HUMANIZED Foxp2 SPECIFICALLY AFFECTS CORTICO-BASAL GANGLIA CIRCUITS

S. REIMERS-KIPPING, W. HEVERS, S. PÄÄBO AND W. ENARD*

Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig, Germany

Abstract—It has been proposed that two amino acid substitutions in the transcription factor FOXP2 have been positively selected during human evolution and influence aspects of speech and language. Recently it was shown that when these substitutions are introduced into the endogenous *Foxp2* gene of mice, they increase dendrite length and long-term depression (LTD) in medium spiny neurons of the striatum. Here we investigated if these effects are found in other brain regions. We found that neurons in the cerebral cortex, the thalamus and the striatum have increased dendrite lengths in the humanized mice whereas neurons in the amygdala and the cerebellum do not. In agreement with previous work we found increased LTD in medium spiny neurons, but did not detect alterations of synaptic plasticity in Purkinje cells. We conclude that although Foxp2 is expressed in many brain regions and has multiple roles during mammalian development, the evolutionary changes that occurred in the protein in human ancestors specifically affect brain regions that are connected via cortico-basal ganglia circuits. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: FOXP2, dendrite length, synaptic plasticity, evolution, pleiotropy.

The genetic basis of human evolution is of cultural as well as medical interest (Enard and Paabo, 2004; Varki et al., 2008). There are approximately 20 million nucleotide sequence differences between currently living humans and the last common ancestor that humans shared with chimpanzees some 6 million years ago (Mikkelsen et al., 2005). Among these "human-specific" changes must be those that built the genetic basis for human abilities that evolved during this time, such as the ability to acquire language and to learn the complex motor movements necessary for speech. Almost nothing is known about this link, but two human-specific amino acid changes in the transcription factor FOXP2 are intriguing candidates for two reasons.

First, FOXP2 is currently the only gene for which a specific link to the development of speech and language is well established (Vargha-Khadem et al., 2005; Fisher and Scharff, 2009). Humans carrying only one functional copy of the gene (Lai et al., 2001; MacDermot et al., 2005) have difficulties in performing complex movements of the mouth and face, show impairments in expressive and receptive language tasks, but show hardly any impairments in other cognitive skills (Vargha-Khadem et al., 1998; Watkins et al., 2002a). Brain imaging studies suggest that the deficits are due to functional impairments in the cortex, the striatum and/or the cerebellum (Vargha-Khadem et al., 1998; Watkins et al., 2002b; Belton et al., 2003; Liegeois et al., 2003). It has been argued that the patients resemble a developmental variant of aphasias that are caused, for example, by lesions of Broca's area (Watkins et al., 2002a).

Second, two amino acid substitutions have occurred during 6 million years of recent human evolution, which is more than expected for a protein that differs only by one amino acid substitution between chimpanzee and mouse. This suggests that FOXP2 has been positively selected specifically during human evolution (Enard et al., 2002; Zhang et al., 2002). This is an intriguing correlation with the emergence of language and speech in humans, but to understand the functional consequences of these evolutionary changes it is necessary to compare the human version of FOXP2 with non-human versions in a relevant model system. We recently took a first step in this direction by creating a mouse in which the two amino acid changes in Foxp2 have been knocked-in (Enard et al., 2009). These mice, "humanized" for Foxp2, are generally healthy and show no phenotypic effects in many organ systems in which Foxp2 is expressed. However, they do show slight changes in pup vocalizations, decreased exploratory behavior, and decreased dopamine concentrations. Furthermore, medium spiny neurons in the striatum have increased dendrite lengths *in vitro* and *in vivo* and show stronger long-term depression (LTD) after high-frequency stimulation. Interestingly, mice heterozygous for non-functional alleles of *Foxp2* show partly opposite effects (Groszer et al., 2008; Enard et al., 2009). An important unanswered question is to what extend the effects of humanized Foxp2 are specific to particular neurons.

Foxp2 is expressed in many cells throughout the body (Lai et al., 2001; Shu et al., 2005, 2007). In the brain it is expressed in a subset of postmitotic neurons, such as in medium spiny neurons, Purkinje cells, neurons of cortical layer VI, and thalamic neurons, but it is, for example, not expressed in hippocampal neurons (Ferland et al., 2003; Lai et al., 2003; Campbell et al., 2009). Here we investigate the neuroanatomical and electrophysiological effects in different brain regions of the humanized *Foxp2* mice in order to better understand the consequences of the two amino acid changes.

EXPERIMENTAL PROCEDURES

Mice

The investigated humanized *Foxp2* (*Foxp2^{hum}*) allele is the *Foxp2^{humΔneo}* allele derived from the embryonic stem (ES) cell clone 5H11 described in (Enard et al., 2009). The *Foxp2* knock-out (*Foxp2^{ko}*) allele is derived from the same ES cell clone and results in a premature stop codon in exon 8 and absence of Foxp2 protein in Western blot analyses (Enard et al., 2009). Since the ES cell line is derived from C57BL/6 mice and all breedings were done with C57BL/6J mice, the genetic background can be considered as pure C57BL/6J. *Foxp2$^{hum/hum}$* and *Foxp2* wildtype (*Foxp2$^{wt/wt}$*) mice were derived from heterozygous crossings and balanced across litters. Mice were housed at a 10 h/14 h dark/light cycle under standard conditions. All animal work was performed in accordance with governmental and institutional guidelines. All efforts were made to minimize the number of animals used and their suffering.

Immunohistochemistry

Foxp2$^{ko/ko}$ and *Foxp2$^{wt/wt}$* embryonic heads were obtained from heterozygous crossings 17.5 days after mating (E17.5) and fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) pH 7.4 at 4 °C for 24 h. Adult *Foxp2$^{hum/hum}$* and *Foxp2$^{wt/wt}$* animals (12 weeks, for all brain regions except cerebellum) and postnatal day 17 (P17; only for cerebellum) animals were perfused with 4% paraformaldehyde in PBS, their brains dissected and postfixed with the same solution for 24 h at 4 °C. Brains were dehydrated in 15% and subsequently in 30% sucrose in PBS for 24 h at 4 °C, embedded in Tissue-Tek OCT compound (Sakura Japan) and cut into 40 μm coronal slices using a cryomicrotome (Microm HM550, Thermo Scientific, Walldorf, Germany). Series of every 10th section were incubated floatingly (adult and P17) or mounted on glass slides (embryonic) for 10 min with an antigen unmasking buffer (10 mM sodium citrate buffer pH 6.0) at 98 °C, cooled for 30 min at room temperature and washed three times with bi-distilled H_2O, three times with Tris buffered saline with 0.1% Tween 20 pH 7.4 (TBST) and afterward blocked for 2 h with 5% normal donkey serum (NDS) and 1% bovine serum albumin (BSA) in TBST. Brain sections were incubated overnight with the primary antibody in the blocking buffer (rabbit anti-Foxp2 antibody HPA000382, Atlas Antibodies, Stockholm, Sweden; 0.12 μg/ml), chicken anti-MAP2 ab5392 (Abcam, Cambridge, UK; 1.9 μg/ml), mouse anti-NeuN biotinylated ab77315 (Abcam; 2 μg/ml), goat anti-DARPP32 sc-8483 (Santa Cruz Biotechnologies Inc., Santa Cruz, CA, USA; 4 μg/ml), mouse anti-Calbindin D-28K, C9848, (Sigma-Aldrich, St. Louis, MO, USA; 3.2 μg/ml), washed three times with TBST and incubated for 8 h with secondary antibodies in blocking buffer (DyLight™ 549-conjugated AffinityPure *F*(ab′)2 Fragment Donkey anti-Chicken IgY++ (H+L); 2 μg/ml, Biotin-SP-conjugated AffiniPure Donkey anti-goat++ IgG (H+L); 2 μg/ml, Biotin-SP-conjugated AffiniPure Donkey anti-mouse; 2 μg/ml, Biotin-SP-conjugated AffiniPure Donkey anti-rabbit (2 μg/ml; all purchased from Jackson ImmunoResearch, West Grove, PA, USA). Sections were washed with TBST three times, incubated over night with streptavidin DyLight conjugates in blocking buffer (1 μg/ml; Jackson ImmunoResearch), washed with TBST twice and once with TBS and subsequently mounted on glass slides using Prolong Antifade (Invitrogen, Paisley, UK). Laser scanning microscopy (LSM 510; Zeiss, Jena, Germany) was performed with appropriate emission and excitation filters for maximal signal separation. For all co-localizations of Foxp2 with neuronal markers, images were acquired at constant exposure times and pinhole settings for all animals and brain regions to ensure comparability. During data collection, the investigator was blind to the genotype of the animals.

Quantification of Foxp2-expressing cells

Foxp2 co-localization with the neuronal marker proteins NeuN (in neocortex, piriform cortex, thalamus, and striatum) and calbindin (in cerebellum) were analyzed quantitatively to determine the fraction of Foxp2-expressing neurons in these regions. Serial sections of three adult *Foxp2$^{hum/hum}$* and *Foxp2$^{wt/wt}$* mice were triple labeled with anti-Foxp2 (Atlas Antibodies), anti-MAP2 and anti-NeuN or anti-calbindin antibodies. Images were acquired as described above. 100 NeuN-positive neuronal nuclei and 40 calbindin-positive Purkinje cell bodies were identified in at least four individual images per animal using the particle analyzer tool of the ImageJ software. To obtain absolute numbers of Foxp2-positive neurons, Foxp2 immunoreactivity was scored as positive for the NeuN or calbindin-positive cell bodies, if the cell body exhibited intact morphology and nuclear FOXP2 staining. Numbers of Foxp2-positive neurons were averaged per animal and the mean calculated across all animals of the same genotype to determine percent co-localization. During data collection, the investigator was blind to the genotype of the animals.

Analysis of dendrite length

For the analysis of dendrite lengths in neurons of the thalamus, the amygdala, the neocortex and the piriform cortex we examined brains of six adult *Foxp2$^{hum/hum}$* and six adult *Foxp2$^{wt/wt}$* littermates, previously stained using the FD Rapid GolgiStain™ kit (FD NeuroTechnologies, Ellicott City, MD, USA) as described (Enard et al., 2009). Total dendritic trees were tracked in 200 μm thick coronal slices using an oil-immersion lense (10×63.5) and Neurolucida software (MBF Bioscience, Williston, ND, USA). Being blind to genotype, we measured three cells per region and animal in five of the six animals per genotype. Only neurons whose dendritic trees were located completely within the section were included in the analysis. For the analysis of dendrites in Purkinje cells, P17 mouse brains were used to ensure the presence of all dendritic arborizations and processed in the same way. Dendrites of three cells per animal were tracked in 200 μm sagittal slices. Measurements of medium spiny neurons, located throughout the striatum, were taken from Enard et al., 2009. All 289 measurements are available upon request. We analyzed the data using SPSS v.16.0 (SPSS Inc., Chicago, IL, USA). For analysis of variances (ANOVAs) we used Levene's test for homoscedasticity and visual inspection of residuals plotted against predicted values to check for violations of assumptions. Since we only tested for an increased length of dendrites in *Foxp2$^{hum/hum}$* mice we report one-sided *P*-values for all genotype-dependent effects. For analyzing all cell types we used the following model: Log$_2$(total dendritic length)=Genotype+Foxp2expression+CellType(Foxp2expression)+Genotype*Foxp2expression+Genotype*CellType(Foxp2expression)+error, whereas the factor *Genotype* has two levels (*Foxp2$^{hum/hum}$* and *Foxp2$^{wt/wt}$*), the factor *Foxp2expression* has two levels (yes when over 20% of the neurons are positive for Foxp2) and the factor *CellType* has 10 levels (the 10 examined neuronal cell types). When analyzing only cell types expressing Foxp2 the same model without the factor *Foxp2expression* was used. When considering the factor *Circuit* (with level yes for the two Foxp2 expressing cell types in layer VI, striatal neurons and thalamic neurons) for these cells, the following model was applied: Log$_2$(total dendritic length)=Genotype+Circuit+CellType(Circuit)+Genotype*Circuit+Genotype*CellType(Circuit)+error. Note that these models assume independence of each measured neuron, despite the fact that the same animals were used for different cell types. The violation of this assumption does not seem to be very problematic because within the same animals we observe no significant correlations of dendritic length across cell types and only very limited correlation within the same cell types (data not shown). Nevertheless, using a fully balanced dataset of four *Foxp2$^{hum/hum}$* animals and five *Foxp2$^{wt/wt}$* animals for which neurons had been measured in all cell types, we ran an ANOVA with animal as random factor that takes into account this dependency: Log$_2$

(total dendritic length)=Genotype+Animal(Genotype)+CellType (Foxp2expression)+Foxp2expression+CellType*Genotype(Foxp2 expression)+Genotype*Foxp2expression+Foxp2expression*Animal (Genotype)+Animal*CellType(Foxp2expression*Genotype)+error. The *Genotype*Foxp2expression* interaction is tested against the error term *Foxp2expression*Animal(Genotype)* and confirms that only cells expressing humanized Foxp2 have increased dendritic length ($F_{1,7}=8.41$, $P_{one-sided}=0.012$). Since different animals were used to measure the dendrite length in Purkinje cells, we also used a "pseudobalanced" dataset to include all measured cells. After matching sex and genotype, we randomly assigned these additional measures to "pseudo-animals." This procedure is expected to artificially increase the variance for the factor *Pseudoanimal(Genotype)*, but since this factor is part of the error term against which we test our relevant hypotheses, this is conservative. Using the same model as for the fully balanced data set above (just using factor *Pseudoanimal* instead of *Animal*), we find again a significant interaction of *Genotype* and *Foxp2expression* ($F_{1,6.2}=3.86$, $P_{one-sided}=0.048$). The marginally significant interaction of *Genotype* and *CellType* ($F_{8,51.5}=1.64$, $P_{one-sided}=0.07$) indicates that the effect might be different among cell types. When considering only Foxp2-positive neurons, this interaction is confirmed ($F_{5,31.8}=2.37$, $P_{one-sided}=0.031$). When splitting Foxp2-expressing cell types into "cortico-basal ganglia circuit" (cortex, striatum, thalamus) and "non-cortico-basal ganglia circuit" (amygdala, Purkinje cells) and add this factor to the model, the *genotype*circuit* interaction is highly significant ($F_{1,6.1}=,11.6$, $P_{one-sided}=0.007$). Hence, all different models, whether accounting for animal as random factor or not, strongly support that the total dendritic length of neurons increases when a neuron expresses humanized Foxp2 and is part of the cortico-basal ganglia circuits.

Electrophysiology

For recordings of medium spiny neurons in the striatum coronal slices of 250 μM thickness were prepared from the medial striatum of 33–53-day-old mice. Medium spiny neurons were identified by their morphology and characteristic electrophysiological properties like negative resting membrane potentials (below −80 mV), slow capacitance transients and low input resistances (Pawlak and Kerr, 2008). In initial experiments we did not suppress GABA$_A$—receptor currents assuming to be close to the chloride reversal potential with a holding potential of −80 mV. Accordingly, adding the GABA$_A$ blocker SR-95531 (Sigma) did not affect the magnitude of LTD (data not shown). Excitatory afferents were stimulated with a theta-glass electrode filled with external saline and placed between the recorded medium spiny neuron and cortex, typically ~100 μm from the cell body. A bipolar voltage pulse of 0.1 ms duration was used, with the stimulus intensity adjusted to yield excitatory postsynaptic potentials (EPSPs) typically about 10 mV without eliciting action potentials or inducing any direct stimulation. Recordings were performed in the current clamp configuration with the bridge mode enabled, filtered at 2.5 kHz and digitized at 10 kHz. Following 15 min of control stimulation at a frequency of 0.33 Hz, we applied four tetani each 3 s long with a frequency of 100 Hz, separated by 30 s. LTD was then measured using the control stimulation for at least 30 min. This resulted in recordings of seven cells each from five *Foxp2hum/hum* and four *Foxp2wt/wt* animals.

For recordings of Purkinje cells, sagittal slices were taken from the cerebellar vermis of 27–53-day-old mice and recordings were performed as described above with the following alterations: all recordings were performed in the presence of 20 μM SR-95531 and cells were recorded in the voltage clamp configuration at a membrane potential of −75 mV with series resistances in the range of 13–18 MΩ compensated routinely by 50%. For paired pulse recordings excitatory parallel fiber inputs were stimulated

near the slice surface close to the Purkinje cell dendrites but without any direct stimulation. Paired pulses were recorded at intervals increasing exponentially from 10 to 300 ms and the paired–pulse ratio calculated as peak current of the second test pulse divided by the first control pulse. Five trials, separated by 2 min, were averaged for each cell (16 cells from four *Foxp2wt/wt* animals and 19 cells from four *Foxp2hum/hum* animals). For the induction of LTD in Purkinje cells an additional theta electrode stimulated the climbing fiber input proximal to the Purkinje cells cell bodies. Parallel fiber input was stimulated at 0.1 Hz for 15 min before and at least 30 min after LTD induction, which was performed by synchronous stimulation of both parallel fiber and climbing fiber pathways for 5 min under current-clamp conditions. This resulted in recordings of 13 and 12 cells from six *Foxp2hum/hum* and eight *Foxp2wt/wt* animals, respectively.

Slices were superfused with an external aCSF (artificial cerebrospinal fluid) containing (in mM): 125 NaCl, 3.5 KCl, 2 CaCl$_2$, 1 MgCl$_2$, 30 NaHCO$_3$, 1.25 NaH$_2$PO$_4$, and 15 glucose, with a pH of 7.3–7.4 when bubbled with 95% O$_2$/5% CO$_2$. Osmolarity was adjusted to 315 mOsmol using H$_2$O/sucrose. For initial preparation and cutting of slices a high sucrose aCSF was used (60 NaCl, 0.5 CaCl$_2$, 6 MgCl$_2$, osmolarity adjusted by sucrose; ~4 °C). Glass electrodes (5–8 MΩ) were filled with a solution containing (in mM): 150 K-gluconate, 10 NaCl, 3 Mg-ATP, 0.3 GTP, 10 HEPES and 0.05 EGTA, adjusted to pH 7.3 and an osmolarity of 310 mOsmol. The liquid junction potential of 15 mV was corrected online. All recordings were performed at room temperature using a EPC-10 amplifier (HEKA, Lambrecht, Germany).

Acquisition and analysis were performed using Patch- and Fitmaster software (HEKA) in conjunction with Origin 8.0 (Origin Lab Corp., Northhampton, MA, USA). Statistical analysis was done in SPSS v.16.0 using repeated measures ANOVA to test genotype-dependent effects. The included time-points for LTD analyses were 3 min averages of the 10 min before LTD induction and 10–30 min after LTD induction. We used the Greenhouse-Geisser correction implemented in SPSS to adjust the degrees of freedom when the sphericity assumption was violated.

RESULTS

Foxp2 immunoreactivity in mouse brains

Previously it was shown that the total length of dendritic trees is increased in medium spiny neurons of mice homozygous for a humanized version of *Foxp2* (*Foxp2hum/hum* mice; Enard et al., 2009). To investigate to what extent this effect depends on Foxp2 expression and neuronal cell type, we first quantified the fraction of Foxp2-positive cells in different brain regions. We evaluated three commercially available antibodies and used one that showed no immunoreactivity in brains of mice homozygous for a knockout allele of *Foxp2* (Fig. 1). Immunoreactivity patterns in adult and embryonic wild type mice were qualitatively very similar to what has been described (Ferland et al., 2003; Lai et al., 2003; Campbell et al., 2009; Enard et al., 2009). For quantitative analyses, we focused on five brain regions with abundant Foxp2 immunoreactivity and two regions with very little Foxp2 immunoreactivity in adult mice. We defined Foxp2-expressing neurons as cell bodies with positive staining for the neuronal marker NeuN- (in Purkinje cells Calbindin-D28k) and a simultaneous nuclear Foxp2 immunoreactivity (Fig. 2). The fractions of Foxp2-
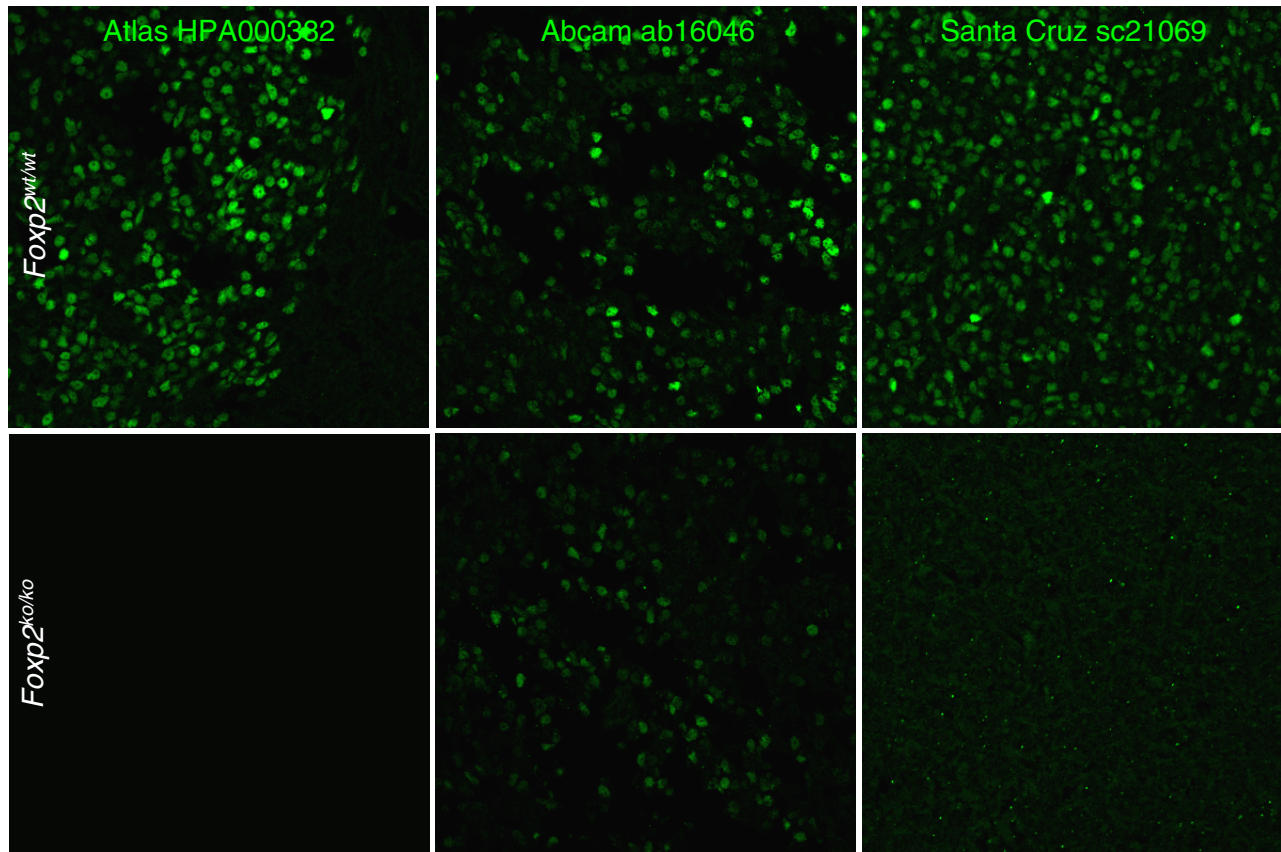
Fig. 1. Specificity of three Foxp2 antibodies. Foxp2 immunoreactivity was localized by DyLight-488 in the dorsal striatum of embryos (E17.5), homozygous for a knockout allele of Foxp2 (*Foxp2^ko/ko*) (Enard et al., 2009) and wildtype (*Foxp2^wt/wt*) littermates. Primary antibodies against Foxp2 are HPA000382 (Atlas Antibodies, against a 150 aa recombinant peptide), ab16046 (Abcam, 15 aa peptide, C-terminus) and sc-21069 (Santa Cruz, N-terminal peptide). Using ab16046 in adult brains, we noticed a weak staining in the upper cortical layers and in the CA1 region of the hippocampus (data not shown). Since these regions are known to express Foxp1, crossreactivity might reflect Foxp1, the closest paralogue of Foxp2. All images were acquired with fixed detector gain, amplifier gain, amplifier offset, and pinhole settings. Image width is 230 μm. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

positive cells in three *Foxp2^hum/hum* mice on the one hand and three *Foxp2^wt/wt* littermates on the other hand were similar in all examined brain regions indicating that the humanized *Foxp2* has no major effect on Foxp2 expression or stability (2-tailed *t*-tests on arcsin-transformed data, all $P>0.1$). On average, we found Foxp2 expression in 0% (0±0% in *Foxp2^wt/wt* and 0±0.3% in *Foxp2^hum/hum*) of the neurons in layer II/III of the primary motor cortex (M1), in 2% (2±1.1%; 2±0.8%) of the deep pyramidal cells of the piriform cortex, in 22% (22±6.5%; 22±2.7%) of neurons in the medial amygdala, in 66% (68±2.2%; 63±2.5%) of M1 layer VI neurons, in 71% (73±6.1%; 68±0.3%) of Purkinje cells, in 91% (91±3.4%; 92±1.3%) of neurons in the dorsal striatum and in 94% (92±3.1%; 95±1.6%) of neurons in the parafascicular nucleus of the thalamus. We further characterized the neurons of M1 layer VI and found, in accordance with a recent study (Hisaoka et al., 2010), that Foxp2 is expressed in a subset of glutamatergic, DARPP-32-positive projection neurons but absent in interneurons (data not shown). Additionally, we performed MAP2, NeuN and Foxp2 co-stainings and classified layer VI neurons by the size and shape of their cell body and the position and orientation of the apical dendrite (Zhang and Deschenes, 1997) as pyramidal cells (78.0% of all NeuN positive cells), bipolar spiny neurons (10.5%), inverted pyramidal cells (5.3%) or local circuit neurons (6.2%). We found that 63% (62±4.0% in *Foxp2^wt/wt* and 65±4.3% in *Foxp2^hum/hum*) of pyramidal cells and 60% (59±4.5%; 61±14.4%) of bipolar spiny neurons expressed Foxp2 while only 2% (4±4%; 0±0%) of inverted pyramidal cells and 2% (4±4%; 0±0%) of local circuit neurons showed Foxp2-positive nuclei (Fig. 3).

Enlarged dendritic trees in the striatum, the thalamus and layer VI

To investigate the effect of humanized Foxp2 on dendrite length in different neuronal cell types, we reconstructed complete dendritic trees of Golgi-Cox impregnated neurons. Only completely impregnated neurons with dendritic trees entirely embedded within the 200 μm section were used for analysis (Fig. 4). In at least four *Foxp2^hum/hum* and four *Foxp2^wt/wt* brains matched for age, sex and litter, we measured the total lengths of the dendritic trees in 2–3 neurons for each of 10 cell types, resulting in a total of 289

	Piriform cortex	M1 layer II/III	M1 layer VI	Cerebellum	Thalamus	Striatum	Amygdala
wt/wt	2% ±1.1%	0% ±0%	68% ±2.2%	73% ±6.1%	92% ±3.1%	91% ±3.4%	22% ±6.5%
hum/hum	2% ±0.8%	0% ±0.3%	63% ±2.5%	68% ±0.3%	95% ±1.6%	92% ±1.3%	22% ±2.7%

Fig. 2. Co-localization of Foxp2 and neuronal markers. Representative images of a triple-immunofluorescence staining of Foxp2 (green), MAP2 (not shown) and NeuN (red) or Calbindin (red, Cerebellum only) are shown. The fraction (±SEM) of Foxp2-positive cells was determined from at least 100 neurons for each of the three individuals per genotype and brain region in adult mouse brains or P17 mouse brains (Cerebellum only). Image width is 230 μm.

measurements. Overall, dendrite lengths for both genotypes were similar to what has been described in the literature (e.g. Martone et al., 2003; Benavides-Piccione et al., 2006).

Fig. 5 shows the average lengths of the dendritic trees in the *Foxp2hum/hum* animals, normalized to wild type levels. In the four neuron types where no or only few cells express Foxp2, dendrite lengths are similar between the two genotypes. Furthermore, dendrite lengths are similar in neurons in the amygdala and in Purkinje cells, although about one quarter and three quarters of the neurons in these brain regions, respectively, express Foxp2. In contrast, dendrite length is increased between 25% and 50% in bipolar spiny neurons (one-sided Wilcoxon rank test, P=0.03) and pyramidal neurons (P=0.1) of cortical layer VI, in neurons in the thalamus (P=0.03) and in medium spiny neurons in the striatum

	Pyramidal	Bipolar	Inverted pyramidal	Local circuit
hum/hum	65% ± 4.3%	61% ± 14.4%	0% ± 0%	0% ± 0%
wt/wt	62% ± 4.0%	59% ± 4.5	4.2% ± 4.2%	4.2% ± 4.2%

Fig. 3. Co-localization of Foxp2, NeuN and MAP2 in layer VI of the primary motor cortex. Representative images of the four neuron types are shown. These were identified by the size and shape of the soma and the position and orientation of the apical dendrite (asterisks). A total of 200 neurons were counted in three *Foxp2wt/wt* and three *Foxp2hum/hum* brains and the fraction (±SEM) of Foxp2-positive neurons was determined for each cell type. All images were acquired with fixed detector gain, amplifier gain, amplifier offset, and pinhole settings. Image width is 50 μm.
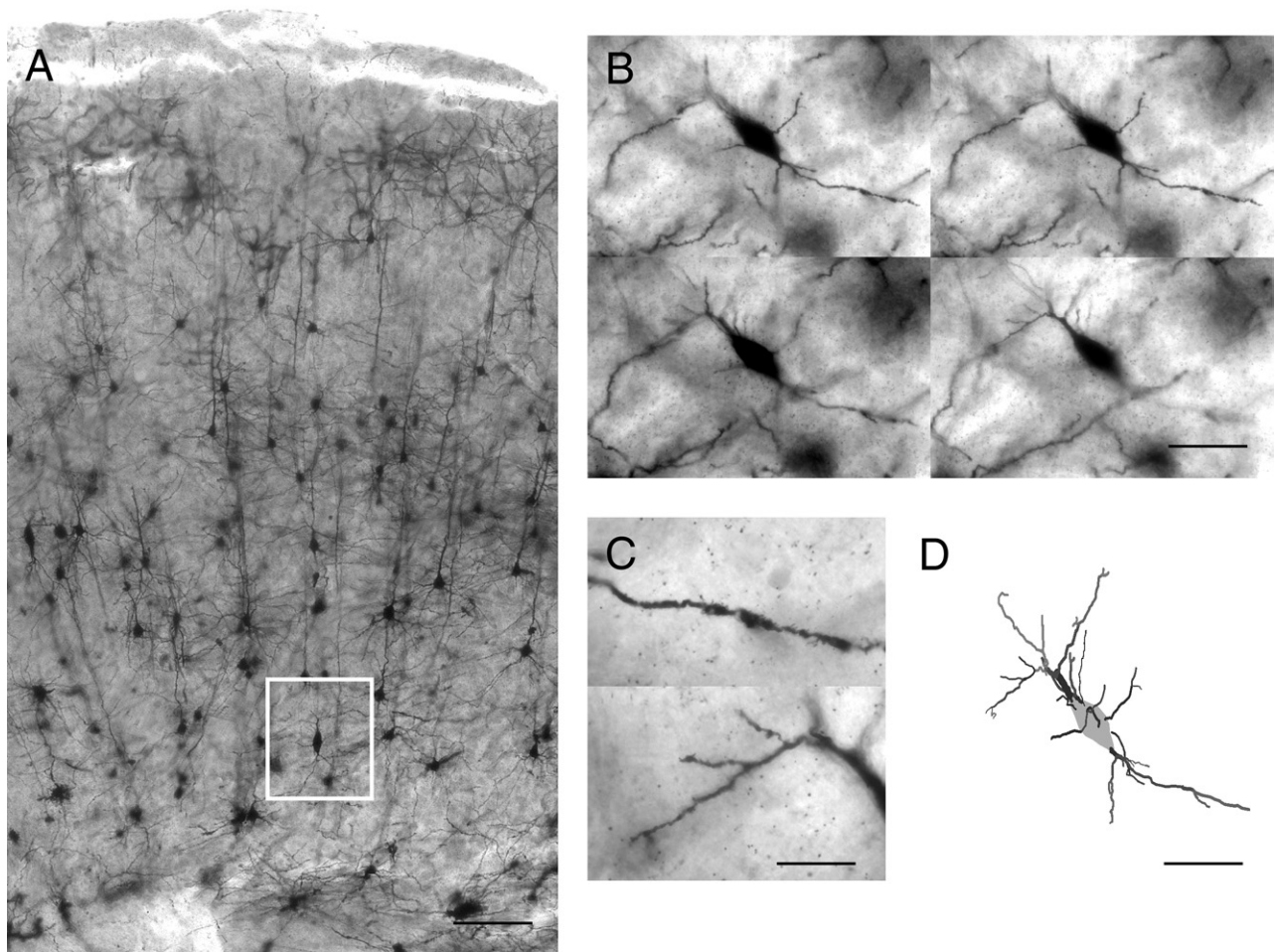
Fig. 4. Representative images of the primary motor cortex, a bipolar neuron in layer VI and its reconstruction. (A) Overview of the primary motor cortex impregnated by the Golgi Cox method and counterstained with Toluidine Blue. (B) The bipolar neuron highlighted in (A) in a 40× magnification and four different z planes. (C) A higher power view of two dendrites of the bipolar neuron. (D) Reconstruction of the bipolar neuron using a 100× oil immersion objective and the Neurolucida software. Scale: (A) 170 μm; (B) 50 μm; (C) 17 μm; (D) 50 μm.

(P=0.009). To statistically evaluate these data further, we used an ANOVA with the factors *Genotype* (with levels *Foxp2*$^{hum/hum}$ and *Foxp2*$^{wt/wt}$), *Foxp2expression* (yes when >20% of this cell type express Foxp2) and *Cell Type* (the 10 cell types shown in Fig. 5) and tested for effects of *Foxp2*$^{hum/hum}$ on increasing dendrite lengths. We found a significant interaction between *Foxp2expression* and *Genotype* ($F_{1,269}$=4.58, $P_{one-sided}$=0.017), supporting that dendrite lengths are increased in *Foxp2*$^{hum/hum}$ mice only in neurons that express Foxp2. When the analysis is restricted to Foxp2-expressing neurons, the effect of *Genotype* is confirmed ($F_{1,166}$=8.277, $P_{one-sided}$=0.002) and a marginally significant interaction of *Genotype* and *Cell Type* ($F_{5,166}$=1.804, $P_{one-sided}$=0.06) suggests that this effect might be different in different cell types. When we group Foxp2-positive neurons in regions that belong to the cortico-basal ganglia circuits (i.e. neurons in the cortex, the striatum and the thalamus) or not (i.e. amygdala and PCs) we find a significant interaction of *Genotype* and *Circuit* ($F_{1,166}$=7.71, $P_{one-sided}$=0.003). Similar results are obtained when using more complex models that take the dependency among neu-

rons within animals into account. Thus, there is strong statistical support for the notion that humanized Foxp2 increases dendrite length in Foxp2-positive neurons that are part of the cortico-basal ganglia circuits but not in neurons in other regions of the brain.

Increased synaptic plasticity in the striatum

It is particularly noteworthy that dendritic trees of the Purkinje cells in the cerebellum are unaffected by the humanized Foxp2 whereas neurons in cortical layer VI, the thalamus and the striatum have larger trees, because cerebellar circuits as well as cortico-basal ganglia circuits have been implicated in the etiology of the FOXP2-dependent speech and language disorder in humans (Watkins et al., 2002b; Belton et al., 2003; Vargha-Khadem et al., 2005). During development of the cerebellum, Foxp2 is certainly important since mice homozygous for non-functional *Foxp2* show a striking reduction of the cerebellar size and foliation (Shu et al., 2005; Fujita et al., 2008; Groszer et al., 2008). Furthermore, in
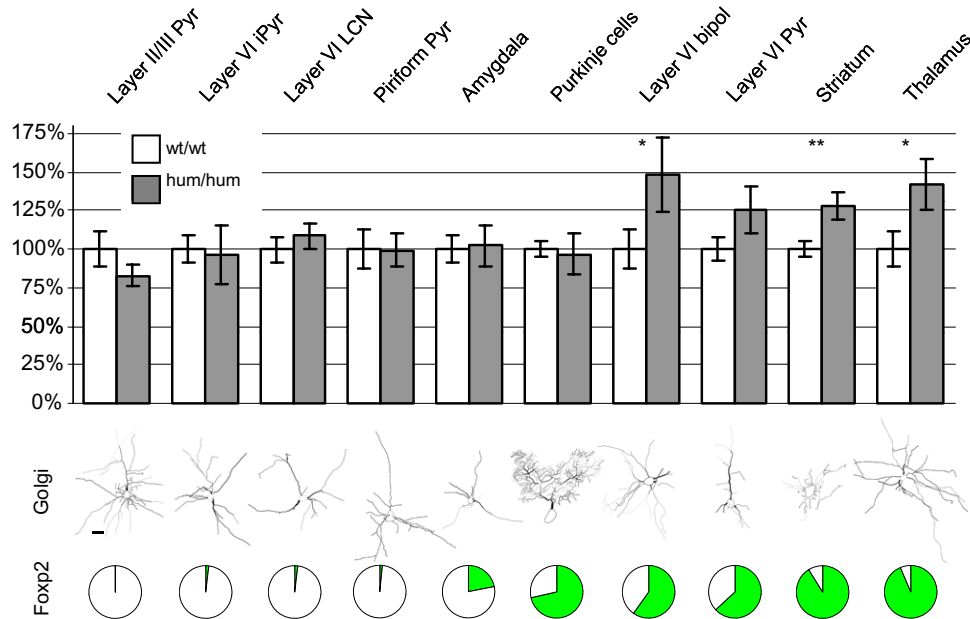
Fig. 5. *Foxp2hum/hum* increases dendrite length only within cortico-basal ganglia circuits. Averages (±SEM) of the total length of dendritic trees normalized to wild type levels ($N=10–21$ per cell type and genotype), representative reconstructions of traced neurons (Golgi) and the fraction of Foxp2-positive cells as determined by immunofluorescence are shown. Stars represent significantly longer dendritic trees in *Foxp2hum/hum* neurons as evaluated by a one-sided Wilcoxon rank test. Pyr, classical pyramidal neuron; iPyr, inverted pyramidal neuron; LCN, local circuit neuron; Bipol, bipolar spiny neuron. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

mice that are—as the human patients—heterozygous for non-functional *Foxp2*, impaired synaptic plasticity has been found in cerebellar circuits and cortico-basal ganglia circuits: in the dorsolateral striatum, medium spiny neurons showed a strongly reduced LTD after high-frequency stimulation. In Purkinje cells, paired-pulse facilitation was enhanced at short interstimulus intervals and LTD induced by co-activation of the parallel fibers and climbing fibers tended to be more rapidly induced (Groszer et al., 2008).

We therefore investigated the effects of humanized Foxp2 on these forms of synaptic plasticity in medium spiny neurons and Purkinje cells. We first studied LTD of medium spiny neurons in the dorsolateral striatum after high-frequency stimulation. 5–10 min after LTD induction, responses in *Foxp2hum/hum* decayed more rapidly in *Foxp2wt/wt* mice and stabilized after 30 min at a level of 68% (±12%) compared relative to before LTD induction. In contrast, in *Foxp2wt/wt* mice responses stabilize at 88% (±4%) (Fig. 6A). Hence, in agreement with our earlier work (Enard et al., 2009), we observed an increased LTD in *Foxp2hum/hum* mice ($N=14$; repeated measures ANOVA with Greenhouse-Geisser correction: *time*genotype*: $F_{2,24}=3.35$; $P_{\text{one-sided}}=0.026$).

We then studied synaptic plasticity in Purkinje cells as in Groszer et al., 2008. After inducing LTD by simultaneous stimulation of parallel- and climbing-fibers, response amplitudes decayed in a similar manner in both genotypes over 30 min and stabilized after 40 min at a plateau of 56% (±9%) and 58% (±7%) in *Foxp2wt/wt* and *Foxp2hum/hum* mice, respectively ($N=25$; Fig. 6B). Hence, we found no genotype-dependent effect on LTD in Purkinje cells (Re-

peated measures ANOVA with Greenhouse-Geisser correction: *time*genotype*: $F_{1.45,33.6}=0.38$; $P=0.54$; *genotype*: $F_{1,23}=0.18$; $P=0.68$). When paired-pulse facilitation in Purkinje cells was induced by stimulating parallel fibers with two pulses separated by 10–300 ms ($N=35$, Fig. 6C), neither single time points (all *t*-tests $P>0.1$) nor all time points considered together (repeated measures ANOVA with Greenhouse-Geisser correction: *time*genotype*: $F_{3.1,102.2}=1.12$; $P=0.34$; *genotype*: $F_{1,33}=2.3$; $P=0.14$) are significantly different between the two genotypes. Hence, humanized Foxp2 influences synaptic plasticity in the medium spiny neurons, but not in Purkinje cells.

DISCUSSION

We have previously shown that when the humanized Foxp2 protein is expressed in mice it appears to have no systemic effects except in the brain, although the protein is expressed in most tissues during development (Enard et al., 2009). Within the brain, the humanized protein changes striatal gene expression patterns, decreases dopamine levels, and increases dendrite length as well as LTD of medium spiny neurons (Enard et al., 2009). Here we explore the specificity of the latter two effects with respect to different brain regions. We find no differences in dendrite lengths in neurons that do not express Foxp2, suggesting that humanized Foxp2 induces this increase not by altering exogenous factors, such as the afferent input to affected neurons, but by altering endogenous factors that control dendrite morphogenesis (Jan and Jan, 2010). Among the examined brain regions that contain a substantial number of Foxp2-positive neurons, we find that
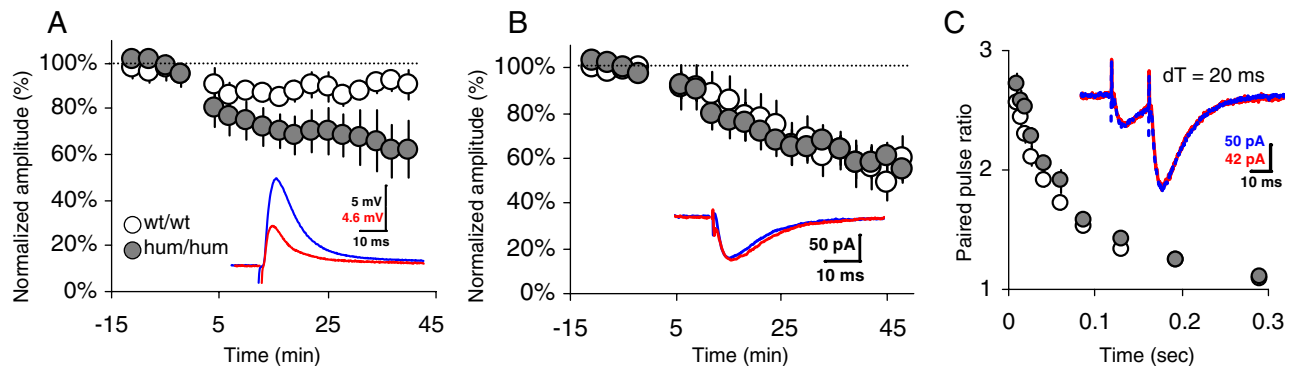
Fig. 6. *Foxp2hum/hum* increases synaptic plasticity only in medium spiny neurons but not in Purkinje cells. (A) LTD in medium spiny neurons was induced by high-frequency stimulation at time zero. Mean amplitudes (±SEM) normalized to baseline levels are shown ($N=7$ cells per genotype). The inset shows the average response from *FOXP2wt/wt* (blue) and *FOXP2hum/hum* (red) cells 35–40 min after stimulation. The previously described increase of LTD in striatum was confirmed (repeated measures ANOVA with Greenhouse-Geisser correction: *time*genotype*: $F_{2,24}=3.35$; $P_{one-sided}=0.026$). (B) LTD in Purkinje cells was induced by synchronous stimulation of parallel fibers and climbing fibers. Mean amplitudes (±SEM) normalized to baseline levels are shown ($N=12$ and 13 cells for *FOXP2wt/wt* and *FOXP2hum/hum*, respectively). No genotype dependent effects were found (repeated measures ANOVA with Greenhouse-Geisser correction: *time*genotype*: $F_{1.45,33.6}=0.38$; $P=0.54$; *genotype*: $F_{1,23}=0.18$; $P=0.68$). (C) Paired-pulse facilitation in Purkinje cells was induced by stimulating parallel fibers with two pulses separated by 0.01–0.3 s. Mean ratios (±SEM) of the induced currents (I_{second}/I_{first}) are shown for the different intervals ($N=16$ and 19 cells for *FOXP2wt/wt* and *FOXP2hum/hum*, respectively). The inset shows averages of five responses from *FOXP2wt/wt* (blue) and *FOXP2hum/hum* (red) cells with an interpulse period of 20 ms. Neither single time points are significantly different between the two genotypes (all *t*-tests $P>0.1$), nor are all time points considered together (repeated measures ANOVA with Greenhouse-Geisser correction: *time*genotype*: $F_{3.1,102.2}=1.12$; $P=0.34$; *genotype*: $F_{1,33}=2.3$; $P=0.14$). For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

the humanized Foxp2 increases dendrite length in medium spiny neurons of the striatum, layer VI neurons of the motorcortex and neurons in the thalamus, but not in Purkinje cells of the cerebellum and not in neurons of the amygdala. Since the cortex, the striatum and the thalamus form cortico-basal ganglia circuits (Middleton and Strick, 2000; Graybiel, 2008) our results suggest that the humanized Foxp2 specifically affects neurons belonging to these circuits. This is supported by our electrophysiological recordings, which in agreement with earlier work showed increased LTD in medium spiny neurons, but failed to find alterations of synaptic plasticity in Purkinje cells.

The changes in dendrite length could be important for the firing patterns of neurons (van Elburg and van Ooyen, 2010) or affect their connectivity (Spruston, 2008). Alterations in synaptic plasticity of medium spiny neurons have been linked to functional changes in several contexts (Di Filippo et al., 2009; Surmeier et al., 2009; Lovinger, 2010). However, even if these changes are not directly functionally relevant, but just correlated with relevant properties caused by humanized Foxp2, their regional specificity has implications for understanding the role of FOXP2 in human evolution and disease.

Humans heterozygous for a non-functional variant of FOXP2 show impairments in the timing and sequencing of movements of mouth and face and a number of deficits in expressive and receptive language skills (Vargha-Khadem et al., 2005) while general cognitive skills are much less affected (Watkins et al., 2002a; Fisher and Scharff, 2009). In addition, altered brain activation patterns and grey matter densities are seen in cortical regions involved in language and speech (e.g. Broca's area), in the putamen, the caudate nucleus and the cerebellum (Vargha-Khadem et al., 1998; Watkins et al., 2002b; Belton et al., 2003; Lieg-

eois et al., 2003). Hence, deficits in cortico-basal ganglia circuits as well as cerebellar circuits, which are both important for the sequencing and fine-tuning of movements (Middleton and Strick, 2000), may be central to the etiology of the speech and language impairment in humans (Vargha-Khadem et al., 2005). Importantly, mice heterozygous for the equivalent non-functional *FOXP2* allele show effects in both circuits for the same electrophysiological assays that have been performed here (Groszer et al., 2008). Our results suggest that cortico-basal ganglia circuits, and not cerebellar circuits, have been modified by the amino acid changes in FOXP2 that occurred during human evolution. Therefore, possible cerebellar contributions to speech, for example, online sequencing of syllables or the temporal organization of internal speech (Ackermann, 2008), are not likely to depend on these evolutionary changes in FOXP2. Furthermore, if the evolutionary changes in FOXP2 affect similar processes that lead to the speech and language impairment in humans— and opposite pattern of effects in humanized mice and mice heterozygous for non-functional Foxp2 hint towards such a possibility (Enard et al., 2009)—then our results indicate that FOXP2-dependent changes in cortico-basal ganglia circuits are responsible for the impairments of learning orofacial movements as well as for a possible enhancement of such learning during human evolution. This is in fact a plausible scenario since cortico-basal ganglia circuits have been implicated in the evolution of speech and human cognition for various reasons (Lieberman, 2006) and recent evidence indicates that synaptic plasticity in these circuits is crucial for learning a sequence of actions (Jin and Costa, 2010).

In this respect it is intriguing that the evolution of vocal learning in birds has led to a specialized cortico-basal

ganglia circuit, the anterior forebrain pathway (Scharff and Nottebohm, 1991; Jarvis, 2004; Mooney, 2009), and that a knockdown of FOXP2 expression in the striatal component of this circuit (AreaX) impairs vocal imitation in zebra finches (Haesler et al., 2007). It is possible that adaptations in cortico-striatal circuits are necessary when vocal learning evolves and that this has occurred in several species independently. In humans, changes in FOXP2 are then likely to have contributed to such adaptations. A better understanding of the mechanisms contributing to vocal learning in birds as well as investigations of vocal learning in other mammalian species such as seals (Fitch et al., 2010) will be important to investigate such a scenario.

Finally, the results are also of relevance for the question to what extent evolutionary changes in genes with many different functions (pleiotropic genes) can have specific functions (Wagner et al., 2008). Foxp2 certainly fulfills many functions since mice homozygous for non-functional *Foxp2* have many different neurological and non-neurological impairments (Shu et al., 2005; Fujita et al., 2008; Groszer et al., 2008) and mice heterozygous for a non-functional allele show a variety of more subtle neurological (Shu et al., 2005; Fujita et al., 2008; Groszer et al., 2008) and non-neurological (Enard et al., 2009) effects. So whereas *Foxp2* is similar to most genes in that it has pleiotropic functions (Mackay et al., 2009), the amino acid changes that occurred on the human lineage seem to have very specific effects within the brain. It has been argued that functional specificity can be achieved most readily by genetic changes in regulatory regions of genes (Carroll, 2000) and that this was especially relevant during human evolution (King and Wilson, 1975). However, this idea has been questioned (Hoekstra and Coyne, 2007; Lynch and Wagner, 2008) and systematic experiments may be necessary to clarify if some general patterns can be discerned (Orr, 2009). In any event, FOXP2 seems to be an example of a gene in which evolutionary relevant amino acid sequence changes do not affect the majority of tissues in which it is expressed (Enard et al., 2009), but rather a subset of neuronal cells in the brain.

CONCLUSION

In conclusion, this study shows that although Foxp2 is expressed in many brain regions and has multiple roles during mammalian development, the evolutionary changes that occurred in the protein in human ancestors specifically affect brain regions that are connected via cortico-basal ganglia circuits.

REFERENCES

Ackermann H (2008) Cerebellar contributions to speech production and speech perception: psycholinguistic and neurobiological perspectives. Trends Neurosci 31:265–272.

Belton E, Salmond CH, Watkins KE, Vargha-Khadem F, Gadian DG (2003) Bilateral brain abnormalities associated with dominantly inherited verbal and orofacial dyspraxia. Hum Brain Mapp 18:194–200.

Benavides-Piccione R, Hamzei-Sichani F, Ballesteros-Yanez I, DeFelipe J, Yuste R (2006) Dendritic size of pyramidal neurons differs among mouse cortical regions. Cereb Cortex 16:990–1001.

Campbell P, Reep RL, Stoll ML, Ophir AG, Phelps SM (2009) Conservation and diversity of Foxp2 expression in muroid rodents: functional implications. J Comp Neurol 512:84–100.

Carroll SB (2000) Endless forms: the evolution of gene regulation and morphological diversity. Cell 101:577–580.

Di Filippo M, Picconi B, Tantucci M, Ghiglieri V, Bagetta V, Sgobio C, Tozzi A, Parnetti L, Calabresi P (2009) Short-term and long-term plasticity at corticostriatal synapses: implications for learning and memory. Behav Brain Res 199:108–118.

Enard W, Gehre S, Hammerschmidt K, Holter SM, Blass T, Somel M, Bruckner MK, Schreiweis C, Winter C, Sohr R, Becker L, Wiebe V, Nickel B, Giger T, Muller U, Groszer M, Adler T, Aguilar A, Bolle I, Calzada-Wack J, Dalke C, Ehrhardt N, Favor J, Fuchs H, Gailus-Durner V, Hans W, Holzlwimmer G, Javaheri A, Kalaydjiev S, Kallnik M, Kling E, Kunder S, Mossbrugger I, Naton B, Racz I, Rathkolb B, Rozman J, Schrewe A, Busch DH, Graw J, Ivandic B, Klingenspor M, Klopstock T, Ollert M, Quintanilla-Martinez L, Schulz H, Wolf E, Wurst W, Zimmer A, Fisher SE, Morgenstern R, Arendt T, de Angelis MH, Fischer J, Schwarz J, Paabo S (2009) A humanized version of Foxp2 affects cortico-basal ganglia circuits in mice. Cell 137:961–971.

Enard W, Paabo S (2004) Comparative primate genomics. Annu Rev Genomics Hum Genet 5:351–378.

Enard W, Przeworski M, Fisher SE, Lai CS, Wiebe V, Kitano T, Monaco AP, Paabo S (2002) Molecular evolution of FOXP2, a gene involved in speech and language. Nature 418:869–872.

Ferland RJ, Cherry TJ, Preware PO, Morrisey EE, Walsh CA (2003) Characterization of Foxp2 and Foxp1 mRNA and protein in the developing and mature brain. J Comp Neurol 460:266–279.

Fisher SE, Scharff C (2009) FOXP2 as a molecular window into speech and language. Trends Genet 25:166–177.

Fitch WT, Huber L, Bugnyar T (2010) Social cognition and the evolution of language: constructing cognitive phylogenies. Neuron 65:795–814.

Fujita E, Tanabe Y, Shiota A, Ueda M, Suwa K, Momoi MY, Momoi T (2008) Ultrasonic vocalization impairment of Foxp2 (R552H) knockin mice related to speech-language disorder and abnormality of Purkinje cells. Proc Natl Acad Sci U S A 105:3117–3122.

Graybiel AM (2008) Habits, rituals, and the evaluative brain. Annu Rev Neurosci 31:359–387.

Groszer M, Keays DA, Deacon RM, de Bono JP, Prasad-Mulcare S, Gaub S, Baum MG, French CA, Nicod J, Coventry JA, Enard W, Fray M, Brown SD, Nolan PM, Paabo S, Channon KM, Costa RM, Eilers J, Ehret G, Rawlins JN, Fisher SE (2008) Impaired synaptic plasticity and motor learning in mice with a point mutation implicated in human speech deficits. Curr Biol 18:354–362.

Haesler S, Rochefort C, Georgi B, Licznerski P, Osten P, Scharff C (2007) Incomplete and inaccurate vocal imitation after knockdown of FoxP2 in songbird basal ganglia nucleus Area X. PLoS Biol 5:e321.

Hisaoka T, Nakamura Y, Senba E, Morikawa Y (2010) The forkhead transcription factors, Foxp1 and Foxp2, identify different subpopulations of projection neurons in the mouse cerebral cortex. Neuroscience 166:551–563.

Hoekstra HE, Coyne JA (2007) The locus of evolution: evo devo and the genetics of adaptation. Evolution 61:995–1016.

Jan YN, Jan LY (2010) Branching out: mechanisms of dendritic ar-
borization. Nat Rev Neurosci 11:316–328.

Jarvis ED (2004) Learned birdsong and the neurobiology of human
language. Ann N Y Acad Sci 1016:749–777.

Jin X, Costa RM (2010) Start/stop signals emerge in nigrostriatal
circuits during sequence learning. Nature 466:457–462.

King MC, Wilson AC (1975) Evolution at two levels in humans and
chimpanzees. Science 188:107–116.

Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP (2001) A
forkhead-domain gene is mutated in a severe speech and lan-
guage disorder. Nature 413:519–523.

Lai CS, Gerrelli D, Monaco AP, Fisher SE, Copp AJ (2003) FOXP2
expression during brain development coincides with adult sites of
pathology in a severe speech and language disorder. Brain
126:2455–2462.

Lieberman P (2006) Toward an evolutionary biology of language.
Cambridge, MA: Harvard University Press.

Liegeois F, Baldeweg T, Connelly A, Gadian DG, Mishkin M, Vargha-
Khadem F (2003) Language fMRI abnormalities associated with
FOXP2 gene mutation. Nat Neurosci 6:1230–1237.

Lovinger DM (2010) Neurotransmitter roles in synaptic modulation,
plasticity and learning in the dorsal striatum. Neuropharmacology
58:951–961.

Lynch VJ, Wagner GP (2008) Resurrecting the role of transcription factor
change in developmental evolution. Evolution 62:2131–2154.

MacDermot KD, Bonora E, Sykes N, Coupe AM, Lai CS, Vernes SC,
Vargha-Khadem F, McKenzie F, Smith RL, Monaco AP, Fisher SE
(2005) Identification of FOXP2 truncation as a novel cause of
developmental speech and language deficits. Am J Hum Genet
76:1074–1080.

Mackay TF, Stone EA, Ayroles JF (2009) The genetics of quantitative
traits: challenges and prospects. Nat Rev Genet 10:565–577.

Martone ME, Zhang S, Gupta A, Qian X, He H, Price DL, Wong M,
Santini S, Ellisman MH (2003) The cell-centered database: a da-
tabase for multiscale structural and protein localization data from
light and electron microscopy. Neuroinformatics 1:379–395.

Middleton FA, Strick PL (2000) Basal ganglia and cerebellar loops:
motor and cognitive circuits. Brain Res Brain Res Rev 31:
236–250.

Mikkelsen T, Hillier L, Eichler E, Zody M, Jaffe D, Yang S, Enard W,
Hellmann I, Lindblad-Toh K, Altheide T, Archidiacono N, Bork P,
Butler J, Chang J, Cheng Z, Chinwalla A, deJong P, Delehaunty K,
Fronick C, Fulton L, Gilad Y, Glusman G, Gnerre S, Graves T,
Hayakawa T, Hayden K, Huang X, Ji H, Kent W, King M, Kulbokas
E, Lee M, Liu G, Lopez-Otin C, Makova K, Man O, Mardis E,
Mauceli E, Miner T, Nash W, Nelson J, Paabo S, Patterson N, Pohl
C, Pollard K, Prufer K, Puente X, Reich D, Rocchi M, Rosenbloom
K, Ruvolo M, Richter D, Schaffner S, Smit A, Smith S, Suyama M,
Taylor J, Torrents D, Tuzun E, Varki A, Velasco G, Ventura M,
Wallis J, Wendl M, Wilson R, Lander E, Waterston R, Consortium
CSA (2005) Initial sequence of the chimpanzee genome and com-
parison with the human genome. Nature 437:69–87.

Mooney R (2009) Neurobiology of song learning. Curr Opin Neurobiol
19:654–660.

Orr HA (2009) Fitness and its role in evolutionary genetics. Nat Rev
Genet 10:531–539.

Pawlak V, Kerr JN (2008) Dopamine receptor activation is required for
corticostriatal spike-timing-dependent plasticity. J Neurosci 28:
2435–2446.

Scharff C, Nottebohm F (1991) A comparative study of the behavioral
deficits following lesions of various parts of the zebra finch song
system: implications for vocal learning. J Neurosci 11:2896–2913.

Shu W, Cho JY, Jiang Y, Zhang M, Weisz D, Elder GA, Schmeidler J,
De Gasperi R, Sosa MA, Rabidou D, Santucci AC, Perl D, Morrisey
E, Buxbaum JD (2005) Altered ultrasonic vocalization in mice with
a disruption in the Foxp2 gene. Proc Natl Acad Sci U S A
102:9643–9648.

Shu W, Lu MM, Zhang Y, Tucker PW, Zhou D, Morrisey EE (2007)
Foxp2 and Foxp1 cooperatively regulate lung and esophagus de-
velopment. Development 134:1991–2000.

Spruston N (2008) Pyramidal neurons: dendritic structure and synaptic
integration. Nat Rev Neurosci 9:206–221.

Surmeier DJ, Plotkin J, Shen W (2009) Dopamine and synaptic plas-
ticity in dorsal striatal circuits controlling action selection. Curr Opin
Neurobiol 19:621–628.

van Elburg RA, van Ooyen A (2010) Impact of dendritic size and
dendritic topology on burst firing in pyramidal cells. PLoS Comput
Biol 6:e1000781.

Vargha-Khadem F, Gadian DG, Copp A, Mishkin M (2005) FOXP2 and
the neuroanatomy of speech and language. Nat Rev Neurosci
6:131–138.

Vargha-Khadem F, Watkins KE, Price CJ, Ashburner J, Alcock KJ,
Connelly A, Frackowiak RS, Friston KJ, Pembrey ME, Mishkin M,
Gadian DG, Passingham RE (1998) Neural basis of an inherited
speech and language disorder. Proc Natl Acad Sci U S A
95:12695–12700.

Varki A, Geschwind DH, Eichler EE (2008) Explaining human unique-
ness: genome interactions with environment, behavior and culture.
Nat Rev Genet 9:749–763.

Wagner GP, Kenney-Hunt JP, Pavlicev M, Peck JR, Waxman D,
Cheverud JM (2008) Pleiotropic scaling of gene effects and the
"cost of complexity." Nature 452:470–472.

Watkins KE, Dronkers NF, Vargha-Khadem F (2002a) Behavioral
analysis of an inherited speech and language disorder: compari-
son with acquired aphasia. Brain 125:452–464.

Watkins KE, Vargha-Khadem F, Ashburner J, Passingham RE, Con-
nelly A, Friston KJ, Frackowiak RS, Mishkin M, Gadian DG (2002b)
MRI analysis of an inherited speech and language disorder: struc-
tural brain abnormalities. Brain 125:465–478.

Zhang J, Webb DM, Podlaha O (2002) Accelerated protein evolution
and origins of human-specific features: Foxp2 as an example.
Genetics 162:1825–1835.

Zhang ZW, Deschenes M (1997) Intracortical axonal projections of
lamina VI cells of the primary somatosensory cortex in the rat: a
single-cell labeling study. J Neurosci 17:6365–6379.