Molecular and functional definition of the developing human striatum

Marco Onorati1,7,8, Valentina Castiglioni1,8, Daniele Biasci2, Elisabetta Cesana3, Ramesh Menon4, Romina Vuono5, Francesca Talpo3, Rocio Laguna Goya3, Paul A Lyons2, Gaetano P Bulfamante6, Luca Muzio4, Gianvito Martino4, Mauro Toselli3, Cinthia Farina4, Roger A Barker5, Gerardo Biella3 & Elena Cattaneo1

The complexity of the human brain derives from the intricate interplay of molecular instructions during development. Here we systematically investigated gene expression changes in the prenatal human striatum and cerebral cortex during development from post-conception weeks 2 to 20. We identified tissue-specific gene coexpression networks, differentially expressed genes and a minimal set of bimodal genes, including those encoding transcription factors, that distinguished striatal from neocortical identities. Unexpected differences from mouse striatal development were discovered. We monitored 36 determinants at the protein level, revealing regional domains of expression and their refinement, during striatal development. We electrophysiologically profiled human striatal neurons differentiated in vitro and determined their refined molecular and functional properties. These results provide a resource and opportunity to gain global understanding of how transcriptional and functional processes converge to specify human striatal and neocortical neurons during development.

Despite pioneering studies on human cerebral cortex development1, our knowledge of the developmental mechanisms underlying brain formation has largely relied on cellular, molecular and functional studies conducted in rodents2. The evidence that specific human neurological and psychiatric disorders may have a neurodevelopmental origin has provided impetus for more detailed studies of human brain development3–5.

Neurons in the striatum represent an important brain hub, and dysfunction of them is associated with a range of disorders, such as Huntington’s disease6. During development, the telencephalon subdivides into two main subregions: dorsally, the pallium gives rise to neocortical structures and, ventrally, the lateral and medial ganglionic eminences (LGE and MGE, respectively) give rise to the main neuronal populations of the striatum and to most neocortical interneurons and pallidial neurons, respectively3,4,7. Previous studies have described neocortical transcriptome organization5,8–10, but little is known about the spatiotemporal regulation of gene expression that specifies early striatal and neocortical progenitors and their progeny in humans.

In this work, we identified the transcriptional dynamics that distinguish the human striatum from the neocortex during a critical early temporal window, between 6 and 11 post-conception weeks (w), poorly investigated before. Differential gene expression analysis and unbiased screening for bimodal genes identified coding and noncoding genes that segregated striatal and neocortical identities. We found also distinct gene coexpression networks that revealed the functional organization of the early striatal and neocortical transcriptome.

We then performed a systematic immunohistochemical analysis of human samples, aged 2 to 20 w, to detect 36 known and newly identified developmental determinants, including 17 transcription factors (TFs). For most of them, we describe their previously unknown expression patterns, starting from the earliest neural fold stage. In addition, we determined the electrophysiological properties of human striatal and neocortical primary neurons. For the first time, to the best of our knowledge, we show the functional activity of human fetal striatal neurons, their neurotransmitter responses and dopamine neuromodulation. Thus, this study describes human striatal and neocortical development, combining transcriptomics, cell-fate determinant coexpression patterns and electrophysiological recordings. It represents a resource for neurodevelopmental disease hypotheses and stem cell modeling.

RESULTS

Global transcriptional signatures of developing striatum and neocortex

The transcriptional dynamics were investigated using total RNA isolated from samples of human brains from 6 to 11 w (LGE/striatum n = 16 and neocortex n = 14; Supplementary Table 1a,b). RNA samples were hybridized on an Illumina HumanHT-12v4 array platform. After quality control, normalization and filtering (Fig. 1a), 26,778 expressed genes were used for further analyses. Principal component analysis and hierarchical clustering of the filtered transcriptome revealed differences between striatum and neocortex (Supplementary Fig. 1a,b).

To determine the differentially expressed genes (DEGs) between...
We identified 145 BEGs out of 26,778 expressed genes (Fig. 1a). The most relevant were represented by 92 genes associated with striatum/neocortex separation, while another relevant class was related to sex separation (Supplementary Table 2b). All striatal and neocortical BEGs were present among the DEGs; GO annotations confirmed their association with neurodevelopmental processes (Supplementary Fig. 1d). Specifically, we found that 51 BEGs were upregulated in striatum and 41 were upregulated in neocortex (Fig. 1a and Supplementary Table 2a). All BEGs exhibited two classes of samples (Fig. 1b).

Bimodal gene landscape of developing striatum and neocortex

In a complementary unbiased approach, we subjected the transcriptional data from all 22 striatal and neocortical samples (both paired and unpaired; Supplementary Table 1a,b) to identification of bimodally expressed genes (BEGs), whose expression pattern may suggest the existence of different classes of samples in a given data set. Traditionally, BEGs have been considered ideal biomarker candidates because they provide sharp decision boundaries to classify samples into different groups. Unlike analysis of DEGs, analysis of BEGs may also reveal the existence of previously unknown classes of samples.

We identified 145 BEGs out of 26,778 expressed genes (Fig. 1a). The most relevant were represented by 92 genes associated with striatum/neocortex separation, while another relevant class was related to sex separation (Supplementary Table 2b). All striatal and neocortical BEGs were present among the DEGs; GO annotations confirmed their association with neurodevelopmental processes (Supplementary Fig. 1d). Specifically, we found that 51 BEGs were upregulated in striatum and 41 were upregulated in neocortex (Fig. 1a and Supplementary Table 2a). All BEGs exhibited two different expression averages in the striatal and neocortical samples (Fig. 1d and Supplementary Table 2b). The striatal BEGs comprised some typical markers of LGE or striatum, such as GAD1, GAD2, SLIC32A1 (also known as VGAT), ISL1, DLX5, EBF1, TAC1 and LMO3 (Supplementary Table 2b). Surprisingly, NKX2-1 was one of the top-rated striatal TFs, at variance with mouse studies that showed Nkx2-1 to be an MGE marker labeling only striatal interneurons. The TF-encoding gene ZFHX3 and the two noncoding RNAs DLX6 antisense (DLX6AS) and the large intergenic noncoding RNA LINC00403 (Supplementary Fig. 1e), never to our knowledge described as human LGE- or striatum-enriched markers, were present among the striatal BEGs. A complementary set of TFs was identified as a strong neocortex discriminant and included DMRTA2, EMX1, EMX2, FEZF2, AP2C and noncoding RNAs such as EMX2OS (EMX2 opposite strand) (Supplementary Table 2b).
Figure 2 Gene network analysis identifies distinct modules of coexpressed genes. (a) Dendrogram from gene coexpression network analysis of human striatal and neocortical tissue samples. Modules of coexpressed genes were assigned colors, indicated by the horizontal bars under the dendrogram. (b, d) Plot of the correlations between gene expression values and tissue of origin for all gene members of module M25 (b) or M3 (d). Each symbol represents one gene; DEGs between striatum and neocortex are represented by colored dots, non-DEGs are represented by gray dots; genes classified as bimodal (BEGs) are represented by a triangle with black borders. BEGs showed particularly high correlation with the module eigengene and tissue of origin. (c, e) Network representation of selected genes from M25 and M3 modules (top 5% genes by intramodular connectivity). The size of each vertex is directly proportional to the intramodular connectivity value for a particular gene. Thickness and no cut of each line are directly proportional to the topological overlap measure between the two connected genes. DEGs between striatum and neocortex are represented in blue (c) or red (e). BEGs are represented by a blue (c) or red (e) triangle and marked in bold text. Genes names are shown for top 3% genes by intramodular connectivity. Names may appear more than once because different probes may interrogate different transcripts from the same gene.
Figure 3 TF expression pattern from neural fold stage. Immunohistochemistry was performed on embryonic sections; slice orientations and developmental stages are indicated at the top of each column. (a,e) At 2 w + 5 d, OTX2 is expressed in the N-cadherin* (N-cad) neural folds (NF). (b,f,j) At 3 w + 3 d and 3 w + 4 d, OTX2 is found in the prosencephalon (Pr). (b,f,j) Note the inverted dorsal-ventral (D-V) orientation of the Pr. (d) Hoechst staining at 7 w + 2 d shows locations of images in panels (h,i,p,t). (f) At 7 w + 2 d, OTX2 is in the VZ and the incipient cortical plate (CP). (i) At 2 w + 5 d, PAX6 is expressed in the closing NF and, later (j,k), in the Pr, excluding the ventral domain (see arrowheads). (l) At 7 w + 2 d, PAX6 is confined to the pallium and excluded from the GSX2+ subpallium. (m-o) FOXG1 expression starts at 3 w + 4 d in telencephalic NEPs. (p) At 7 w + 2 d, FOXG1 is expressed in the VZ. (q) At 2 w + 5 d, NKX2-1 is expressed in the closing NF. (r,s) After 3 w + 3 d, NKX2-1 is restricted to the ventral telencephalon. (t) At 7 w + 2 d, NKX2-1 is expressed in the VZ and SVZ of the medial ganglionic eminence (MGE). CX, neocortex; Di, diencephalon; Me, mesencephalon; SV, supraventricle; CPP, cephalic preplacode; PM, primordial mesenchyme; LGE, lateral ganglionic eminence; PSB, pallial-subpallial boundary; SVZ, subventricular zone; IZ/SP, intermediate zone and subplate; OV, optic vesicle. Scale bars, 50 μm (insets: 25 μm).

Gene network analysis identifies coexpressed gene modules

We next investigated the functional organization of the transcriptome. A weighted gene coexpression network analysis\(^1\) (WGCNA) identified 44 modules (Fig. 2a and Supplementary Table 3). A trait-module correlation analysis was performed to identify potential attributes associated with the different modules. Notably, modules M3, M25 and M35 were correlated with the tissue of origin of the samples. M25 members were highly expressed in striatum, while members of M3 and M35 showed higher expression in neocortex (Fig. 2b and Supplementary Fig. 2a,b). We also report the correlation between module eigengenes and potential confounders (for example, array batch, hybridization date, sample collection date, operator and sample age). The correlation between modules eigengene and these potential confounders was not statistically significant (M25 and M35, $P \geq 0.12$ and $P = 1$, respectively) or was smaller than the correlation with the biological variable of interest (M3; Supplementary Fig. 2a).

To further dissect the correlation between modules and tissue of origin, we represented the genes members of each module in a two-dimensional scatterplot (Fig. 2b,d). In M25, genes with the highest degree of connectivity, termed hub genes, also showed high correlation with tissue of origin (Fig. 2c). Notably, a subset of DEGs and BEGs lay at the core of the module, probably representing a set of genes subject to tight regulation during the transcriptional program that differentiates striatum from neocortex. These genes included the GABA receptor GABRB3, the guanine nucleotide exchange factor PSD2, GAD1 and LMO3 (Fig. 2c). Furthermore, GO enrichment analysis showed that genes in M25 were enriched in the categories synaptic transmission and nervous system development (http://people.ds.cam.ac.uk/db544/onorati2014/blue/). A separate GO analysis on M25 BEGs revealed enriched processes such as neuron fate commitment and regulation of transcription (http://people.ds.cam.ac.uk/db544/onorati2014/blue/).

We analyzed the genes list for M3 similarly (Fig. 2d). M3 was primarily enriched for GO processes such as DNA metabolic process and cell-cycle process (http://people.ds.cam.ac.uk/db544/onorati2014/red/), compatibly with the presence in the neocortex of still-proliferating progenitors. Also in M3, a subset of DEGs and BEGs was found in the module core; among them, the chromatin assembly factor 1 p60 (CHAF1B), WNT5B and EMX2OS were hub genes (Fig. 2c). GO analysis on M3 BEGs revealed enriched processes such as cerebral neocortex regionalization and forebrain neuroblast division (http://people.ds.cam.ac.uk/db544/onorati2014/red/). Also, M35 was significantly correlated ($P \leq 4.7 \times 10^{-5}$) with neocortical origin (Supplementary Fig. 2b) and included important DEGs: for example, NEDD9, PAX6 and NEUROD1, which appeared as highly interconnected genes in the M35 network (Supplementary Fig. 2c). In conclusion, the WCGNA identified a significant spatial association between individual modules and striatum/neocortex ontology and also pivotal DEGs and BEGs as the most interconnected genes in the module networks.

TF expression profile from the human neural fold stage

We investigated the coherence between the analyses of gene expression and the emergence of striatal identity through protein expression study. Our immunohistochemical analyses started in a rare 2-w+5-d embryo (Supplementary Table 1c), when the neural plate invaginations give rise to the neural folds. Gene profiling identified the homeobox gene OTX2 as a DEG. In mouse, this gene is expressed...
in the anterior neuroectoderm, demarcating the prosencephalic and mesencephalic compartments. In the 2 w + 5 d embryo, OTX2 was expressed in the N-cadherin+ neural folds (Fig. 3a,e) and in the more posterior, closing neural tube (Supplementary Fig. 3a–d). One week later, the neural tube comprised proliferating SOX2+ neuroepithelial progenitors (NEPs; Supplementary Fig. 3e–g) organized into a pseudostratified neuroepithelium (Fig. 3b,c,f,g and Supplementary Fig. 3h,i). OTX2 was also detected in prosencephalic structures and in the cephalic preplacodal epithelium (Fig. 3c,g and Supplementary Fig. 3j). At 3 w, OTX2+ NEPs surrounded the large telencephalic sylvian ventricle (Fig. 3d,h).

PAX6 is the earliest neuroectodermal marker expressed in the developing human central nervous system (CNS), and at later stages it becomes a general pallial marker. Consistently with this, PAX6 was a neocortex-enriched DEG. We detected PAX6 expression in the neural folds of the 2 w + 5 d embryo (Fig. 3i) and, starting at 3 w + 3 d, in the prosencephalon (Fig. 3j), cephalic preplacodes and developing eye structures (Fig. 3k and Supplementary Fig. 3k,l). At 3 w + 4 d, PAX6 was expressed in the entire neuraxis, except in the mesencephalon (Supplementary Fig. 3m–p). At 7 w (Fig. 3i) it was restricted to the pallium, while the subpallium expressed the striatum-enriched DEG product GSX2. At this stage, PAX6 and GSX2 defined the pallial-subpallial boundary (PSB) (Fig. 3i).

As expected, FOXG1 was not a DEG or BEG, as this gene identifies the entire telencephalic anlage. We report its earliest prosencephalic expression at 3 w + 4 d (Fig. 3m–o). At 7 w, FOXG1 expression persisted in the entire telencephalon, particularly in the NEPs of the ventricular zone (VZ) (Fig. 3p).

As rostro-caudal regionalization develops, dorso-ventral patterning is also under way. Nkx2-1 is a key TF involved in ventralization, and NKX2-1 was seen to be a pivotal striatum-enriched DEG and BEG. Through immunofluorescence, we found its earliest expression in the anterior neuroectoderm of the 2 w + 5 d embryo (Fig. 3q). At 3 w + 3 d, its expression was restricted to the most ventral part of the prosencephalon (Fig. 3r,s), suggesting that PAX6 and NKX2-1 had acquired complementary expression domains. At 7 w, NKX2-1 clearly marked the MGE germinative zones (Fig. 3t). Together, these analyses reveal the developmental expression pattern of pivotal TFs in a critical period of early human neurodevelopment.

A core of regional TFs defines early fetal LGE/striatum domains

At the beginning of the fetal stages (8 w), the telencephalon is largely occupied by a sylvian ventricle. The ventral telencephalon has a thick, postmitotic FOXP1+ mantle zone (MZ) (Fig. 4a), where the internal capsule begins to appear. We analyzed TF coexpression pattern to distinguish progenitor, precursor and mature neuron compartments within the human LGE. ASCL1 was a striatum-enriched DEG and ASCL1 was expressed in the VZ and subventricular zone (SVZ) of the LGE, up to the PSB, where TR2 pallial expression began (Fig. 4b,c). Other expression patterns demarcated the PSB, including the mutually exclusive
expression of ASCL1 and PAX6 (Fig. 4d) or of PAX6 and GSX2 (Supplementary Fig. 4a). However, although GSX2 displayed mainly LGE VZ expression (Supplementary Fig. 4b), ASCL1 was expressed in the SVZ and scattered in the FOXP2+ MZ (Fig. 4c–e), with an accumulation at the VZ/SVZ boundary (Supplementary Fig. 4c).

We then confirmed that the dorsal LGE marker, SP8, demarcated a distinct region, adjacent to the GSX2, ASCL1 and PAX6 domains (Supplementary Fig. 4d–g). At 8 w, we found the striatum-enriched DEG and BEG ISL1 expressed in the SVZ and the MZ (Fig. 4f), coexpressed with FOXP1, CTIP2 (also known as BCL11B) and FOXP2 (Fig. 4g,h and Supplementary Fig. 4h). This compartment-restricted TF expression was also demonstrated by the mutually exclusive expression of GSX2 and CTIP2 (Supplementary Fig. 4i).

Medium spiny neurons (MSNs) represent >90% of striatal neurons in rodents and are mainly identified by coexpression of CTIP2 and DARPP-32 (also known as PPP1R1B) (20). At 8 w, some CTIP2+/DARPP-32+ neurons were detected in the ventral MZ (Fig. 4i). We confirmed CTIP2 expression in the cortical plate (CP) in GABA+ cells (Supplementary Fig. 4m–o). We also report here the expression of DARPP-32 in the human fetal CP at 8 w (Supplementary Fig. 4n).

Upon finding that NKX2-1 was a human striatal DEG and BEG, we probed its mRNA distribution in the developing ventral forebrain (Supplementary Fig. 5a–c) and confirmed its expression in both MGE and LGE by western blotting (Supplementary Fig. 5d). Furthermore, we found that NKX2-1 was coexpressed with ISL1 and CTIP2 (Fig. 5). This compartment-restricted TF expression was also demonstrated by the mutually exclusive expression of ASCL1 and PAX6 in the LGE and MZ at 8 w (Supplementary Fig. 5d).

As early as 7 w, GABAergic cells were detected in the MZ of the LGE and in the intermediate zone and subplate of the neocortex (Supplementary Fig. 6d). At 11 w, we found an abundance of GABA+ cells, both in the MZ (scattered in the VZ and SVZ) and in neocortex (Fig. 5). This was consistent with the tangential migration of neocortical GABAergic interneuron recently reported (3,4,6). Notably, some GABAergic cells were also located in the pallial VZ/SVZ (Fig. 5) and Supplementary Fig. 7b). In conclusion, during the early fetal period, the MZ in the LGE enlarged gradually and acquired a code that included ISL1, FOXP1, FOXP2, NKX2-1, EBF1, CTIP2 and DARPP-32 (Fig. 5k).

**TF code maintenance in the early fetal human striatum**

During early fetal human development, the telencephalic suprventricular expands substantially up to 11 w and a massive SVZ overlies the developing FOXP1+ striatum, separated into the caudate putamen by the internal capsule (Fig. 5a). At this stage, the FOXP1+ and OTX2+ LGE VZ is delimited by the underlying SVZ and by the pallial VZ at the PSB, as evidenced by the localized expression of PAX6, ASCL1 and GSX2 (Fig. 5b,c and Supplementary Fig. 7a–c). The 11-w striatum showed wide CTIP2 expression in the MZ (Fig. 5d). DARPP-32 became widely expressed in the striatum, too, particularly in the putamen (Fig. 5e), where it was expressed with CTIP2 (Fig. 5f). At 11 w, the CP clearly remained DARPP-32+/CTIP2+ (Fig. 5g). ISL1 and EBF1 were found in the LGE SVZ and then in the caudate putamen (Fig. 5h and Supplementary Fig. 7d,e), where FOXP1 and FOXP2 were widely distributed (Supplementary Fig. 7f). In the striatum, we still found a clear signal for NKX2-1, which was coexpressed with CTIP2 (Fig. 5i).

**Figure 5** TF expression in striatal domains is maintained at the end of the first trimester. Immunohistochemistry performed on 11-w sections. (a) FOXP1 staining of telencephalic coronal hemisection. (b,c) PAX6 and ASCL1 expression in the neocortex and LGE VZ, respectively, is interrupted at the PSB. (d–g) CTIP2 and DARPP-32 are coexpressed by the MSNs in the MZ and in the CP. (h) ISL1 is expressed in the SVZ and MZ of the LGE. Higher magnification of the boxed area in h shows ISL1 expression in the caudate-putamen. (i) CTIP2+ striatal neurons still express NKX2-1. (j) GABA immunodetection in the LGE striatum and pallial compartments. (k) Expression map at 11 w. LGE, lateral ganglionic eminence; PSB, pallial-subpallial boundary; VZ, ventricular zone; SVZ, subventricular zone; MZ, mantle zone; I.C., internal capsule; Ca, caudate; Pu, putamen; MSNs, medium spiny neurons; IZ, intermediate zone; SP, subplate; CP, cortical plate; CX, neocortex; ST, striatum; Th, thalamus; LV, lateral ventricle. Scale bars, 50 µm (insets: 2.5 µm).
Redefinition of TF patterns in late midfetal stage

During midfetal development, a general increase is observed in the cerebral wall, with large VZ/SVZ regions overlying the FOXP1+ and CTIP2+ striatum, where neurogenesis continues (Fig. 6a–c). At 20 w, we observed dynamic changes in the expression pattern of several TFs. For example, ASCL1 expression continued in the SVZ, but it did not accumulate at the VZ/SVZ boundary (Supplementary Fig. 8a). PAX6 expression spread in the LGE VZ (data not shown), as reported by Ma et al.3. Furthermore, ISL1 expression, previously detected in the SVZ, was restricted to the caudate-putamen in fewer cells than at 8 w (24.9 ± 4.2% at 20 w versus 74.1 ± 2.1% at 8 w, respectively) (Fig. 6d and Supplementary Table 4a,b). Notably, some ISL1+ cells did not coexpress FOXP1, CTIP2 or FOXP2 at this stage (Fig. 6b–d), which suggested that they may have entered an interneuronal fate, as observed in rats, where ISL1 is initially expressed in all striatal precursors and later restricted to cholinergic interneurons23.

FOXP2 underwent the greatest variation in expression. We observed a transition from a wide FOXP2 expression in the CTIP2+ MZ at 8 w (80.4 ± 5.3% FOXP2+ cells) to FOXP2 expression in only 25.2 ± 2.6% of the striatal cells at 20 w (Fig. 6g and Supplementary Table 4a,b). Remarkably, some cells showed high FOXP2 expression and were ISL1−, CTIP2low and EBF1+ (Fig. 6d, Supplementary Fig. 8b,c and Supplementary Table 4b).

At 20 w, NKX2-1 was restricted to a few scattered cells (6.3 ± 2.7%) (Fig. 6e–g and Supplementary Table 4b). Only around 4% of the striatal cells remained CTIP2+/NKX2-1+ (which represents the 56.4 ± 9.3% of total NKX2-1+ cells in the striatum), but two other subsets emerged that were CTIP2+/NKX2-1− and CTIP2−/NKX2-1−, respectively (Fig. 6e–g and Supplementary Table 4b). Moreover, a nearly complete segregation of NKX2-1 and ISL1 expression was now evident (Fig. 6f). We reasoned that the rare NKX2-1+/ISL1+ cells (Fig. 6f) could represent cholinergic interneuronal precursors, as we did not detect choline acetyltransferase at this stage (data not shown).

In the 20-w striatum, CTIP2+ cells generally displayed a GABAergic identity (Fig. 6h) and broad CALB coexpression (Supplementary Fig. 8d and Supplementary Table 4b). A coexpression study demonstrated that the majority of the CTIP2+, FOXP1+ and FOXP2+ neurons also expressed DARPP-32, while only 20% of the NKX2-1+ cells were DARPP-32+ (Fig. 6i, Supplementary Fig. 8e–h and Supplementary Table 4c). Notably, we observed that most ISL1+ neurons were also DARPP-32+ (86.6 ± 1.2%); Fig. 6j and Supplementary Table 4c), in contrast with observations in rats, where no ISL1+ DARPP-32+ neurons are detected23. Moreover, we found IZK1 expression in a subset of caudate-putamen neurons (Supplementary Fig. 8i). Our findings define a core of striatal developmental factors, whose coexpression establish striatum identity during human neurodevelopment (Fig. 6k and http://www.neurostemcellrepair.org/protected/IMG_Comparator.zip).

The striatum is the main synaptic target of dopaminergic inputs from the substantia nigra pars compacta. Tyrosine hydroxylase (TH+) fibers were detected as early as 7 w in the ventral MZ, where the first FOXP1 and DARPP-32 signal appeared (data not shown). At 20 w, we found extensive dopaminergic innervation in the striatum. The majority of CTIP2+ MSNs were in contact with TH+ neurites (Supplementary Fig. 8j). We then investigated the striatal interneuron population, which represents about 5% of total adult striatal neurons24. Striatal interneurons are mainly GABAergic (a small population of cholinergic interneurons is also present), with different histochemical phenotypes: for example, positive for calretinin, parvalbumin, somatostatin (SST), or neuropeptide Y (NPY) and nitric oxide synthase24. Distinct subpopulations of...
neocortical interneurons have been reported to express different TFs, such as SOX6, COUP-TFI and SP8 (ref. 3). While SOX6 and COUP-TFII were expressed in the 20-w striatum, mutually exclusively with CTIP2 (Supplementary Fig. 9a,b), we did not detect SP8 expression in the caudate-putamen (data not shown). We found that SST+ interneurons were COUP-TFII-, NKX2-1-, FOXP2- and CTIP2-, but SOX6+ (Supplementary Fig. 9c–h and Supplementary Table 4d). In contrast, the majority of CR+ interneurons coexpressed NKX2-1 (Supplementary Fig. 9i–m and Supplementary Table 4d). NPY+ interneurons did not express CTIP2 and COUP-TFII, but were SST+ (Supplementary Fig. 9n,o). Higher confocal magnification revealed that some cells had begun to send out processes (Supplementary Fig. 9p), but most interneurons at this stage lacked conspicuous neurites.

As noted above, some typical LGE/striatum determinants showed a dynamic expression pattern that spread into the neocortex. At 20 w, we detected FOXP1 expression in the CP (Fig. 7a–c). FOXP2+ cells were found in the deeper CP (dCP) and subplate (Fig. 7d). NKX2-1+ cells were present in the CP of the dorsolateral frontal cortex (Fig. 7e), as previously reported. In the CP, we also observed some CALB+ and CR+ interneurons (Supplementary Fig. 10a,b).

As neocortical CTIP2+ cells in the upper CP were found not to be GABAergic at this developmental period (Fig. 7f), we aimed to identify them by detecting the coexpression of CTIP2 and layer-specific neocortical markers (Supplementary Fig. 10c). CTIP2 did not colocalize with SATB2 or TBR1, and DARPP-32 was expressed in the dCP and subplate (Fig. 7g–i). Double immunolabeling revealed broad coexpression of DARPP-32 and FOXP2 (Fig. 7j). We detected some DARPP-32+/FOXPI+ and rare CTIP2+/DARPP-32+ neocortical neurons (Fig. 7k and Supplementary Fig. 10d,e). However, DARPP-32/SATB2 coexpression was never observed (Fig. 7l). Furthermore, unlike MSNs, neocortical DARPP-32+ neurons were not GABAergic (Fig. 7m). Taken together, these findings define the expression pattern of a core of cell-fate determinants in the 20-w neocortex and their dynamic changes over time (Fig. 7n and Supplementary Fig. 10f–k).

Functional properties of human striatal and neocortical primary neurons

We next profiled the functional properties of the developing striatum and neocortex by studying human primary neuron (hPN) electrophysiology (Supplementary Table 1e). We differentiated striatal and neocortical hPNs up to 30 days in vitro (DIV) to obtain mature MSNs and glutamatergic pyramidal neurons, respectively (Supplementary Fig. 11a–e) and performed whole-cell patch-clamping at 3–5, 14–20 and 20–30 DIV. We then postlabeled the recorded neurons with biocytin and examined them for DARPP-32 or VGLUT1 expression to confirm their identity (Supplementary Fig. 11f–i).

After 20 DIV, a large percentage of striatal hPNs displayed either single action potentials (APs) or repetitive firing (Fig. 8a and Online Methods), as expected from mature neurons. A similar pattern of activity was detected in neocortical hPNs (Supplementary Fig. 11j and Online Methods). The ability to elicit APs in hPNs was correlated with the activation of sizeable tetrodotoxin-sensitive Na+, and delayed-rectifier K+ voltage-gated currents (Fig. 8b,c and Supplementary Fig. 11k). The shape of the mean neocortical Na+ current density/voltage relationship (Supplementary Fig. 11l) largely overlapped with that obtained from striatal hPNs (Fig. 8d). Individual striatal hPNs also exhibited coexisting high- (HVA) and low-voltage-activated (LVA) Ca2+ channels (Fig. 8e,f). By comparing the current/voltage relationships of striatal and neocortical neurons, we found a significant
difference in the mean current density amplitude (P < 0.046, unpaired t-test), suggesting that HVA channels were more abundant in the striatal hPNs (Fig. 8f, Supplementary Fig. 11m and Online Methods). Functional HVA Ca$$^{2+}$$ channel current is critical for neurotransmitter release, but LVA Ca$$^{2+}$$ channel current might underlie activation of Ca$$^{2+}$$ spikes in these cells (Fig. 8a).

Striatal hPNs exhibited K$$^+$$ outward currents with both fast and slow inactivating components (panel 2, Supplementary Fig. 11n), delayed-rectifier K$$^+$$ currents (panel 3, Supplementary Fig. 11n) and fully inactivating A-type K$$^+$$ currents (K$$\text{A}_\text{channel}) (Fig. 8g). The average current density/voltage relationship for K$$\text{A}$$_\text{channel}$ in striatal hPNs (Fig. 8h) displayed an activation threshold and amplitude similar to those obtained in neocortical hPNs (Supplementary Fig. 11o). The K$$\text{A}_\text{channel}$ plays a crucial role in regulating the firing pattern and the initial latency of APs. The selective K$$\text{A}_\text{channel}$ blocker 4-aminopyridine (4AP) reversibly suppressed the K$$\text{A}_\text{channel}$ current (Fig. 8i, top panel) and reduced the latency between the current stimulus and the first AP peak (Fig. 8i, bottom traces and Supplementary Fig. 11p).

Next we verified whether striatal hPNs expressed functional ligand-gated channels. We found that 19 of 20 striatal hPNs responded to GABA application (mean: −996 ± 165 pA) and 5 of 6 responded to coapplication of quinpirole, a selective D2-receptor agonist, which significantly (P < 0.037, paired t-test) and reversibly reduced the peak of the GABA$\text{A}_\text{channel}$-evoked current (49 ± 12%; Fig. 8j). This inhibition was expected for a mature MSN with functional dopaminergic receptors. At 30 DIV, 6 of 9 striatal hPNs exhibited inward currents in response to glutamate (mean: 79 ± 43 pA) (Fig. 8k). Furthermore, glutamate depolarization exceeded the threshold for generating
a classical AP (Fig. 8k). Sizeable GABA_A currents were evoked in 5 of 5 neocortical hPNs (mean: −1,067 ± 431 pA; Supplementary Fig. 11q). A glutamatergic response to AMPA was also recorded in 9 of 14 neocortical hPNs (mean, 50 ± 7 pA) (Supplementary Fig. 11r). AMPA depolarized the neurons above the AP threshold and generated trains of APs (Supplementary Fig. 11r). Finally, spontaneously GABAergic post-synaptic currents (sPSCs) were recorded in all tested striatal hPNs (4 of 4; Fig. 8l), suggesting that these cells were connected with axonal projections from neighboring neurons and formed GABAergic synapses.

By culturing GFP-labeled striatal cells with tdTomato-labeled neocortical cells, we rebuilt and analyzed, in vitro, a cortico-striatal circuit model. Under these conditions, we recorded glutamatergic sPSCs from 2 of 6 striatal hPNs (Fig. 8l). Likewise, in 9 of 64 (14%) of neocortical hPNs in monoculture, we detected glutamatergic sPSCs (Supplementary Fig. 11s).

We asked whether the electrophysiological hallmarks found in hPNs could be reflected by a transcriptional enrichment in voltage-gated ion channel subunits and receptor-associated molecules. To this end, we measured transcriptomes for three striatal and three neocortical hPN samples. To highlight function-associated traits in hPNs that were not yet present in the original, not fully differentiated tissue of origin, we performed DEG analysis between 30-DIV striatal hPNs and 8-w striatal brain samples (hPNs were derived from 8-w tissues; thus, 8 w was considered DIV 0) (Supplementary Table 1b). The same approach was used for neocortical hPNs. We identified 186 DEGs that clearly discriminated between striatal tissue and striatal hPNs; similarly, 1,018 DEGs discriminated between neocortical tissue and neocortical hPNs (Supplementary Fig. 12a,b and Supplementary Table 5a,b).

GO enrichment analysis on striatal hPN DEGs revealed terms such as synaptic vesicle coating, neurotransmitter secretion and synaptic transmission (Supplementary Table 5c), and SYN2 and NCAM2 were significantly enriched (Supplementary Fig. 12c). Also, consistent with these cells’ GABAergic identity and electrophysiological response, we found enrichments of GABARAPL2 (Supplementary Fig. 12c). A plethora of neuronal function-related GO classes were also found for neocortical hPNs (Supplementary Table 5d). In agreement with the neocortical hPN identity, we found enrichments in glutamate- and GABA-receptor-encoding genes (Supplementary Fig. 12c). Our analyses also revealed enrichments in electrophysiology-related molecules such as SCN2A, consistent with the presence of inward Na^+ currents in neocortical hPNs (Supplementary Fig. 12c). Together, these results define the functional signature of striatal hPNs (Supplementary Fig. 11t) and provide a detailed electrophysiological description of neocortical hPNs.

**DISCUSSION**

As yet, we lack a detailed understanding of the molecular basis of CNS development, an understanding which may in turn also speak to diseases unique to the human brain. Here we have attempted to fill this gap by cross-analyzing multiple aspects of the early developing human striatum and neocortex, including global gene expression, functional transcriptome organization, spatiotemporal dynamics of instrumental developmental factors and electrophysiological descriptions of hPNs.

**Transcriptional landscape of developing human striatum and neocortex**

Previous studies have described global transcription in the developing human brain by analyzing brain samples from early fetal stages to adulthood$^{5,8,10,27,28}$. However, no study has specifically assessed the transcriptional architecture of the developing striatum and neocortex in the embryonic and early fetal life (6–11 w) that is pivotal for human CNS formation.

We have shown that gene expression clearly distinguishes the developing human striatum from the neocortex. DEGs and BEGs provided both well described (ASCL1, DLXs, LHXs, GSX2) and unexpected (NKX2-1, ZFHX3) striatum-enriched genes. Our gene expression data were corroborated by the Allen Brain Atlas; for example, clear NKX2-1 expression was seen in the two 8- to 9-w LGE samples present in the data set and in the late fetal and adult striatum.

Our investigation of the early period, 6–11 w, has added to the report from Kang et al.$^8$, in which did not identify some of the pivotal DEGs, including GSX2, ASCL1, DLX1 and LMO3, as differentially expressed in the striatum, highlighting the need for a larger temporal window for studies on human brain development. In addition, we found striatum-enriched noncoding RNAs, such as DLX6AS and LINC00403, whose expression is confirmed by the Allen Brain Atlas.

**Molecular map of TFs in human headfold to midfetal period**

We show here a set of TFs that is expressed by early neuroectoderm as it acquires ventral or dorsal telencephalic fates. In this regard, early embryonic samples (2–6 w) were particularly informative because of their extreme rarity and because they were not included in the transcriptional analysis. Our survey started with the description of the expression profile of OTX2, PAX6 and NKX2-1 in the closing neural folds at 2 w + 5 d. Notably, our first description of NKX2-1 expression at headfold stage was consistent with its expression in mouse anterior neural plate$^{31}$. Five days later, we demonstrated the beginning of the dorso-ventral patterning by the complementary expression of PAX6 and NKX2-1 in the prosencephalon. Conversely, FOXG1 immunoreactivity started from 3 w + 4 d and represents the first molecular sign of telencephalic determination.

Second, we analyzed the coexpression of 22 striatal determinants to distinguish LGE domains and delineate the spatiotemporal expression map at the protein level. The proliferating LGE VZ progenitors expressed OTX2, FOXG1, GSX2 and ASCL1, while the SVZ precursors expressed ASCL1, ISL1 and EBF1. Finally, at 8–11 w, striatal neurons were defined by the coexpression of ISL1, FOXP1, FOXP2, CTIP2, EBF1, GABA, DARPP-32 and NKX2-1. In the late midfetal stage, anatomical changes were accompanied by modifications in the expression pattern of many TFs. ISL1 was expressed only in the caudate-putamen and FOXP2 expression was drastically restricted. This analysis provided the exact positions of striatum-enriched gene products in a precise map, which differs in notable aspects from that which was previously accepted. For instance, in mouse, Nkx2-2 is strongly expressed in the MGE, preoptic area and part of the septum, but not in the LGE$^{14}$, and in humans NKX2-2 was classified as an MGE-origin cell marker$^{3,4,32,33}$. Thus, it was surprising to find expression NKX2-2 in the human LGE, thus highlighting that mouse development is not entirely predictive of that in the human CNS. The identity of NKX2-2* striatal cells has not to our knowledge been investigated before. We show here coexpression of NKX2-1 with the striatal markers.
ISL1 and CTIP2. Furthermore, despite the decreased number of ISL1+ striatal cells at 20 w, many retained DARPP-32 expression, in contrast with observations in rats.

Even though DARPP-32 expression in layer V1 was described in adult rats, we found a marked coexpression of CTIP2 and DARPP-32 in the human CP at 8–11 w. This observation should be considered when monitoring in vitro the differentiation of human pluripotent stem cells toward MSNs. Collectively, these extensive coexpression studies in striatum and neocortex define the MSN signature. Our work systematically defines the prosencephalic ontogeny, from the appearance of the CNS primordium to the midfetal stage.

**Molecular and functional signature of striatal and neocortical hPNS**

Preserved, fixed tissues cannot reveal the distinctive traits of dynamic neuronal behavior. Thus, we performed a detailed electrophysiological analysis of hPNS. We defined a number of hallmarks, which included repetitive firing; Na+, K+ and Ca2+ currents; neurotransmitter responses; and spontaneous synaptic activity. In the case of striatal neurons, we described a remarkable response to dopamine neuromodulation and the appearance of functional glutamatergic synapses in the presence of cocultured neocortical hPNS. We then linked electrophysiological properties to the transcriptional enrichment of relevant voltage-gated ion channel subunits and receptor-associated molecules, such as K+ and Na+ channel family members, and also GABA and glutamate receptors. Our results on hPNS supported the limited data available from recordings on developing human neocortical neurons in slice cultures and from transcriptional profiling of human neural cultures.

Striatal hPNS show a MSN phenotype and share many functional properties with those observed in MSNs of different animal species. Furthermore, according to the Allen Brain Atlas, the expression of specific physiology-relevant genes (for example, SCN2A, KCNJ12, KCND2 and DRD2) increases from 8 to 12 w, which correlates well with specific ionic and synaptic conductances and dopaminergic modulation. We found that these neurons showed a quite depolarized resting membrane potential (mean = –47.5 ± 4.3 mV, n = 15), as usually found in cultured human neurons, in contrast with the expected value for a fully differentiated MSN in vivo (near ~90 mV). These results could be explained by a low expression level of the K+ inward rectifier channel (as in the striatum at 8–12 w, according to the Allen Brain Atlas) and by the fact that we did not record any K+ inward-rectifier current (n = 7, data not shown). So far, culture conditions do not reflect the in vivo milieu and improvements are needed. Successfully generating authentic human forebrain neurons from cultured stem cells is based on exploiting developmental mechanisms identified in vivo. We anticipate that our study of hPNS may provide a valuable reference for advancing stem cell research.

To the best of our knowledge, this is the first attempt to molecularly define human developing striatal anatomy. We provide a comprehensive transcriptomic analysis of the early human striatum in comparison with neocortex. This strategy, combined with protein expression study from the earliest moments of CNS formation, constitutes a valuable approach for deciphering the dynamics of striatum determination. The emerging functional properties measured in ex vivo striatal and neocortical cultures provide a new perspective on the physiological properties of human neurons. These findings advance our basic understanding of human brain development and provide a constellation of genes, functional transcriptome data, TF maps and electrophysiological parameters for future studies. These data may also promote studies on the genetic and neurodevelopmental basis of human neurological disorders.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Accession codes.** The MIAME-compliant gene expression data have been deposited in the Array Express database under accession code E-MTAB-1918.

**Note:** Any Supplementary Information and Source Data files are available in the online version of the paper.

**ACKNOWLEDGMENTS**

We thank N. Sestan and S. Piccolo for critical reading of the first version of the manuscript. We also thank C. Laterza for assistance with Illumina chip hybridization, M. Caiazzo for micrograph acquisition assistance, V. Broccoli for providing tdTomato and GFP lentiviral constructs, M. Binetti for help in sample collection, M. Molinari for the interactive striatum map, and M. Ascagni, from the Centro Interdipartimentale Microscopia Avanzata of the Department of Bioscience, University of Milan, for assistance in confocal imaging. We also thank the families of our Huntington’s disease patients for their support. This study received funding from NeuroStemcell (EU Seventh Framework Programme, grant agreement no. 222943), from Cure Huntington’s Disease Initiative (CHDI, U.S.A., ID: A-4529), from the Ministero dell’Istruzione dell’Università e della Ricerca (MIUR 2010-MMELY_001, Italy), to E. Cattaneo; from NeuroStemcellRepair (European Union Seventh Framework Programme, grant agreement no. 602278) to E. Cattaneo and R.A.B.; from Fond per gli Investimenti della Ricerca di Base (FIRB, RBFR10A01S, Italy) to M.O.; and partially from TargetBrain (EU Framework 7 project HEALTH-F2-2012-279017) to G.M. D.B. was supported by a Marie Curie Fellowship (TransSVIR FP7-PEOPLE-ITN-2008 #238756, EU). We acknowledge the contribution of Tavola Valdese (2010–2013) and support from UniCredit Banca S.p.A. (2010–2011, Italy).

**AUTHOR CONTRIBUTIONS**

D.B., E. Cesana and R.M. contributed equally to this work. M.O., V.C. and E. Cattaneo designed the research program and wrote the manuscript. M.O. and V.C. performed the experiments that comprise the main body of this work. D.B., R.M., C.F., G.M. and P.A.L. assisted in transcriptional study design and acquired and interpreted transcriptional data. E. Cesana, F.T., M.T. and G.B. did the electrophysiological analysis and acquired and interpreted experimental data. R.V., R.L.G., R.A.B. and G.P.B. provided and processed the human specimens and helped in their staging. L.M. performed in situ hybridization experiments. All authors contributed to the revision of the manuscript up to its final form. E. Cattaneo provided guidance and conceptual support and approved the final version of the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.


ONLINE METHODS

Human tissue. Human fetal tissues were collected from patients that requested pregnancy terminations and autopsy diagnostic procedures. All procedures were approved by the local research ethics committees and Research Services division of the University of Cambridge and Addenbrooke’s Hospital in Cambridge and by the Ethics Committee of San Paolo Hospital, Milan. The ethics were fully reviewed and approved in the UK, in accordance with the Polkinghorne Report and Department of Health guidelines. Both documents were submitted to the Ethics Committee of the University of Milano, and ethics approval was obtained.

Definition of human developmental periods. Human development is a highly dynamic process. The developmental staging of human nervous system was previously described8,9,34–36, and our study aimed to elaborate on previous findings. We used post-conception weeks (w) because this is the proper term, as based on current literature, to indicate the developmental age of the human embryo (also termed post-fertilization or post-ovulatory age). According to Kang et al.8, we used the following developmental periods to describe the samples employed in this study:

Embryonic development, 2 w to 8 w
Early fetal development, 8 w to 13 w
Early midfetal development, 13 w to 19 w
Late midfetal development, 19 w to 24 w

Tissue collection. This study was conducted with postmortem human brain specimens from tissues collected at the John van Geest Centre for Brain Repair, University of Cambridge, Cambridge, UK. Additional specimens were procured from the San Paolo Hospital, Milan, IT. Specimens ranged in age from 2 w + 5 d to 20 w. Embryonic and fetal age was extrapolated based on the date of the mother’s last menstruation, crown to rump length (CRL) and visual inspection.

Depending on the condition and period of the procured specimens, three different dissection methods were used. Supplementary Table 1a provides a complete list of all tissue samples included in this study. Both left and right brain regions of interest were collected from fresh tissues.

For each experiment, the sample size was determined by tissue availability in the collection. Each human brain represents a unique sample information resource, because it is area- and stage-specific.

Brain sampling for RNA extraction. Brains were chilled on ice during dissection and placed onto a chilled plate on ice. The brainstem and cerebellum were removed from the cerebral by making a transverse cut at the junction between the diencephalon and midbrain. Next the cerebrum was divided into left and right hemispheres by cutting along the midline. The LGE/striatum and the neocortex were dissected with a scalpel blade. In particular, the LGE at early stage was dissected following the lateral border of the LGE and a line following the putative MGE/LGE sulcus ventrally into the striatum, resulting in a curved ‘finger’ of tissue. The dissected samples were placed in RLT buffer (Qiagen) and immediately frozen in dry ice. Dissected samples were stored at −80 °C for later RNA extraction.

Brain sampling for immunostaining. Specimens were chilled on ice during dissection and placed onto a chilled plate on ice. For samples between 2 w and 4 w, the whole embryo was processed. For the samples older than 4 w, the entire brain (when possible) was removed and fixed for 2–4 d in 4% formaldehyde (in PBS) at 4 °C. Samples were then cryoprotected in 30% sucrose (in PBS), embedded in Tissue-Tek, stored at −80 °C and then cryosectioned (12–30 μm).

Brain sampling for primary neuronal cultures. Brains were chilled and dissected as described for RNA extraction. The LGE/striatum and the neocortex were dissected with a scalpel blade, immediately placed in prechilled Hibernate-E (Life Technologies) and stored at 4 °C for further shipment and processing.

RNA isolation and cRNA synthesis. Total RNA was extracted from LGE/striatal and neocortical human fetal tissues or from 30-DIV differentiated striatal and neocortical hPNs (Supplementary Table 1b) with an RNaseq kit (Qiagen), according to the manufacturer’s instructions. Optical density values of extracted RNA were measured with NanoDrop (Thermo Scientific) to confirm an A260/A280 ratio ≥ 1.9. 500 ng of total RNA from each sample were used for cRNA preparations.

cRNA was prepared on the Illumina TotalPrep RNA Amplification Kit (Ambion); 750 ng of labeled cRNAs were hybridized on Illumina HumanHT-12 v4 arrays (Illumina).

Microarray analysis and differential gene expression. The raw transcriptome data from Illumina HumanHT-12 v4 arrays were cubic-spline normalized and exported with GenomeStudio GX software (Illumina Inc.). Detection was considered positive for probes with detection P values < 0.05 in at least 5 out of 26 samples (12 paired striatal and neocortical samples and two single striatal samples). No outlier samples were found in principal component analyses and hierarchical sample cluster analysis. To identify DEGs among 12 pairs of striatal and neocortical samples, a paired t-test was performed with the MeV package46. We used criteria of an FDR (false discovery rate)-corrected P value < 0.05 and fold-change threshold of 1.7. A hierarchical clustering analysis (Pearson’s correlation) on the resultant probes revealed two distinct sample clusters, in striatal and neocortical samples. However, we also found a separate, mixed cluster containing four samples (one striatum and neocortex pair, one unpaired neocortex and one unpaired striatum sample) (Supplementary Table 1b). Removal of the three relative sample pairs that contributed to the mixed cluster increased the DEGs discovery rate by nearly 30%; this procedure resulted in 1,306 DEGs.

Three striatal and three neocortical hPN samples were then measured and added to the tissue data set. After filtering, 28,700 probes were considered detected (detection P value < 0.05 in at least three samples). Differential expression analysis was performed between in vitro differentiated striatal or neocortical hPNs and the five related 8-w striatal or neocortical tissue samples, respectively. This analysis used an unpaired t-test with the MeV package46. The criteria were an FDR-corrected P value < 0.05 and fold-change threshold of 1.7. Gene ontology and pathway enrichment analyses were conducted with the MetaCore tool (Thomson Reuters Corporation) with the criteria of the FDR and corrected P value < 0.05.

Bimodally expressed genes. Bimodally expressed genes (BEGs) were identified by calculating a bimodality score for each expressed gene in the data set. Let G be the P50 gene in the data set and let $E_G = \{ G_1, G_2, \ldots, G_n \}$ the set of expression values for the gene G, measured in n different samples. The bimodality score for the gene G was calculated by performing univariate clustering on the set of expression values $E_G$ using the Mclust function in the R package mclust47 and iterating over multiple bootstrap iterations with the boot function in the R package boot48.

Weighted gene coexpression network analysis (WGCNA). WGCNA was performed on the entire data set (22 samples) using the WGCNA R package49. The similarity matrix was computed using Pearson’s correlation coefficient (function cov). The adjacency matrix was calculated as adjacency = ((similarity + 1) × 0.5) with $\theta = 8$. The choice of $\theta$ was made according to the scale-free topology criterion15. The topological overlap matrix (TOM) was calculated using the adjacency matrix as input for the TOMsimilarity in the WGCNA R package. Modules were identified using the cutreeDynamic function (minClusterSize = 10, cutHeight = 0.925), and similar modules were merged using the function mergeCloseModules (cutHeight = 0.25). For each module, GO enrichment analysis was performed using GOrilla tool50 (last update 19 July 2014). The background list for the enrichment analysis included all genes represented on the Illumina Human HT-12 v4 array, with a detection P value < 0.05 in at least three samples. GO data are accessible at http://people.dcs.cam.ac.uk/db544/onorati2014.html. Network representations of the modules were obtained using the R package igraph51.

Immunofluorescence. Cells were fixed in 4% formaldehyde, permeabilized with 0.5% Triton X-100 and blocked with 5% PBS for 1 h at room temperature. Brain sections were rehydrated for 10 min with PBS, then permeabilized with 0.5% Triton X-100 and blocked with 5% PBS for 1 h at room temperature. Cells or brain sections were incubated overnight at 4 °C with primary antibodies (α-tubulin, 1:10,000, T9026, Sigma; ASCI1, 1:500, 556604, BD; β-catenin, 1:500, 9652, Cell Signaling; β3-tubulin, 1:1,000, T8660, Promega; β2-tubulin, 1:1,000, PRB-435P, Covance; calbindin, 1:300, CB-038, Swant; calretinin, 1:300, 610908, BD; GABA, 1:550, A2052, Sigma; GAD65/67, 1:300, AB1511, Millipore; COUPTF-II, 1:300, PP-H7147-00, Perseus Proteomics; CTIP2, 1:550, ab18465, Abcam; DARPP-32, 1:200, ab40801, Abcam; DARPP-32, 1:100, SC-135877, Santa Cruz; EBF1, 1:500, SC-137065, Santa Cruz; FOXG1, 1:1,000, NCEFB, StemCulture; FOXP1, 1:1,000, ab16645, Abcam; FOXP2, 1:2,000, ab16046, Abcam; GABA, 1:550, A2052, Sigma; GAD65/67, 1:300, AB1511, Millipore; α-tubulin, 1:10,000, T9026, Sigma; ASCI1, 1:500, 556604, BD; β-catenin, 9652, Cell Signaling; β3-tubulin, 1:1,000, T8660, Promega; β2-tubulin, 1:1,000, PRB-435P, Covance; calbindin, 1:300, CB-038, Swant; calretinin, 1:300, 610908, BD; COUPTF-II, 1:1,000, PP-H7147-00, Perseus Proteomics; CTIP2, 1:550, ab18465, Abcam; DARPP-32, 1:200, ab40801, Abcam; DARPP-32, 1:100, SC-135877, Santa Cruz; EBF1, 1:500, SC-137065, Santa Cruz; FOXG1, 1:1,000, NCEFB, StemCulture; FOXP1, 1:1,000, ab16645, Abcam; FOXP2, 1:2,000, ab16046, Abcam; GABA, 1:550, A2052, Sigma; GAD65/67, 1:300, AB1511, Millipore;
Striatal or neocortical tissues in hPNs were transduced with PBS to obtain a single-cell suspension. Differentiation of striatal and neocortical sections were developed in a dark room, counterstained with Hoechst 33342 and applied in a dark room, according to manufacturer's instructions. After 1 week, rinsed in 5× SSC for 5 min then washed in formamide 50% SSC 2X for 30 min at (pH 8) for 5 min, then 400 before receiving H

...and then processed with Adobe Photoshop. Confocal images were acquired with a Leica TCS SP2 microscope (Leica Microsystems) and then processed with Adobe Photoshop.

Cell counting. Brains from 8-w and 20-w samples were selected for quantification (Supplementary Table 4). For quantification of CTP2, ISL1, FOXP1, FOXP2, NKX2-1, CB and DARPP-32 at least three coronal sections through the intermediate brain were selected. For quantification of striatal interneurons (Supplementary Table 4) sections were taken by confocal microscope. We then counted interneurons that expressed transcription factors both in the caudate and putamen. The data are presented as the average ± s.d.

Western blot analysis. LGE and MGE tissues were lysed by adding 300 μl of RIPA buffer (TRIS-HCL, pH 8, 80 mM, NaCl 150 mM, SDS 0.1%, NP40 1%) supplemented with PMSF (1:250; Sigma) and protease inhibitors (1:100; Sigma). After centrifugation at 10,000g for 5 min, the supernatant was collected and the protein concentration was determined by BCA assay (Thermo Scientific). Proteins were separated by SDS-PAGE and then transferred to a nitrocellulose membrane using a Trans-Blot Turbo System (Bio-Rad). The membranes were next probed with primary antibodies, followed by washing and probing with horseradish peroxi-dase-conjugated secondary antibodies (1:3,000; Bio-Rad). Bands were visualized with enhanced chemiluminescence (Pierce) and imaged with the ChemiDoc MP Imaging System (Bio-Rad).

In situ hybridization. In situ hybridization experiments were performed on 15- to 25-μm-thick brain cryosections upon post fixation in 4% formaldehyde. Sections were then washed three times in PBS and incubated in 0.5 mg/mL of proteinase K in 100 mM Tris-HCl (pH 8) and 50 mM EDTA for 10 min at 30 °C and then 15 min in 4% formaldehyde. Slices were washed three times in PBS before receiving H2O washes. Sections were incubated in triethanolamine 0.1 M (pH 8) for 5 min, then 400 μL of acetic anhydride was added twice for 5 min each. Finally, sections were rinsed in H2O for 2 min and air-dried. Hybridization was performed overnight at 60 °C with 33P riboprobes at concentrations ranging from 106 to 107 counts per minute per slide. The following day, sections were rinsed in 5× SSC for 5 min then washed in formamide 50% SSC 2X for 30 min at 60 °C, before receiving RNase-A (Roche) treatment (20 mg/mL in 0.5M NaCl, 10 mM Tris-HCl pH 8, and 5 mM EDTA 30 min at 37 °C). Sections were further washed in 50% formamide, 2× SSC for 30 min at 60 °C and then rinsed 2 times in 2× SSC. Slides were dried by using ethanol series. NTB (Kodak) emulsion was applied in a dark room, according to manufacturer's instructions. After 1 week, sections were developed in a dark room, counterstained with Hoechst 33342 and mounted with DPX (BDH) mounting solution. The NKz1-j probe corresponds to the entire coding region of the gene, kindly provided by A. Joyner ( Sloan-Kettering Institute).

Cell culture and neuronal differentiation. Striatal or neocortical tissues in Hibernate-E were plated within 48 h of termination of pregnancy. The tissue samples were mechanically separated into small pieces and gently triturated in cold PBS to obtain a single-cell suspension. Differentiation of striatal and neocortical precursors was performed in monolayer conditions by plating onto poly-D-lysine (10μg/ml)/laminin (3μg/ml) coated wells at a density of 130,000 cells/cm² and 75,000 cells/cm², respectively. The medium was as follows: DMEM/F12 (Gibco) supplemented with N2+1:100 (Gibco), B27+1:50 (Gibco), BDNF (30 ng/ml, R&D Systems), 1% penicillin/streptomycin (Euroclone) and 1% GlutaMAX (Gibco). For neocortical differentiation, we used B27 minus vitamin A (Gibco). For a better recovery, FGF 2 (10 ng/ml) was added during initial plating. Medium was partially replaced every 3–4 d. Cells were differentiated for 3 to 32 DIV, then used for further analysis.

Lentiviral transduction for coculture system. hPNs were transduced with AAVS1-hPGK-GFP-pA donor or AAVS1-hPGK-tdTomato-pA lentiviral expression constructs containing the human phosphoglycerate kinase 1 (PGK1) promoter. Lentivirus production was obtained by packaging lentiviral particles in 293T cells. Two days after transfection, the supernatant was filtered and then subjected to ultracentrifugation (~70,000g, swinging bucket rotor, 2 h, 4 °C). The viral pellet was resuspended in PBS (2 h at 4 °C), and aliquots of virus were stored at −80 °C.

To assess the cortico-striatal coculture system, freshly dissociated striatal cells were infected with the lentiviral GFP construct for 3 h at 37 °C in hiberna-tE; neocortical cells were infected with the lentiviral tdTomato construct. The cells were washed twice in PBS for 3 min in 200g, mixed 1:1 and then plated for neuronal differentiation.

Electrophysiological recordings. Cells were visualized with an inverted microscope (Eclipse TE200, Nikon) as previously described. Recordings were performed in the whole-cell patch-clamp configuration at room temperature, both in voltage- and current-clamp modes, with an Axopatch 200B amplifier (Axon Instruments) and a Digidata 1322A AD/DA converter (Axon Instruments). Signals were low-pass filtered at 10 kHz and acquired at 10–50 kHz with Clampex software (Molecular Devices). Fast capacitive transients were reduced online with analog circuitry, and residual capacitive and leak currents were removed by P4 subtraction. To appreciate the morphology, cells were filled with biocytin (3 mg/ml) and processed with immunofluorescence labeling after recording. Intracellular and extracellular saline solutions were different, depending on the type of experiments. To record families of currents and to perform experiments in current-clamp mode, the extracellular solution contained the following (in mM): NaCl (140), MgCl2 (1), CaCl2 (2), KCl (3), glucose (10), HEKES (10) at pH 7.4 with NaOH. Pipettes were filled with the following solution (in mM): potassium glutonate (130), NaCl (4), MgCl2 (2), EGTA (1), HEKES (10), CP (5), Na2-ATP (2), Na2-GTP (0.3) at pH 7.3 with KOH.

Alternately, to record GABA-evoked currents, the intracellular solution was substituted with the following solution (in mM): CaCl2 (135), NaCl (3), MgCl2 (1), EGTA (10), HEKES (10), CaCl2 (0.5), Na2-ATP (4), Na2-GTP (0.3), lidocaine-t-ethylamide (5) at pH 7.2 with CsOH. To isolate sodium currents pipettes were filled with the following solution (in mM): CsCl (120), NaCl (10), tetraethylammonium chloride (20), EGTA (10), HEKES (10), MgCl2 (2) at pH 7.3 with CsOH and the following extracellular solution was used (in mM): NaCl (140), KCl (3), HEKES (10), tetraethylammonium chloride (10), 4AP (5), MgCl2 (1), CaCl2 (1) at pH 7.4 with NaOH. To isolate potassium currents the following extracellular solution was used (in mM): NaCl (155), KCl (3), HEKES (10), MgCl2 (1), CaCl2 (1), tetrodotoxin (0.001) at pH 7.35 with NaOH. Pipettes were filled with the following solution (in mM): KCl (128), NaCl (10) MgCl2 (2), CaCl2 (1), EGTA (11), HEKES (10), Mg-ATP (4) at pH 7.25 with KOH.

To isolate calcium currents, the following extracellular solution was used (in mM): NaCl (130), HEKES (10), 4-AP (1), TEA chloride (10), BaCl2 (10), MgCl2 (2), tetrodotoxin (0.001), glucose (10) at pH 7.4 with NaOH. Pipettes were filled with the following solution (in mM): CaCl2 (120), tetraethylammonium chloride (20), EGTA (10), HEKES (10), MgCl2 (2), Mg-ATP (4), pH 7.4 with CsOH. The liquid junction potential between the bath and the pipette solution was measured and subtracted from the membrane potential values. The subtracted values are reported throughout the text. Pipette series resistance was constantly monitored during experiments.

In the voltage-clamp mode, passive membrane properties were measured immediately after the break-in. The input resistance (Rm) was calculated from the last part of the current trace (mean of the last 5 ms) at the end of a 180-ms-long voltage step from −70 mV to −80 mV. With the same protocol, the membrane capacitance (Cm) was measured by subtracting the time integral of

NATURE NEUROSCIENCE

doi:10.1038/nn.3860
the steady-state current from the time integral of total current developing during
the same voltage step and dividing by the voltage step (10 mV).

sPSCs were analyzed with Clampfit (Molecular Devices). For detection, cur-
rent traces were baseline subtracted. The peak of a candidate sPSC was defined
as the mean current over 300 µs around a local minimum. A sPSC was
recognized when this peak was twice the s.d. of the baseline current noise.

Quinpirole, SCH23390 and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]iso-
quinoline-7-sulfonamide disodium salt (NBQX) were from Tocris Bioscience;
tetrodotoxin was from Alomone Labs; all other chemicals were from Sigma. Stock
solutions were prepared in water and stored at −20 °C; during the experiment,
 aliquots were diluted in extracellular saline solution and locally applied with a
multibarrel system, positioned near the recorded cell soma.

Data are reported as mean ± s.e.m. For statistical comparisons, unpaired het-
eroscedastic t-test (two sided) was performed using Microcal Origin software.

Functional benchmarking details. Passive membrane properties of striatal hPNs
assessed during the differentiation. We measured the membrane capacitance (\(C_m\)),
the input resistance (\(R_{in}\)) and the resting membrane potential (\(V_r\)) at the three
time points (DIV 3–5, 13–20 and >20). We found for striatal hPNs: \(C_m\): 26.1 ±
12.4, 29.5 ± 3.4, 27.9 ± 2.7 pF; \(R_{in}\): 1.8 ± 0.5, 1.38 ± 0.1, 1.3 ± 0.1 GΩ; \(n = 4, 41, 59\)
cells, respectively; \(V_r\): −53.1 ± 17.5, −42 ± 1.8, −47.5 ± 4.3 mV; \(n = 2, 11, 15\) cells,
respectively; at the three time points. This analysis was repeated on neocortical
hPNs: \(C_m\): 20.5 ± 2.3, 27.7 ± 2.6, 36.3 ± 2.7 pF; \(R_{in}\): 1.4 ± 0.2, 0.9 ± 0.1, 0.9 ± 0.1
GΩ; \(n = 9, 39, 45\) cells; \(V_r\): −41.1 ± 3.6, −43.4 ± 2.4, −46.3 ± 2.8; \(n = 7, 26, 18\) cells,
respectively; at the three time points.

Firing properties for the hPNs. At 13–20 DIV, 40% of striatal hPNs (6/15) dis-
played abortive APs, 27% (4/15) a single AP and 27% (4/15) repetitive firing. 70% of
neocortical hPNs (21/30) were able to generate a single spike, 16.7% repetitive
firing (5/30) and only 10% abortive APs (3/30). After 20 DIV 36% of striatal hPNs
(9/25) and 81% of neocortical hPNs (22/27) generated single APs. 12% (3/25) of
striatal hPNs and 14.8% (4/27) of neocortical hPNs displayed repetitive firing.

Individual striatal hPNs exhibited coexisting high- (HVA) and low-voltage-
activated (LVA) Ca\(^{2+}\) channels. By comparing the current/voltage relationships of
striatal and neocortical hPNs at a holding potential of 0 mV the mean current density
was greater in striatal hPNs than in neocortical hPNs (−7.8 ± 2.5 pA/pF; \(n = 11\) versus
\(-3.9 ± 1.1\) pA/pF; \(n = 3\), \(P < 0.05\), t-test); while at −40 mV no significant difference was found
\((-1.9 ± 0.4\) pA/pF; \(n = 11\) versus \(-1.5 ± 0.5\) pA/pF; \(n = 3\)).

A Supplementary Methods Checklist is available.

(CRC Press, 2008).
44. Letinic, K., Zoncu, R. & Rakic, P. Origin of GABAergic neurons in the human
45. Sidman, R.L. & Rakic, P. Neuronal migration, with special reference to developing
47. Fraley, C., Scrucca, A.R. & Murphy, T.B. mclust: normal mixture modeling for
model-based clustering, classification, and density estimation. [https://www.stat.
washington.edu/research/reports/2012/tr597.pdf](https://www.stat.washington.edu/research/reports/2012/tr597.pdf) (Technical Report 597, Dept. of
48. Canty, A.J. An S-Plus Library for Resampling Methods (Fairfax, Interface Foundation
49. Langfelder, P. & Horvath, S. WGCNA: an R package for weighted correlation network
50. Eden, E., Navon, R., Steinfeld, I., Lipson, D. & Yakhini, Z. GOrilla: a tool for
discovery and visualization of enriched GO terms in ranked gene lists.
51. Csardi, G. & Nepusz, T. The igraph software package for complex network research,
52. Delli Carri, A. et al. Human pluripotent stem cell differentiation into authentic