Humanized Foxp2 accelerates learning by enhancing transitions from declarative to procedural performance

Christiane Schreiweis[a,b,1], Ulrich Bornschein[b,1], Eric Burguière[a,c], Cemil Kerimoglu[b,d], Sven Schreiter[b,e], Michael Dannemann[b], Shubhi Goyal[a], Ellis Rea[f], Catherine A. French[g,h], Rathi Puliyadi[h], Matthias Groszer[i], Simon E. Fisher[j,k], Roger Mundry[l], Christine Winter[f], Wulf Hevers[b], Svante Pääbo[b,2], Wolfgang Enard[b,d,2,3], and Ann M. Graybiel[a,2,3]

[a]McGovern Institute for Brain Research and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139; [b]Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, 04103 Leipzig, Germany; [c]Institut National de la Santé et de la Recherche Médicale U 1127, Centre National de Recherche Scientifique Unité Mixte de Recherche 7225, Sorbonne Universités, Université Pierre et Marie Curie, Université Paris 06 Unité Mixte de Recherche S 1127, Institut du Cerveau et de la Moelle épinière, F-75013 Paris, France; [d]Laboratory of Anthropology and Human Genetics, Department of Biology II, Ludwig-Maximilians University Munich, 82152 Martinsried, Germany; [e]DFG Research Center for Regenerative Therapies, Technical University Dresden, 01307 Dresden, Germany; [f]Department of Psychiatry and Psychotherapy, Faculty of Medicine Carl Gustav Carus, Technical University Dresden, 01187 Dresden, Germany; [g]Champalimaud Neuroscience Programme, Champalimaud Centre for the Unknown, 1400-038 Lisbon, Portugal; [h]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, United Kingdom; [i]Institut du Fer à Moulin, Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche S839, Université Pierre et Marie Curie, 75005 Paris, France; [j]Department of Language and Genetics, Max Planck Institute for Psycholinguistics, 6525 XD, Nijmegen, The Netherlands; [k]Donders Institute for Brain, Cognition and Behaviour, Radboud University, 6525 EN, Nijmegen, The Netherlands; and [l]Departments of Developmental and Comparative Psychology and Primatology, Max Planck Institute for Evolutionary Anthropology, 04103 Leipzig, Germany

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The acquisition of language and speech is uniquely human, but how genetic changes might have adapted the nervous system to this capacity is not well understood. Two human-specific amino acid substitutions in the transcription factor forkhead box P2 (FOXP2) are outstanding mechanistic candidates, as they could have been positively selected during human evolution and as *FOXP2* is the sole gene to date firmly linked to speech and language development. When these two substitutions are introduced into the endogenous *Foxp2* gene of mice (*Foxp2*^{hum}), cortico-basal ganglia circuits are specifically affected. Here we demonstrate marked effects of this humanization of Foxp2 on learning and striatal neuroplasticity. *Foxp2*$^{hum/hum}$ mice learn stimulus–response associations faster than their WT littermates in situations in which declarative (i.e., place-based) and procedural (i.e., response-based) forms of learning could compete during transitions toward proceduralization of action sequences. Striatal districts known to be differently related to these two modes of learning are affected differently in the *Foxp2*$^{hum/hum}$ mice, as judged by measures of dopamine levels, gene expression patterns, and synaptic plasticity, including an NMDA receptor-dependent form of long-term depression. These findings raise the possibility that the humanized Foxp2 phenotype reflects a different tuning of corticostriatal systems involved in declarative and procedural learning, a capacity potentially contributing to adapting the human brain for speech and language acquisition.

dorsomedial striatum | dorsolateral striatum | T-maze | cross maze | learning strategy

The gene encoding the transcription factor forkhead box P2 (FOXP2) is a promising candidate for investigating the evolutionary basis of human speech and language capabilities. Humans carrying only one functional copy of this transcription factor experience difficulties in learning and performing complex orofacial movements and have receptive and expressive deficits in oral and written language, whereas other cognitive skills are less affected. These speech and language deficits are associated with functional impairments in cortico-basal ganglia and cortico-cerebellar circuits (1). Since the time that the human and chimpanzee lineages separated, approximately 6 Mya, two amino acid substitutions have occurred in FOXP2, a higher rate of change than expected given its conservation in mammals (2, 3). Mice in which the endogenous *Foxp2* gene has been "humanized" for these two amino acid changes (*Foxp2*$^{hum/hum}$ mice) exhibit prominent neurochemical, neurophysiological, and neuroanatomical alterations in the striatum and related cortico-basal ganglia circuits (4, 5). These circuits are known to be essential for acquiring habits and other motor and cognitive behaviors (6), including vocal learning in songbirds (7) and speech and language capabilities in humans (8). However, whether learning behavior depending on these circuits is affected in *Foxp2*$^{hum/hum}$ mice has so far not been investigated.

A key functional distinction has been made between subregions of the striatum that underlie modes of learning also considered to be crucial for speech and language development and performance: declarative learning and procedural learning (9–12). These learning modes were first distinguished in human cognitive studies to differentiate between a conscious form of

Significance

The human form of forkhead box P2 (*FOXP2*) is the leading genetic candidate for human speech and language proficiency. We demonstrate that the introduction of the amino acid changes that occurred during human evolution into murine *Foxp2* (*Foxp2*^{hum}) profoundly affects learning and striatal neuroplasticity. *Foxp2*$^{hum/hum}$ mice learn stimulus–response associations more rapidly than WT mice when declarative (i.e., place-based) and procedural (i.e., response-based) forms of learning could interfere with one another. Dopamine levels, gene expression patterns, and synaptic physiology are oppositely affected in the striatal districts underpinning these learning forms, paralleling the behavioral change. We hypothesize that the human FOXP2 evolution led to differential tuning of corticostriatal systems involved in declarative and procedural learning and thus contributed to adapting the human brain for speech and language acquisition.

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[1]C.S. and U.B. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: paabo@eva.mpg.de, enard@bio.lmu.de, or graybiel@mit.edu.

[3]W.E. and A.M.G. contributed equally to this work.

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learning that can be "declared" and nonconscious forms of learning that require repetitive exposure (13). Equivalents for these two forms of learning have been suggested for animals in many pioneering studies, and terminology has been adapted depending on whether the motivational drive (action–outcome vs. stimulus–response; goal-directed vs. habit) or the task objective (place-based vs. response-based) is more central to the learning. In rodents, the two learning systems are often probed by tasks requiring motor learning, a type of learning thought to be mainly procedural, or by navigational maze tasks in which place-based learning is suggested to correspond to declarative learning and response-based learning is representative of procedural learning (13–17).

These systems are thought to interact dynamically to optimize behavior (17–22). Evidence suggests that these interacting systems have the capacity to compensate for each other if key components are pathologically affected (23, 24), but can also compete with each other under normal circumstances (14, 15, 17, 19, 25). In situations in which such competition occurs, learning is lessened but can be facilitated by attenuating one of the two competing learning strategies (19, 25). In a novel context, a fact-oriented, declarative type of learning predominates as the new environment is explored. With extended training, as beneficial behaviors are acquired, the procedural system becomes predominant.

Early suggestions that declarative learning solely depends on the temporal lobe and hippocampus, and procedural learning solely on the striatum and cerebellum, have been replaced by evidence that these functions are distributed. Within the striatum, moreover, strong evidence indicates that the declarative system operates early during learning in circuits engaging the dorsomedial striatum, when action–outcome associations are formed, whereas the eventual automatization or proceduralization of the behavior engages circuits interconnected with the dorsolateral striatum (17, 20–22, 26, 27). In brain imaging studies of humans lacking one functional copy of *FOXP2*, contrasting activation patterns have been reported for regions that are considered to be homologous to the dorsomedial and dorsolateral striatum in rodents (28, 29).

We took advantage of these findings by developing a panel of behavioral learning protocols adapted for mice to determine how humanized *Foxp2* influences these two striatal learning systems.

Results

Motor Skill Learning Is Normal in Humanized Foxp2 Mice. We first evaluated motor skill learning, given that mice lacking one functional allele of murine *Foxp2* are reported to exhibit learning deficits on an accelerating rotarod and a tilted running wheel (30, 31). However, mice homozygous for humanized Foxp2 ($Foxp2^{hum/hum}$) performed at levels equivalent to those of their WT ($Foxp2^{wt/wt}$) controls when tested by these two tasks (n = 9–10 per genotype; Figs. S1 and S2), extending earlier findings based on different protocols (4). Hence, these types of motor skill learning are impaired in heterozygous murine Foxp2 KO mice (31), but they are not detectably affected by humanizing the Foxp2 protein in mice.

Learning Is Enhanced in Humanized Foxp2 Mice When Declarative and Procedural Systems Can Be Active. We next performed a series of navigational maze experiments to probe declarative and procedural learning in the $Foxp2^{hum/hum}$ mice. We began by assessing learning in a context allowing place-based/declarative and response-based/procedural forms of learning. We trained $Foxp2^{hum/hum}$ and $Foxp2^{wt/wt}$ mice on a conditional T-maze task, in which distinctive learning-related activity patterns have been found in the dorsomedial and the dorsolateral striatum (22, 32). The mice were required to associate each of two sensory stimuli—a rough or smooth tactile flooring surface—with a food reward that could be found at either goal-arm of a T-maze. In addition, we surrounded the T-maze with salient spatial cues (Fig. 1*A*).

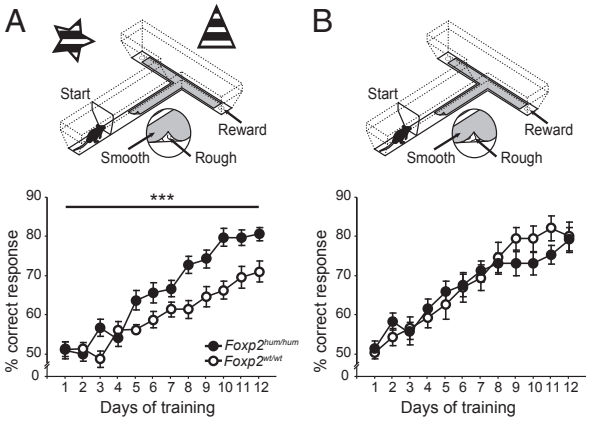

Fig. 1. $Foxp2^{hum/hum}$ mice learn more rapidly than WT littermates in a conditional T-maze paradigm when spatial cues are present. (*Upper*) T-maze task with spatial cues (*A*), promoting place-based/declarative learning; or without spatial cues (*B*), promoting response-based/procedural learning. (*Lower*) Average percent correct responses for $Foxp2^{hum/hum}$ mice (black) and their WT littermates (white) in the two environments. Error bars indicate ± SEM (***$P < 0.001$).

The $Foxp2^{hum/hum}$ mice clearly learned faster than their WT littermates [n = 21–22 per genotype; repeated-measures ANOVA (RMA) days 1–12: $F_{1,41} = 14.94$, $P_{GT} < 0.001$; $F_{7.2,41} = 3.99$, $P_{day*GT} < 0.001$; generalized linear mixed model days 1–12, $z = -3.9$, $P_{day*GT} < 10^{-4}$; Fig. 1*A*, *SI Materials and Methods*, and Table S1]. Moreover, this faster learning in the $Foxp2^{hum/hum}$ mice was specific to the acquisition phase of training. Performance during overtraining, as correct performance was reached and then maintained at greater than 72.5%, did not differ between genotypes (n = 14–15 per genotype; RMA overtraining days 1–10: $F_{1,27} = 0.11$, $P_{GT} = 0.74$; $F_{9,27} = 1.14$, $P_{day*GT} = 0.34$; Fig. S3).

We designed experiments to determine whether this enhancement of learning speed in the $Foxp2^{hum/hum}$ mice reflected enhanced place-based/declarative learning, enhanced response-based/procedural learning, or an altered interaction of these learning systems. An altered interaction, for example, caused by an attenuated declarative system, could enhance performance by accelerating the transition toward the procedural system, an interaction that has been proposed to occur during striatum-dependent learning tasks (17, 18, 21, 22, 27). In the original T-maze surrounded by spatial cues, the mice were provided with at least three learning possibilities. They could associate a sensory stimulus (rough or smooth) with a reward at a constant place (place-based/declarative learning), associate the stimulus with a body turn (procedural/response-based strategy), or shift from a declarative to a procedural strategy during the course of the training. We tested these three alternatives individually.

First, we changed the T-maze task to favor procedural learning by removing extramaze spatial cues (Fig. 1*B*), and we tested acquisition in new, naïve cohorts of mutant and WT mice. In this context, the $Foxp2^{hum/hum}$ and WT mice learned equally well (n = 13–14 per genotype; RMA days 1–12: $F_{1,25} = 0.07$, $P_{GT} = 0.795$; $F_{11,25} = 1.439$, $P_{day*GT} = 0.156$; Fig. 1*B* and Table S2). Analyses of the combined data for the two task paradigms showed that the presence of spatial cues had clearly a different effect on learning in $Foxp2^{hum/hum}$ mice and their WT controls (RMA days 1–12: $F_{7.85,68} = 4.04$, $P_{day*GT*setup} < 0.001$). This difference appears to reflect less efficient learning by WT mice in the presence of spatial cues (Fig. 1). This possibility is in accord with reports of less efficient learning in an environment in which the two learning strategies of declarative/place-based and procedural/response-based learning can interact competitively

(25) and that WT C57BL/6 mice are "essentially place learners" (33–35). By this view, the abundance of spatial cues in the original maze task did not impair the performance of the *Foxp2*$^{hum/hum}$ mice, which might have dealt more effectively with competition between the two available learning strategies.

Given this result, we turned to a cross-maze task often used to discriminate place-based from response-based learning (15, 17, 25). We chose a Tolman variation of the task (16, 36), tailored for our purposes, because the cross-maze variation by Packard and McGaugh (15) has been reported to be difficult for mice (33–35). In this cross-maze paradigm, we were able to test declarative/place-based learning and procedural/response-based learning separately as well as to challenge the interaction between them by testing the ability to change between place-based and response-based learning. The mice started from either of two opposing arms of the maze (north or south), with reward available after a specific response (e.g., right turn; Fig. 2*A*, *Left*) or at a fixed place (e.g., east arm; Fig. 2*A*, *Right*).

Remarkably, we did not observe enhanced learning by the *Foxp2*$^{hum/hum}$ mice in the response-based task or the place-based task. The *Foxp2*$^{hum/hum}$ and WT mice learned both tasks equally rapidly (response-based, $n = 7$–8 per genotype, RMA: $F_{1,13} = 0.43$, $P_{GT} = 0.53$; $F_{4.6,13} = 0.56$, $P_{day*GT} = 0.72$; place-based, $n = 19$–20 per genotype, RMA: $F_{1,37} = 0.45$, $P_{GT} = 0.51$; $F_{6.2,37} = 0.83$, $P_{day*GT} = 0.55$; Fig. S4). Thus, *Foxp2*$^{hum/hum}$ mice did not learn faster when the mice were required to use only place-based learning or only response-based learning to solve the task, despite exhibiting accelerated learning when both strategies could be used.

Prompted by this finding, we tested whether the enhanced performance of the *Foxp2*$^{hum/hum}$ mice resulted from an altered interaction between the two learning systems, attenuating the declarative and favoring the procedural system. We required mice that previously had acquired both tasks without significant difference in performance to shift from place-based learning to response-based learning. We expected to find a difference only during the first days after the task switch, when the two learning systems would likely be in direct competition with each other. To control for general effects on memory or behavioral flexibility, we additionally tested the mice on the opposite direction of transition, measuring learning speeds during the first days after a shift from response-based to place-based learning.

For the transition from place-based to response-based learning, the *Foxp2*$^{hum/hum}$ mice switched significantly more rapidly ($n = 7$–8 per genotype; RMA: $F_{1,13} = 5.68$, $P_{GT} = 0.03$; Fig. 2*B* and Table S3). By contrast, their learning rates did not differ from those of their WT littermates after the opposite, response-to-place transition conditions ($n = 7$–8 per genotype, RMA: $F_{1,13} = 0.19$, $P_{GT} = 0.67$; Fig. 2*C*). These findings suggest that it is specifically the transition from declarative/place-based learning to procedural/response-based learning that is enhanced by the introduction of the humanized form of Foxp2, and not either one of these learning systems alone. The findings further suggest that the competitive interaction between these systems could be lessened in mice with humanized Foxp2, therefore facilitating the transition from declarative to procedural learning that is proposed to occur during striatum-dependent habit learning (18, 20–22).

By contrast, we did not detect differences between *Foxp2*$^{hum/hum}$ mice and their WT siblings in either of these learning systems when they were tested individually. The two genotypes exhibited equivalent procedural/response-based learning as assessed with the accelerating rotarod protocol, the tilted running wheel test, the T-maze protocol in which extramaze cues had been removed, and the procedural/response-based version of the cross-maze task. We also did not observe a difference in the place-based learning of the *Foxp2*$^{hum/hum}$ mice, which we tested in the declarative/place-based version of the cross-maze task. Only when both learning systems could be engaged in parallel and could interact during the early acquisition phase of learning, as in the T-maze task with extramaze cues, did the humanized Foxp2 mice exhibit more efficient learning. By challenging this interaction between the learning systems with the abrupt shift from declarative/place-based to procedural/response-based learning in the cross-maze task, we found that the more rapid learning in the humanized Foxp2 mice could reflect a faster transition from declarative to response learning.

We next tested the possibility that such a change in learning dynamics could reflect differential effects of the Foxp2 humanization on the dorsomedial and dorsolateral striatum, nodes in circuits that differently support these learning forms.

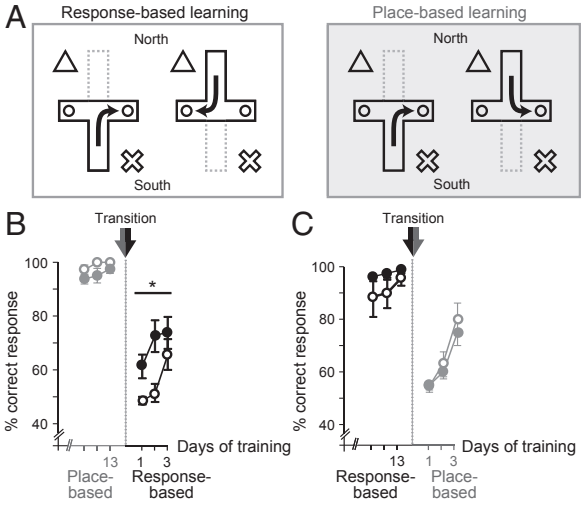

Fig. 2. *Foxp2*$^{hum/hum}$ mice exhibit enhanced ability to make transitions from a declarative to a procedural mode of learning. (*A*) Response-based/procedural (*Left*) and place-based/declarative (*Right*) versions of the cross-maze task. (*B* and *C*) Average percent correct responses (±SEM) for *Foxp2*$^{hum/hum}$ (filled dots) and *Foxp2*$^{wt/wt}$ (open dots) mice successively trained on the two cross-maze task versions and tested on the switch to response-based/procedural (*B*) or place-based/declarative version (*C*) (*$P < 0.05$).

Differential Effects of Humanized Foxp2 on mRNA Expression Profiles in the Dorsomedial and Dorsolateral Striatum. To test the possibility that humanized Foxp2 might influence the dorsomedial and the dorsolateral striatum differently, we isolated striatal samples from each subregion by laser capture microdissection in adult *Foxp2*$^{hum/hum}$ mice and WT littermates ($n = 11$–12 per genotype) and obtained profiles of mRNA expression with >20 million RNA-Sequencing (Seq) reads per sample. We found many differences between the mRNAs in the two regions [5,895 of 25,259 detected genes with a false discovery rate (FDR) < 0.05; $P_{permutations} < 0.001$], but no single gene differed between genotypes (no genes with an FDR < 0.1; $P_{permutations} = 0.17$). This result indicated that the introduction of humanized Foxp2 does not produce massive changes in the expression profile of striatal cells at the level of single genes.

We did detect a significant effect of humanized Foxp2 at the level of functional gene categories, in particular, a down-regulation of genes in the dorsomedial striatum (1,485 of 3,930 categories at an FDR < 0.05; $P_{permutations} = 0.013$; Dataset S1). The most significant category affected was "signaling," and the strongest enrichment was found for "neurotransmitter transporter activity" and many categories involved in synaptic regulatory processes (Fig. S5 and Dataset S1). Effects in the dorsolateral striatum were often smaller and nonsignificant (914 of 3,930 categories at an FDR < 0.05; $P_{permutations} = 0.08$). Thus, we detected differential effects of humanized Foxp2 on genes involved in synaptic regulatory processes in the two striatal regions. These subtle molecular effects could reflect important physiological alterations, if present in a

subset of cells or if produced by differential inputs to the two striatal districts.

Humanized Foxp2 Influences Dopamine Levels Differently in the Dorsomedial and Dorsolateral Striatum. To explore such potential physiological consequences of the Foxp2 humanization, we next analyzed striatal dopamine levels, which are known to be related to learning and to be reduced in striatal samples spanning the dorsomedial and dorsolateral regions in *Foxp2*$^{hum/hum}$ mice (4). Dopamine levels in the dorsomedial striatum of the *Foxp2*$^{hum/hum}$ mice were reduced to 70% of those found in WT control mice (n = 10–22 per genotype; t test, $t_{30} = 3.7$; $P_{GT} = 0.001$), whereas dopamine levels in the dorsolateral striatum were similar in the two genotypes (n = 9–22 per genotype; t test, $t_{29} = 0.7$; $P_{GT} = 0.5$). Thus, humanized Foxp2 influences dopamine levels differently in the sensorimotor and associative regions of the dorsal striatum, reducing them dorsomedially (RMA, $F_{1,29} = 5.73$, $P_{GT*region} = 0.02$; Fig. 3*A*).

Humanized Foxp2 Influences Induction of LTD Differently in the Dorsomedial and Dorsolateral Striatum. To explore potential electrophysiological effects of the Foxp2 humanization, we measured in acute brain slices the induction of dopamine-dependent long-term depression (LTD) after high-frequency stimulation (HFS) in medium spiny neurons (MSNs) located in the dorsolateral and dorsomedial striatum (n = 9–19 cells per genotype and striatal region). In the *Foxp2*$^{hum/hum}$ mice, LTD in the dorsolateral striatum was stronger than that in WT controls (Fig. 3*D*), in accordance with previous results (4, 5). However, in the dorsomedial striatum, LTD tended to be weaker in the *Foxp2*$^{hum/hum}$ mice relative to that in WT controls (Fig. 3*C*), indicating again the presence of a region-specific effect of humanized Foxp2 (n = 9–19; ANOVA, $F_{1,52} = 5.9$, $P_{GT*region} = 0.02$; Fig. 3*B*).

To determine the mechanistic basis of the stronger LTD in the dorsolateral striatum of the *Foxp2*$^{hum/hum}$ mice, we first compared

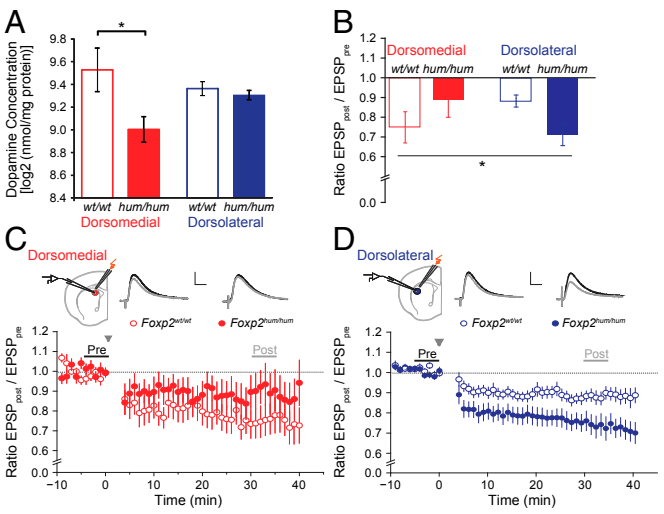

Fig. 3. *Foxp2*$^{hum/hum}$ mice exhibit differential effects of dopamine levels and synaptic plasticity in the dorsomedial and the dorsolateral striatum. (*A*) Average (±SEM) concentrations of dopamine in dorsomedial (red) and dorsolateral (blue) striatal biopsies of *Foxp2*$^{hum/hum}$ mice (*hum/hum*) relative to WT (*wt/wt*) levels (*P < 0.05). (*B*) Averaged excitatory postsynaptic responses (±SEM) in dorsomedial and dorsolateral MSNs in mutant and WT mice 30–40 min after HFS to induce LTD, normalized to baseline levels (*P < 0.05). (*C* and *D*) Recording location, representative traces, and time course of LTD induction (post; mean amplitudes ± SEM), normalized to baseline levels (pre) and after stimulation in the dorsomedial (*C*) and dorsolateral (*D*) striatum. (Scale bars: 2 mV and 10 ms.)

our protocol, involving a modest −70-mV depolarization during induction, vs. the commonly used HFS-LTD protocol in which stronger depolarization to −15 mV (37) favors the activation of voltage-gated calcium channels (38, 39). When we used the strong depolarization, the genotype difference disappeared. We also observed robust LTD in WT mice (n = 7–17 per LTD protocol; ANOVA, $F_{1,22} = 10.1$, $P = 0.004$; Fig. 4 *A* and *B*), and the magnitude of this LTD was similar to that in the *Foxp2*$^{hum/hum}$ mice (n = 7–8 per genotype; ANOVA, $F_{1,13} = 0.28$, $P = 0.6$). This result indicates that LTD is more readily inducible in MSNs of the dorsolateral striatum of the *Foxp2*$^{hum/hum}$ mice and requires less depolarization than LTD in the corresponding region of the WT.

We next tested whether the readily inducible LTD in *Foxp2*$^{hum/hum}$ mice is based on the dopamine D2 receptor (D2R)-dependent striatal mechanism that has been consistently described for LTD in WT mice (38, 40). Applying the D2R antagonist sulpiride to the slice bath eliminated LTD induction in the *Foxp2*$^{hum/hum}$ mice (n = 6–19 per treatment; ANOVA, $F_{1,22} = 5.5$, $P = 0.03$; Fig. 4 *C* and *D*), suggesting that the effect of humanized Foxp2 on striatal LTD depends on D2R-associated mechanisms.

We tested the alternative possibility that the LTD difference could be the result of a confounding effect of long-term potentiation (LTP) present only in WT mice. LTP in striatal MSNs is considered to be mediated by NMDA receptors and is consistently reported to be blocked by APV (38, 41). Therefore, we antagonized NMDA receptors by adding extracellular APV (50 μM) to the bath solution under the modest −70-mV depolarization conditions. The responses in the dorsolateral striatum were not lowered by APV application in the WT mice, excluding the possibility of a confounding LTP effect (n = 5–17; ANOVA, $F_{1,20} = 0.32$, $P = 0.58$; Fig. 4 *C* and *D*). By contrast, in the dorsolateral striatum of the *Foxp2*$^{hum/hum}$ mice, NMDA receptor inhibition abolished the readily inducible, weak-depolarization LTD, so that the response in humanized mice was no longer distinguishable from WT (n = 10–17 per genotype and treatment; ANOVA, $F_{1,25} = 0.42$, $P = 0.52$; Fig. 4 *C* and *D*).

To determine whether this extracellular NMDA receptor blockade in the *Foxp2*$^{hum/hum}$ mice resulted from effects at the presynaptic level or from the actions of postsynaptic receptors on the MSNs themselves, we added the NMDA channel blocker MK801 (1 mM) to the intracellular solution. This treatment blocked the readily inducible LTD in humanized MSNs (n = 5–19 per treatment; $F_{1,22} = 4.3$, $P = 0.04$; Fig. 4*D*), suggesting that, under low-depolarization conditions, postsynaptic NMDA receptor activation accounts for LTD induction in the *Foxp2*$^{hum/hum}$ mice. Our findings thus implicate the humanized form of Foxp2 in enhancing a mechanism of LTD induction in the dorsolateral striatum by means of postsynaptic NMDA receptors. At present, we do not assume a specific increase in NMDA receptors to be responsible for this increased modulation, as the ratio of NMDA to AMPA currents remains unaltered in the *Foxp2*$^{hum/hum}$ mice (Fig. S6*B*).

Discussion

Our findings suggest a striking selectivity in the effects of humanized Foxp2 on behavioral learning dynamics as well as on striatal dopamine levels, gene expression levels, and synaptic plasticity. Based on our experimental findings, we suggest as a working hypothesis that humanized Foxp2 differentially influences the functional contributions of the associative and sensorimotor striatum to learning dynamics (Fig. S7). In this view, the *Foxp2*$^{hum/hum}$ mice exhibited an altered interaction between the declarative and procedural learning strategies, favoring the procedural system when both learning systems were engaged as indicated by their more rapid transition toward procedural behavior in the cue-enriched conditional T-maze task and in the place-to-response switching cross-maze task. This condition

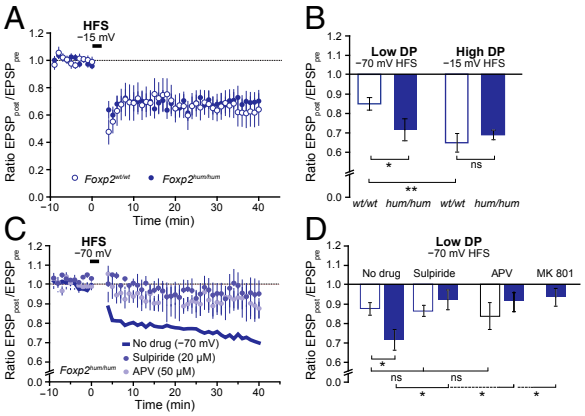

Fig. 4. The enhanced LTD in dorsolateral MSNs of *Foxp2*$^{hum/hum}$ mice is specific for LTD induction under low depolarization (DP) conditions and depends on D2Rs and NMDA channels. (*A*) HFS (gray arrow) with depolarization to −15 mV instead of weaker depolarization of −70 mV (Fig. 3 *B*–*D*) induced comparable LTD in control and mutant mice during the 40 min post-HFS period. (*B*) Changing from HFS with weak depolarization (−70 mV) to high depolarization conditions (−15 mV) enhanced mean LTD levels measured at 30–40 min after the HFS in WT but not in mutant mice. Error bars indicate SEM (**P* < 0.05 and ***P* < 0.005). ns, not significant. (*C*) Readily inducible LTD under low depolarization conditions in dorsolateral MSNs of *Foxp2*$^{hum/hum}$ mice is abolished by the D2R antagonist sulpiride or by external application of the NMDA receptors antagonist APV. Shown are excitatory postsynaptic potential amplitudes (post), normalized to the mean baseline levels (pre), after HFS in the low depolarization condition of −70 mV (gray arrow) in the presence of sulpiride (20 μM) or APV (50 μM). (*D*) In mutant mice, the readily inducible LTD measured 30–40 min after HFS under low depolarization conditions can be reversed to WT levels by blocking D2Rs by sulpiride or NMDA receptors with extracellular APV or intracellular MK801 (1 mM; electrode solution). Recordings in the presence of sulpiride, APV, or MK801 were not different from control recordings obtained without HFS stimulation (n = 5–17; ANOVA, P = 0.32–0.98; Fig. S6*A*). Error bars indicate SEM (**P* < 0.05). ns, not significant.

would contrast with WT conditions, in which the declarative system is thought to dominate and render the naturally occurring transition toward the procedural learning system less than maximally efficient (17, 19, 25).

How this behavioral change in the *Foxp2*$^{hum/hum}$ mice is brought about is not clear. However, the modest effects of humanized Foxp2 on gene expression patterns suggest that generalized molecular or cellular reconfigurations of striatal MSNs are not involved. The region-specific effects of humanized Foxp2 on dopamine content and synaptic plasticity could reflect mechanisms directly related to the behavioral effects, given the differential function of the dorsomedial and dorsolateral striatum in place-based/declarative and response-based/procedural forms of learning. Our electrophysiological recordings indicate a region-specific enhancement of readily inducible LTD in the *Foxp2*$^{hum/hum}$ mice. This form of LTD followed the D2R-dependent mechanism identified for classical strong-depolarization induction protocols (40), but required the activation of NMDA receptors. Such a mechanism has been described in other brain regions (42), but, in the striatum, has been linked mainly, but not exclusively, to the induction of LTP, not LTD (38, 40, 41, 43, 44). Given that the unaltered ratio between NMDA and AMPA currents indicated no increase in NMDA receptors of *Foxp2*$^{hum/hum}$ mice, and that dopamine is critical for striatal synaptic plasticity, one alternative is that an altered dopamine-dependent modulation of NMDA receptors could be responsible for the humanized effect we observed in these mice (45–47).

The contrasting effects in the dorsomedial and dorsolateral striatum of *Foxp2*$^{hum/hum}$ mice are striking given that different regional brain-imaging activation patterns have been reported for what are considered as homologous striatal districts in humans lacking one functional copy of *FOXP2* (28, 29). How these findings relate to the effect of humanized version of Foxp2 in shaping the development of a human brain to enable traits such as language and speech acquisition is unknown. The relation between declarative and procedural learning strategies and language learning is itself unclear (10–12). One possibility raised by our findings is that efficient proceduralization might accelerate probabilistic learning of language features (10) by chunking single speech and language-related actions into sequences, a chunking function that has been suggested to be a core property of the striatum in experimental work (48, 49). If so, such a process could free up declarative capacities by implementing procedural components at earlier time points. Our findings prompt the intriguing speculation that the humanization of this gene imparted a facilitated ability to use procedural forms of learning and therefore to shift more rapidly from declarative to procedural forms of learning, a change that could have been important for the emergence of proficient language and speech.

Materials and Methods

Additional description of study materials and methods is provided in *SI Materials and Methods*.

Animals. A total of 303 *Foxp2*$^{hum/hum}$ mice [5H10 line (4); 1.8–15.2 mo; postnatal day (P)21–P53 for electrophysiological experiments] and WT littermates (160 for behavioral tests, 23 for gene expression assays, 32 for dopamine measurements, and 88 for electrophysiology experiments) were used, and they were balanced for genotype and sex in each experiment. Behavioral procedures were approved by the Committee on Animal Care at the Massachusetts Institute of Technology, and other procedures were in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986 and guidelines of the Max Planck Institute for Evolutionary Anthropology and federal regulations of Saxony, Germany.

Behavioral Experiments. Rotarod and tilted running wheel experiments were conducted as previously described (31). For the maze experiments, mice were food-restricted and were habituated to apparatus and reward (chocolate milk). They were then trained on a T-maze (40 trials each day) to obtain reward on the correct goal arm as instructed by tactile conditional cues (rough or smooth floor surface) or on a cross maze (10 trials each day) to go to a specific goal (place-based version) or to make a particular turn (response-based version) to receive reward. Statistical analysis was performed by using RMA and generalized linear mixed models (*SI Materials and Methods*).

Laser Capture Microdissection and RNA Sequencing. The dorsomedial and dorsolateral striatum of adult mice was dissected from brain slices by using a laser microscope (P.A.L.M. System; Zeiss). Twenty-five nanograms total RNA were used to construct barcoded mRNA-Seq libraries that were sequenced on a Genome Analyzer IIx platform as described earlier (50). Gene expression analysis was performed by the multifactor model of the R package for differential expression analysis for sequence count data (51). Effects of humanized Foxp2 were summarized by the π-value that multiplies the magnitude and significance of genotype effect (52). This ranking was used for the Wilcoxon rank test implemented in FUNC (https://func.eva.mpg.de/) (53) to identify enriched Gene Ontology categories. Permutations of genotype labels were used to assess global significance ($P_{permutations}$).

Dopamine Content. Tissue samples from 1 mm cryocut slabs of the dorsomedial and the dorsolateral striatum were homogenized, and their protein content was measured. Dopamine was detected at an electrode potential of 0.8 V. Statistical analyses were performed on log2-transformed dopamine amounts per milligram of protein normalized per region, sex, and batch.

In Situ Electrophysiology. Coronal striatal slices (250 μm) were prepared from P21–P53 mice, and responses of MSNs to stimulation of cortical afferents (0.33–0.2 Hz) were measured during periods before (15 min) and after (40 min) a tetanic HFS (4 × 100 Hz, at −70 or at −15 mV) in the presence of the GABA(A) receptor blocker SR95531 (GABAzine) by using a whole-cell patch-clamp setup. We applied one- and two-way ANOVAs to test region- and genotype-specific effects.

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