304$, five animals; Figs. 1(a–c) and 2]. About 78% of the newly generated cells expressed the pan-neuronal marker Hu (Barami et al., 1995) ($n = 231$, three animals; Fig. 2). Together, this suggests that around 85% of the new neurons recruited to Area X express FoxP2. FoxP2 has previously been shown to be expressed in spiny neurons (Haesler et al., 2004). Indeed, we found that (78.2% ± 4.6%) of the $FoxP2^+$ cells also contained DARPP32 ($n = 970$, three animals), confirming that this cell population expresses FoxP2 [Figs. 1(d–f) and 2]. Moreover, confocal analysis revealed that (50.4% ± 2.3%) of the BrdU-stained cells were also $DARPP32^+$ ($n = 134$, three animals) demonstrating that spiny neurons are constitutively replaced or added in adulthood [Figs. 1(g–i) and 2]. The fact that 78% of the new cells express a neuronal marker implies that around 64% of the new neurons are spiny neurons. To identify if other striatal neuron types are also recruited to adult Area X we analyzed the presence of different striatal markers in the newly generated cells [Fig. 3(a)]. No $BrdU^+$ cells were immunopositive for parvalbumin [Fig. 3(b)] or choline acetyltransferase [Fig. 3(c)] suggesting that Area X interneurons are not renewed in adulthood ($n = 131$, four animals and $n = 232$, three animals respectively). The only known target of Area X is the dorsal lateral nucleus of the medial thalamus (DLM, for brain nomenclature see, Reiner et al., 2004b). To determine if the DLM-projecting neurons are replaced in adulthood, we injected a rhodamine-conjugated retrograde tracer in birds that had received BrdU injections 1 month prior [Fig. 3(d)]. Five days after the tracer injection into DLM, red fluorescent cell bodies were visible in Area X [Fig. 3(e)]. However, retrogradely labeled projecting neurons did not colocalize with $BrdU^+$ cells [Fig. 3(f), three animals], confirming that the DLM-projecting neurons are also not renewed in adult zebra finch (Sohrabji et al., 1993).

Since phenotypic maturation of the new neurons might take longer than 4 weeks, we also analyzed new Area X neurons 6 weeks after BrdU injection. However, we did not find an increase of new cells expressing both striatal spiny neuronal markers at this later time point, with (63.3% ± 3.4%) of the $BrdU^+$ cells expressing FoxP2 ($n = 404$, five animals) and (60.5% ± 5.6%) of the $BrdU^+$ cells expressing DARPP32 ($n = 307$, five animals). Together, these results establish that the majority of the newly generated neurons in Area X belong to the spiny neuron population and express FoxP2.

FoxP2 Expression in the Adult Germinal Layers

To specify if progenitor cells already express FoxP2, we analyzed the VZ of adult songbirds. Clusters of proliferating cells in the VZ were identified by immu-

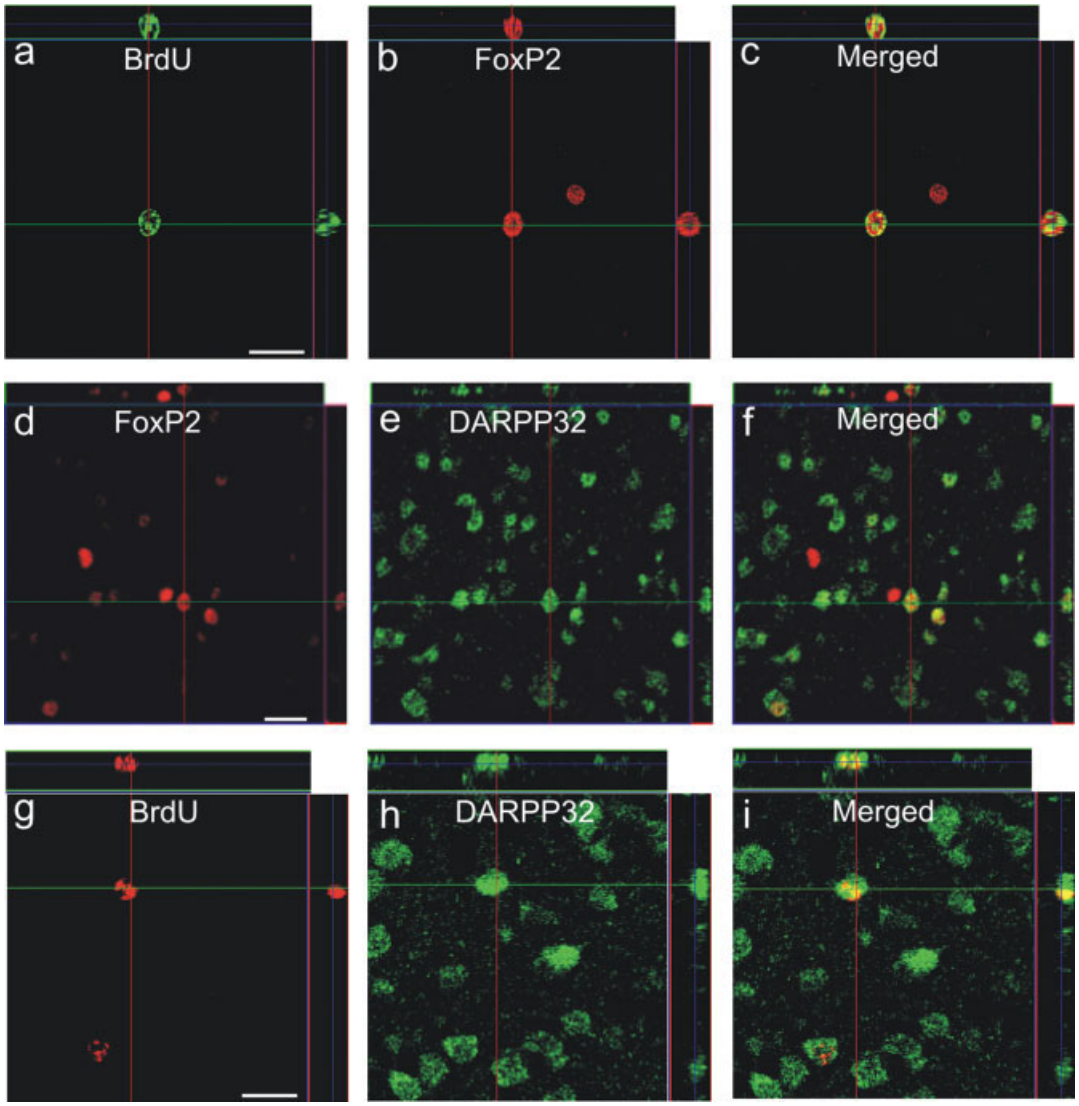

Figure 1 New neurons in Area X express FoxP2 and develop a spiny neuron phenotype. (a–c) Confocal 3D reconstruction of Area X sections labeled for BrdU (green) and FoxP2 (red). (d–f) Confocal 3D reconstruction of Area X sections after immunostaining for FoxP2 (red) and DARPP32 (green). (g–i) Confocal 3D reconstruction of a newly generated cell $BrdU^+$ (red) stained with DARPP32, a marker of the spiny neurons (green). Reconstructed orthogonal projections are presented as viewed in the *x-z* (top) and *y-z* (right) planes. Scale bar: 20 μm.

nostaining against BrdU, 4 h after its injection. FoxP2 was widely expressed in the ventrolateral wall of the ventricle [Fig. 4(a–c)], near the striatum but not in the dorsolateral part of the ventricle (data not shown). About 5% of the $BrdU^+$ cells expressed FoxP2 ($n = 629$, two animals), when analyzed confocally. Since FoxP2 is expressed in a very conserved pattern in all vertebrates studied (Teramitsu et al., 2004; Scharff and Haesler, 2005) and since the antibody we used had previously been employed to detect the murine FoxP2 protein (Ferland et al., 2003), we also investigated whether FoxP2 was present in the mouse subventricular zone of the lateral ventricle and the subgranular zone of the hippocampus, where adult murine neurogenesis occurs. However, these areas did not express FoxP2 [$n = 3$; Fig. 4(d,e)]. Moreover, although FoxP2 is expressed in the glomerular cell layer of the olfactory bulb, the target of the neuroblasts born in the subventricular zone, newly incorporated $BrdU^+$ neurons did not express it [Fig. 4(f)]. Thus, the adult avian, but not the murine neurogenic niches, express FoxP2.

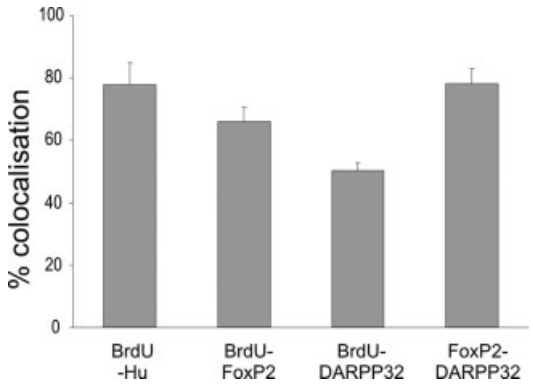

Figure 2 Quantification of the percentage of $BrdU^+$ cells expressing one of several neuronal markers, i.e. Hu, FoxP2 or DARPP32, as well as the percentage of $FoxP2^+$ cells expressing DARPP32. Data are presented as means ± s.e.m.

Recruitment of FoxP2 Expressing New Neurons to Area X Varies Developmentally

Previous work has shown that, during the period of song learning in zebra finches (PHD25-90, of which the first ~10 days are purely sensory and thereafter sensory-motor learning, Roper and Zann, 2006) *FoxP2* expression increases transiently in Area X, measured as the optical density of mRNA signal per unit area (Haesler et al., 2004). During this period, the total number of neurons in Area X also increases (Nordeen and Nordeen, 1988; Nordeen et al., 1989; Burek et al., 1991). Since we found that new neurons express FoxP2, we wondered whether the elevation of *FoxP2* expression observed by Haesler et al. (2004) was due to an increased recruitment of new $FoxP2^+$ neurons into Area X. However, the density of $FoxP2^+$ neurons in Area X during development did not change [Fig. 5(a); D25-D100, ANOVA, $F = 0.9$, $p = 0.5$], arguing that the previously observed elevation of *FoxP2* mRNA signal was not due to more cells expressing it, but instead to an increased *FoxP2* expression per cell. We then specifically analyzed the recruitment of new neurons in Area X and their phenotype during all developmental stages. At day 25, we observed a 5-fold higher density of new $FoxP2^+$ neurons in Area X than at later stages [Fig. 5(b)]. This probably reflects overall elevated perinatal levels of neurogenesis at PHD4, when BrdU was injected for the birds sacrificed at PHD25. This is supported by our observation that $BrdU^+$ cells were abundant at this age throughout the entire brain. At the subsequent time point, PHD 35 and 50, the density of new $FoxP2^+$ neurons was significantly higher than at day 100 [Fig. 5(b)]. Interestingly, the proportion of non-neuronal new cells ($BrdU^+/Hu^-$) added to Area X also varied strikingly throughout development, being highest at 25 and 35 PHD and then decreased steadily [Fig. 5(b)].

Since the total number of cells in Area X, i.e. the number of cells "working together", is probably the most biologically relevant variable, we also analyzed the volume of Area X throughout development [Fig. 5(c)]. There was no significant difference between PHD 25 and 35, after which the volume increased and by PHD 75 Area X reached its maximal size, being almost twice as big as at PHD 35. By PHD 100, Area X was somewhat smaller again. Integrating the volume data with the density of new neurons [Fig. 5(d)] reveals that during the song learning period, Area X incorporates most neurons in the sensory-motor phase, i.e. during plastic song (PHD 50 and 75) rather than during the earliest vocalizations, i.e. subsong (PHD 35) or after song has become crystallized (PHD100).

To substantiate the validity of our volume and cell counts, we performed a separate analysis, quantifying the borders of Area X in a total of 12 sections (90 μm apart) per bird in the five groups (D25, D35, D50, D75, and D100) in DAB-stained material. We then counted all $BrdU^+$ cells within those borders at 10×. Subsequently we extrapolated the number of $FoxP2^+$ cells among the $BrdU^+$ cells by quantifying the percentage of colocalization of $FoxP2^+/BrdU^+$ cells within ~1 μm^2 per animal at 40× at the confocal microscope. This analysis yielded equivalent results (data not shown).

DISCUSSION

Phenotype of the New Neurons

Here we demonstrate that postembryonically generated neurons recruited to Area X of male zebra finches express DARPP32, a marker of striatal spiny neurons (Reiner et al., 1998). In addition, our data suggest that none of the other known neuron types in Area X are generated after hatching, which was previously only known for the pallidal projection neurons (Sohrabji et al., 1993). These results thus reveal that within Area X of adult zebra finches, neurogenesis probably gives rise exclusively to spiny neurons (see below). Interestingly, recent studies indicate that neurogenesis of adult medium spiny neurons can also occur in adult primates, albeit only after experimental BDNF-treatment (Bedard et al., 2005). In the avian basal ganglia, like in mammals, two different kinds of $DARPP32^+$ striatal spiny neurons exist: neurons coexpressing substance P belonging to the direct

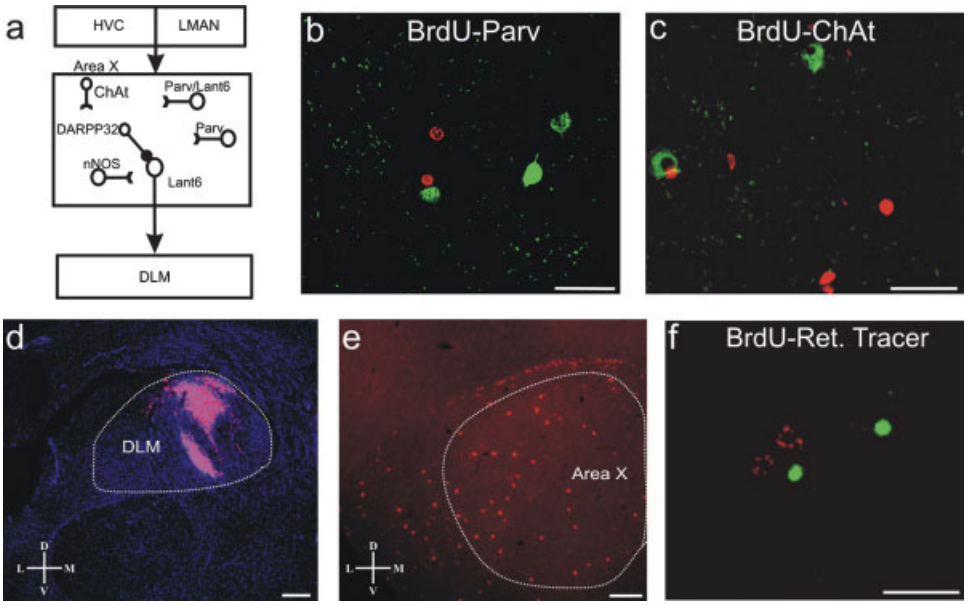

Figure 3 The interneurons and the projection neurons of Area X are not renewed in the adult zebra finch. (a) Diagram illustrating the different neuronal populations of Area X (based on the results of Carrillo and Doupe, 2004 and Reiner et al., 2004a). (b,c) Confocal photomicrographs after immunolabeling for BrdU (red) and two markers of interneuron: parvalbumin and choline acetyltransferase (green, b and c respectively). (d) Coronal section of a zebra finch brain illustrating the injection site (red) in DLM after counterstaining with DAPI (blue). (e) Coronal section of Area X containing sparsely distributed backfilled projection neurons after tracer injection into DLM. (f) Confocal photomicrograph showing $BrdU^{+}$ cells (green) and projecting neurons (red) identified by the presence of rhodamine beads after their injection in DLM. Scale bar: b, c, f, 40 μm; d–e, 100 μm.

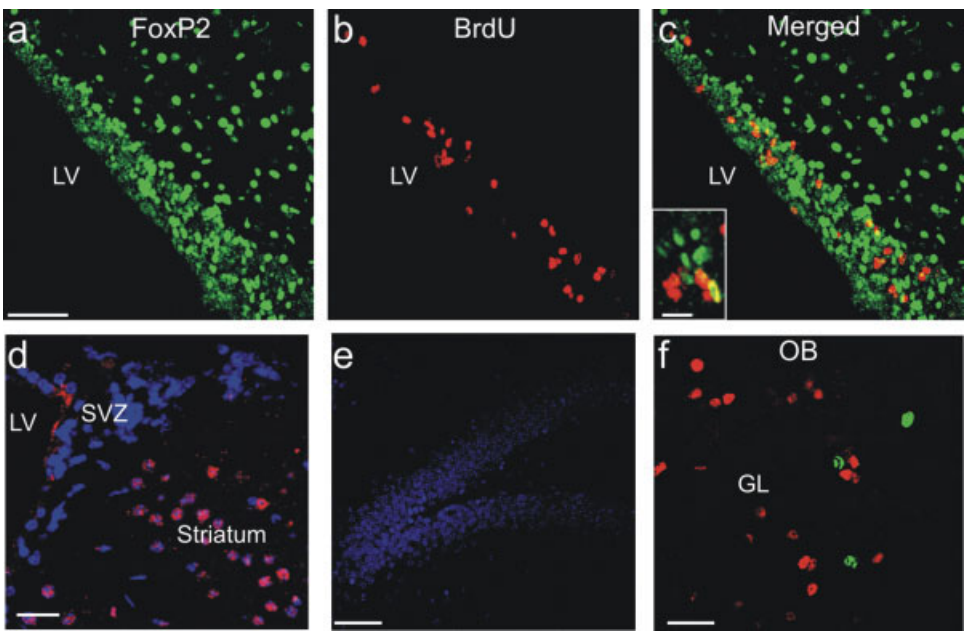

Figure 4 FoxP2 is expressed in the germinal zones of adult songbirds but not of adult mice. (a–c) Confocal photomicrographs of VZ sections in bird striatum after staining for FoxP2 (green) and BrdU (red). (d) Confocal photomicrograph of the subventricular zone of an adult mouse after FoxP2 immunostaining (red) and DAPI counterstaining (blue). (e) Confocal photomicrograph of the dentate gyrus of the hippocampus of an adult mouse after FoxP2 immunostaining. (f) Confocal photomicrograph of a section of olfactory bulb in an adult mouse after staining for FoxP2 (red) and BrdU (green). Scale bar: a–c, 60 μm; d–f, 30 μm; inset: 10 μm. GL, glomerular layer; LV, lateral ventricle; OB, olfactory bulb; SVZ, subventricular zone.

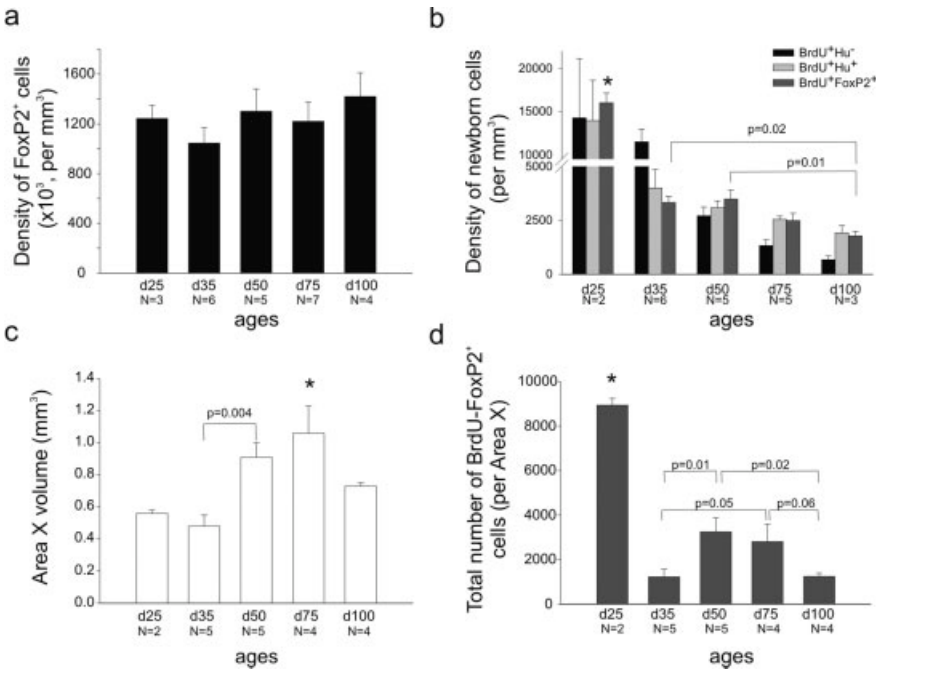

Figure 5 Recruitment of new neurons expressing FoxP2 varies during song development. (a) Density of $FoxP2^+$ neurons at different ages post hatching in Area X. (b) Density of BrdU, BrdU/Hu, and BrdU/FoxP2 cells at different ages post hatching in Area X. (c) Volume of Area X at different ages post hatching. (d) Total number of BrdU/FoxP2 cells in Area X at different ages post hatching. Data are presented as means ± s.e.m., asterisk indicates significant difference with all other groups.

pathway and neurons coexpressing enkephalin belonging to the indirect pathway (Doupe et al., 2005; Farries et al., 2005). Since both populations express DARPP32 (Reiner et al., 1998) our current data cannot distinguish whether recruitment of postembryonically generated neurons affects both or only one of these populations.

For some newly generated neurons in Area X it was not possible to assert their phenotype. We can think of two explanations; maybe some aspiny, fast firing (AF) cells (Farries et al., 2005) might be recruited posthatch, but might belong to the non-DLM-projecting population proposed by Farries et al. (2005). Alternatively, new AF neurons could be of the DLM-projecting kind but were not retrogradely labeled because they had not connected yet, or were not efficiently backfilled. Conceivably the unidentified new neurons could also belong to a class of cells that hasn't been described yet in Area X. For instance, in the striatum of adult rabbits a subclass of GABAergic new interneurons express calretinin (Luzzati et al., 2006), but these newly-generated neurons are very rare and represent only 0.7% of the $BrdU^+$ cells (Luzzati et al., 2006) and the existence of $calretinin^+$ neurons in Area X have not been reported so far.

Plasticity of the Spiny Neurons

Interestingly, we found that the newly-generated spiny neurons express FoxP2, a transcription factor related to speech in humans (Lai et al., 2001) and to vocal learning in songbirds (Scharff and Haesler, 2005). The present report together with previous observations suggests a high degree of plasticity of these cells. Like mammalian medium spiny neurons, spiny neurons in Area X integrate pallial excitatory with ascending nigral dopaminergic 'reward' signals (Reiner et al., 2004a). In zebra finch Area X slices, dopamine can influence the excitatory inputs to spiny neurons (Ding et al., 2003) and also modulates their excitability directly (Ding and Perkel, 2002). Consistent with this, the spiny neurons express DARPP32, which is a key integrator of neurotransmitter and neuromodulator signals, particularly dopamine and glutamate and involved in several aspects of neural plasticity related to learning and memory (Haesler et al., 2004; Thomas and Huganir, 2004; Fernandez et al., 2006). Furthermore, spiny neurons in Area X show long term potentiation starting around D50 in juvenile birds which could underlie sensory-motor learning and song maintenance (Ding and Perkel, 2004). Taken to-

gether, recruitment of FoxP2 expressing spiny neurons into the neural ensemble of Area X is well suited to be among the candidate mechanisms that allow for behavioral plasticity in response to concerted auditory, song motor efference, and reinforcement information.

Recruitment of New Neurons During Song Learning

Consistent with previous studies showing an addition of neurons in Area X during the period of vocal learning (Nordeen and Nordeen, 1988; Nordeen et al., 1989; Burek et al., 1991), we found a transient increase in the number of new neurons expressing FoxP2 in Area X during the late sensory-motor phase of vocal learning (D50-D75). Previous work using *in situ* hybridization found that *FoxP2* expression was most strongly expressed at PHD 35 and 50 in Area X (Haesler et al., 2004), i.e. preceding the increased neurogenesis we report at PHD 50 and 75. In adult canary, increased neurogenesis is also observed during the months following higher expression of *FoxP2* in Area X (Kirn et al., 1994; Haesler et al., 2004). It is thus conceivable that increased *FoxP2* expression, if translated to protein, affects the subsequent incorporation of incoming neurons during the sensory motor phase of learning.

Both songbirds and mice produce vocalizations that contain characteristics of song, with different syllable types and temporal structure (Holy and Guo, 2005), but the necessity to learn song has been described only for vocal learners like songbirds, parrots and hummingbirds. The fact that FoxP2 is expressed both in newly generated Area X neurons and in the VZ of songbirds but is absent from the murine germinal areas (present data and Ferland et al., 2003) could either suggest that FoxP2 is primarily a lineage marker e.g. for spiny neurons, which are not undergoing neurogenesis in mammals, or that FoxP2 regulates some steps of neurogenesis in the birds specifically. Interestingly, previous studies have described the role of FoxP2 in regulating important aspects of development in several tissues including neuronal migration and maturation processes (Shu et al., 2001, 2005). Lending further support to this hypothesis is the recent discovery that FoxG1, another member of the forkhead family transcription factors, has been implicated in the regulation of the adult hippocampal neurogenesis in rodents (Shen et al., 2006).

The striatal medium spiny neurons are specifically affected in Huntington disease. Here we show that they undergo neurogenesis in songbirds. Avian basal ganglia share anatomical, physiological, and functional similarities to the mammalian basal ganglia. Since no postnatal neurogenesis of the medium spiny neurons has been described in the mammalian striatum, songbirds offer a unique model to understand the regulation of the proliferation of striatal progenitors and the recruitment and survival of the striatal spiny neurons in the adult striatum. Understanding these mechanisms could provide new insights into treatment for this neurodegenerative disorder.

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