Developmental Activation of the Proteolipid Protein Promoter Transgene in Neuronal and Oligodendroglial Cells of Neostriatum in Mice

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Key Words

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Abstract

Prior studies suggest that non-canonical proteolipid protein (*PLP*) gene expression occurs during development in non-myelinating neurons as well as myelinating oligodendroglia in mammalian brain. To assess this possibility in neostriatum, a region of uncertain *PLP* gene expression in neurons, morphological and electrophysiological tools were used to determine phenotypes of cells with activation of a *PLP* promoter transgene during the early postnatal period in mice. *PLP* gene expression is evident in both neuronal and oligodendroglial phenotypes in developing neostriatum, a conclusion based on three novel observations: (1) An enhanced green fluorescent protein (EGFP) reporter of *PLP* promoter activation was localized in two distinct populations of cells, which exhibit collective, developmental differences of morphological and electrophysiological characteristics in accord with neuronal and oligodendroglial phenotypes of neostriatal cells found during the early postnatal period in both transgenic and wild-type mice. (2) The EGFP reporter of

PLP promoter activation was appropriately positioned to serve as a regulator of *PLP* gene expression. It colocalized with native *PLP* proteins in both neuronal and oligodendroglial phenotypes; however, only soma-restricted *PLP* protein isoforms were found in the neuronal phenotype, while classic and soma-restricted *PLP* protein isoforms were found in the oligodendroglial phenotype. (3) As shown by EGFP reporter, *PLP* promoter activation was placed to regulate *PLP* gene expression in only one neuronal phenotype among the several that constitute neostriatum. It was localized in medium spiny neurons, but not large aspiny neurons. These outcomes have significant implications for the non-canonical functional roles of *PLP* gene expression in addition to myelinogenesis in mammalian brain, and are consistent with potentially independent pathologic loci in neurons during the course of human mutational disorders of *PLP* gene expression.

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Introduction

Alternative splicing of the proteolipid protein (*PLP*) gene encodes two main ‘classic’ protein isoforms, *PLP* and *DM20*, constituents in myelin membrane of oligodendroglia that may have additional functions suggest-

ed by localization in neural precursors [1, 2], myocardium, spermatozoa, and thymus [3–7]. In mice, alternative splicing of the cryptic 1.1 exon of the *PLP* gene also encodes two ‘soma-restricted’ protein isoforms, sr-PLP and sr-DM20, expressed by both neurons and oligodendroglia and associated with trafficking/recycling of endocytotic vesicles [8–11]. As shown by immunohistochemistry, neuronal expression of sr-isoforms (collectively termed sr-PLP) is complicated by distinct dense versus (vs.) sparse patterns of intracellular label accumulation in various neuronal populations and brain sites, particularly during early postnatal development [10]. Despite substantial evidence for label specificity, sparsely labeled sr-PLP neurons are so numerous and widely distributed that their occurrence remains questionable, and they have received little experimental attention.

Mutant mice that localize enhanced green fluorescent protein (EGFP) reporter under control of activated *PLP* promoter transgenes now allow this problem to be addressed with alternative, intrinsically generated molecular probes that can lead and signify *PLP* gene expression with fewer technical difficulties than immunohistochemical detection of low levels of native proteins [12]. In this report, we test the hypothesis that oligodendroglia, but not neurons, activate a *PLP* gene promoter (EGFP+ vs. EGFP– cells of respective lineage phenotypes) in neostriatum, a brain site that before and during myelinogenesis contains both densely labeled PLP and sr-PLP cells (possible oligodendroglia) and sparsely labeled sr-PLP cells (possible premyelinating stages of oligodendroglia and/or neurons) [pers. obs., 10]. The investigated period is early postnatal development, when the label density distinction is established for sr-PLP cells. The outcome may be clinically relevant – duplicative mutations of human *PLP1* gene in Pelizaeus-Merzbacher X-linked leukodystrophy (PMD) lead to progressive dysmyelination and neuronal degeneration in striatonigral components of basal ganglia [10, 13, 14]. Improved realization of the regional and cellular neuroanatomy of *PLP* gene expression may also contribute to uncovering the true range of its functional roles. For example, medullary neurons in myelin-deficient mutant rats accumulate abnormal PLP proteins, which disrupt potassium channels, alter rhythmic function of central pattern generators and yield lethal respiratory dysfunction [15, 16].

Methods

Animal use protocols were approved by the UCLA Animal Research Committee consistent with guidelines of the National Institutes of Health. Acute experiments were performed on brain slices obtained from homozygous male and female transgenic mice (founder genotype, EGFP10 with high levels of reporter expression, shown by Southern blots, progeny genotype shown by positive generation of EGFP reporter in brain under control of a known *PLP* promoter sequence [see 12 for construct details]). Observations were obtained from 51 mice (4–16 days of postnatal age, P4–P16) in electrophysiological studies, 21 mice (P4–P20) in immunohistochemical studies, and materials from our anatomical collection. As detailed elsewhere [17], acute slices for electrophysiological recording were prepared from brains of mice anesthetized with isoflurane, decapitated and brains removed and placed rapidly in ice-cold, oxygenated slicing solution. Coronal slices (300 μ m thickness) were cut by Vibratome, stored in oxygenated bicarbonate buffer recording solution (BBS) at 30°C for 30 min, then stored at room temperature (RT) before experiments.

Slicing solution contained (in mM): 26 NaHCO₃, 1.25 NaH₂PO₄, 10 glucose, 125 NaCl, 3 KCl, 5 MgCl₂, 1 CaCl₂. BBS contained (in mM): 26 NaHCO₃, 1.25 NaH₂PO₄, 10 glucose, 125 NaCl, 3 KCl, 2 MgCl₂, 2 CaCl₂. These solutions were bubbled with 95% O₂/5% CO₂ at RT (20–23°C), with pH and osmolality adjusted to 7.3 and 300–310 mOsm/kg, respectively. Voltage and current clamp recordings used a K⁺-based electrode solution that contained (in mM): 112.5 K-gluconate, 4 NaCl, 17.5 KCl, 0.5 CaCl₂, 1 MgCl₂, 0.5 Mg-ATP, 0.05 EGTA, 1 Na-HEPES, 0.1 tris-GTP, 0.1 leupeptin, 0.5 phosphocreatine (di-tris). The electrode solution also contained biocytin (0.05%), with pH and osmolality adjusted to 7.2 and 290 mOsm/kg, respectively. Tetrodotoxin (Sigma-Aldrich, St. Louis, Mo., USA) stock solution was prepared in distilled water (1 mM), and diluted to a final concentration of 1–0.5 μ M in BBS. 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Sigma-Aldrich) stock solution was prepared in dimethyl sulfoxide (20 mM), and diluted to a final concentration of 20 μ M in BBS. Both drugs were applied to slices via the perfusion system.

EGFP+ cells in neostriatum were identified by epifluorescent illumination for patch clamp recording, and G Ω seals were obtained under visual control by differential interference contrast illumination [17]. Recording pipettes (3–6 M Ω) were made from glass capillary tubes (World Precision Instruments, Sarasota, Fla., USA) filled with K⁺-based electrode solution (junction potential = 12 mV, corrected off-line). Signals were amplified by an Axopatch 1D amplifier (Molecular Devices, Sunnyvale, Calif., USA), digitized at 10 kHz and filtered at 5 kHz. Signals were grounded with an AgCl bath electrode, and data acquisition was performed with pClamp version 9.0 (Molecular Devices). Cell membrane and recording parameters (capacitance, input resistance, access resistance, holding current, via pClamp) were monitored regularly during each experiment. In voltage clamp experiments, data were discarded if cells displayed access resistances $\geq$ 30 M Ω . Mean ($\pm$ standard error of the mean, X $\pm$ SEM) access resistances for neostriatal neurons were: single action potential (AP) = 20.04 $\pm$ 1.31 M Ω and multiple AP = 20.88 $\pm$ 0.80 M Ω . Series resistance compensation was not applied, and leak and capacitance currents were subtracted digitally during voltage clamp using a P/N protocol. Na⁺ and K⁺ currents were activated from a holding potential of –82 mV with a series of depolarizing voltage commands (–82

to +58 mV, 10 mV steps with a duration of 150 ms). Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded at holding potential of -82 mV in BBS, and analyzed off-line using Mini-Analysis (Synaptosoft Inc., Fort Lee, N.J., USA). Current-voltage (I-V) relationships, AP thresholds, kinetics and after-hyperpolarizations (AHPs) were determined for current clamp experiments by a pulse protocol series of depolarizing current steps (-0.1 to 0.2 nA, 0.1 nA steps, 150 ms duration).

For immunohistochemical preparations and analyses of patch-clamped cells, tissue was fixed by immersion in 4% paraformaldehyde, blocked and permeabilized in phosphate-buffered saline (PBS) containing 10% normal goat serum (NGS) and 0.2% Triton X100, and incubated overnight (4°C with agitation) in primary antibodies diluted in PBS with 10% NGS (carrier) [17]. Slices were subsequently washed in PBS with 0.01% Triton X100, incubated in secondary antibody diluted in carrier (4–6 h, 1:800, RT with agitation), rinsed, and incubated in streptavidin-AMCA for detection of biocytin-filled cells (2 h, 1:400, RT with agitation; Vector Laboratories Inc., Burlingame, Calif., USA). Sections were air dried, mounted on glass slides and coverslipped with aqueous media (Aquamount, Lerner Laboratories, Pittsburgh, Pa., USA; EGFP was an intrinsic fluorescent label that could be visualized in aqueous media mounts on glass slides, with coverslips). The following primary antibodies were tested: mouse monoclonal anti-NeuN (1:800, Millipore, Billerica, Mass., USA); mouse monoclonal anti-MAP2 AP-20 clone (1:800, Sigma-Aldrich); rabbit monoclonal anti-DARPP-32 (1:400, Cell Signaling Technology, Boston, Mass., USA); rabbit polyclonal anti-NG2 (1:300, Millipore), rat monoclonal anti-PDGFR α (1:200, BD Biosciences, San Jose, Calif., USA); rat anti-PLP/DM20 clone AA3 (gift from Steven Pfeiffer, University of Connecticut Health Science Center, Conn., USA); rabbit polyclonal anti-srPLP (1:400). For sequential, multiple labeling studies, immunolabeling of primary antibodies was also produced by Alexa Fluor®-594 conjugated goat IgG antibodies raised against appropriate host species (Invitrogen, San Diego, Calif., USA). In all cases, specific labeling was abolished when these antibodies were deleted from primary incubation. Images were acquired with an inverted microscope (Olympus IX81) equipped with a spinning disc confocal setup.

Biocytin-filled cells were labeled, then characterized by photomicrography and camera lucida drawings [17]. Digital photomicrographs of cells were derived from 35-mm color slide film (Nikon Inc., Melville, N.Y., USA).

For immunohistochemistry on perfusion-fixed tissue sections, mice were deeply anesthetized with sodium pentobarbital and perfused transcardially with ice-cold 4% paraformaldehyde. Brains were postfixed overnight, mounted in agarose, and sectioned in the coronal plane (50 μ m thickness) by Vibratome. Methods for immunolabeling these sections were similar to those for thick slices with alterations in blocking/permeabilization solution (0.1% Triton X100 and 2% NGS), reduced duration of blocking step (2 h RT), and reduced duration of incubation in secondary antibody (1.5 h RT) [17].

Quantitative measurements of EGFP+ and immunoreactive cells were based on cell counts from confocal images acquired and displayed in SlideBook™ 4.0 software (Intelligent Imaging Innovations, Inc., Denver, Colo., USA). Three sections were analyzed for each condition, with counts obtained from six individual fields in each section. Positive cell counts were checked by examination of immunolabel and EGFP signal in multiple optical sec-

tions. Classic PLP expression was quantified using SlideBook™ 4.0 software. EGFP+ cells were selected in regions of interest, and software was used to measure average PLP signal intensity. Electrophysiological data were analyzed using Clampfit 8 (Molecular Devices). Prior to current measurement, traces were normalized to the average holding current from the period preceding the voltage step. Na⁺ currents were measured as peak inward response evoked during the first 10 ms of a depolarizing step to -22 mV. K⁺ currents were measured during the steady-state phase between 100 and 150 ms after onset of a depolarizing voltage command to +58 mV. AP slope and half-width were measured using pClamp, while AP and sEPSC frequencies were analyzed with Mini-Analysis. Normal distributions were tested in each data set using Kolmogorov-Smirnov tests. For data with normal distributions, single between-group comparisons were made by t tests, and multiple comparisons were investigated by one-way ANOVA followed by Bonferroni's multiple comparison tests to detect pair-wise between-group differences. When normal distributions were absent, nonparametric Mann-Whitney U tests were used to make comparisons, and Kruskal-Wallis tests were used for multiple group comparisons, with between-group differences checked by Dunn's multiple comparisons test. All statistical tests were computed with Prism 4.0 for Macintosh (Graphpad Software, Inc., El Camino Real, Calif., USA). A fixed value of $p_{\alpha} < 0.05$ for one-tailed tests was the criterion for reliable differences between groups. Cited values were Xs + SEMs unless otherwise noted.

Results

Morphological Characteristics of Neostriatal Cells with Activated PLP Promoter

EGFP+ cells with activated PLP promoter were widely distributed throughout immature forebrain throughout the period of development under investigation. At the beginning of this period (surveyed at low magnification for a P6 transgenic mouse in fig. 1), these cells were particularly prominent within neostriatum due to their close compaction and extensive distribution across the entire thickness of sections and slices. They had comparable arrangements and high cellular density throughout neostriatum. No consistent evidence of macroscopic 'striosomal' (i.e. cell island vs. matrix) compartmentation was seen in serial sections. At this early age, fiber fascicles of the internal capsule were clearly apparent in neostriatum, but contained few EGFP+ cells, consistent with their coincident low density of interfascicular oligodendroglia and paucity of myelin.

Neostriatal EGFP+ cells were categorized into three interspersed structural groups at this early postnatal stage: group I constituted the majority of EGFP+ cells, with spherical cell bodies and medium (sized) diameters >10 μ m. Group II had bipolar (spindle-shaped) cell bodies and small diameters <10 (mostly 7–8) μ m. Group III

Color version available online

Fig. 1. Colocalization of activated *PLP* promoter transgene and NeuN neuronal lineage marker in neostriatum. **a, b** Coronal fore-brain sections of rostral, middle and caudal levels of neostriatum in a P6 transgenic mouse. At low resolution ($\times 2.5$ objective), neostriatum is well defined by green fluorescent EGFP+ reporter label (left). EGFP+ elements in cerebral cortex are so infrequent, dispersed and weakly fluorescent as to escape recognition. Unlabeled, irregular territories in neostriatum are fiber fascicles of internal capsule. At higher resolution ($\times 20$ objective), EGFP+ label is localized in cell bodies (upper middle). Red fluorescent NeuN+ label (lower middle) in nuclei in same sections is colocalized in EGFP+ cells in merged images (green + red fluorescence in cell bodies and their nuclei, right). **c** Distinct regional patterns of EGFP+ label in

neostriatum and globus pallidus. At low resolution ($\times 2.5$ objective, left), EGFP+ label is located in both brain sites. At higher resolution ($\times 20$ objective), different cellular distributions occur in each site (middle left). NeuN+ label colocalizes with EGFP+ label in neostriatum (upper middle left, middle right and right), but not in globus pallidus (lower middle left, middle right and right), where EGFP+ label is mainly diffuse and not colocalized in cell bodies with NeuN+ nuclei. Dashed lines demarcate brain sites (left): neostriatum (Ns), globus pallidus (GP), cerebral cortex (Cx), hippocampus (Hc), lateral ventricle (Lv); scale bars = 400 μm in left panels. White boxes indicate magnified regions (middle and right). Scale bar = 100 μm in middle (**a, b**) and right panels (**c**). **a, b** Scale bar = 50 μm in right merged image panels.

had spherical cell bodies and small diameters <10 (mostly 7–8) μm . Group I EGFP+ cells matched settled medium-sized neostriatal neurons in conventional histology and Golgi reports [18]. Group II EGFP+ cells, which might have overlapped with group III due to neostriatal anisotropy, matched neural progenitors (NPCs, most

likely neuroblasts), migrating immature neurons and small oligodendroglial progenitor cells (OPCs) described in white matter tracts [12, 17]. Group III, which became much more frequent with age, matched immature and mature interfascicular oligodendroglia described in white matter tracts [19]. Structural groups extended the

Fig. 2. *PLP* promoter transgene activation in neuronal and oligodendroglial phenotypes of neostriatal cells during the first post-natal week (P4). **a** EGFP+ neuronal group. Green fluorescent EGFP+ cells have medium spherical cell bodies with red fluorescent NeuN+ label in nuclei. Green and red labels colocalize in merged images. **b** EGFP+ oligodendroglial (OPC) group. EGFP+ cells have small bipolar cell bodies (prominent perinuclear label) with PDGFRα+ label in perikaryal cytoplasm. Labels colocalize in merged images. **c** EGFP+ oligodendroglial (OPC) group. EGFP+ cells have small bipolar and spherical cell bodies (prominent perinuclear label) with NG2+ label in perikaryal cytoplasm.

Labels colocalize in merged images. Note lack of PDGFRα and NG2 labels in medium spherical EGFP+ cells adjacent to doubly labeled OPCs in **b** and **c** (arrows). **d** A significantly larger proportion of EGFP+/NeuN+ cells (30 of 30 cells) displays spherical cell bodies in comparison to EGFP+/PDGFRα+ (12 of 21 cells) or EGFP+/NG2+ (7 of 13 cells) profiles (NeuN+ vs. PDGFRα+: $\chi^2 = 31.22$, d.f. = 1; NeuN+ vs. NG2+: $\chi^2 = 32.18$, d.f. = 1). **e** A significantly larger proportion of EGFP+ cells contain NeuN ($69 \pm 11\%$) than PDGFRα+ ($10 \pm 4\%$) or NG2 [$8 \pm 3\%$; $F(28.2) = 44.85$; reliable NeuN vs. PDGFRα and NeuN vs. NG2 contrasts; * $p_{\alpha} < 0.05$]. **a–c** Scale bar for merged profiles = 10 μm .

full depth of thick brain slices, and were not due to partial EGFP+ cell body profiles at slice faces.

Neostriatal EGFP+ cells were further delineated from P4–P16 by colocalization of cell lineage markers. NeuN, a pan-neuronal lineage marker, was located in frequent doubly labeled EGFP+/NeuN+ neostriatal cells in immunoreactive section faces (fig. 1). Comparable, but less densely aggregated, doubly labeled neurons were found in cerebral cortex, amygdala and hypothalamus. Among neostriatal NeuN+ neurons, 93% were also EGFP+, a finding cross-validated with the more mature neuronal lineage marker Map2. Approximately 76% of neostriatal Map2+ neurons were also EGFP+. Differences between proportions of doubly labeled neurons obtained with these two lineage markers might reflect different age-related schedules of accumulation of the targeted antigens,

variations in their preservation, and/or distinct tissue penetrances of antibodies. Such neurons were not apparent in globus pallidus, although it contained substantial numbers of EGFP+ cells (fig. 1c). At P6, EGFP+/NeuN+ neurons were group I medium spherical cells (fig. 2a). PDGFRα and NG2, lineage markers of early and later developmental stages of OPCs, were also located in all brain sites containing EGFP+ cells [17, 20, 21]. At P6, EGFP+/PDGFRα+ and EGFP+/NG2 OPCs were small bipolar and spherical cells in groups II and III. EGFP+ cells in globus pallidus were akin to groups II and III (fig. 2b, c).

Allometric associations between form, size and lineage marker expression were evident for neostriatal EGFP+ cells during the P4–P16 study period. Form was quantified as minimum somatic diameter/maximum somatic diameter. Among EGFP+ cells, spherical form had values >0.5

Fig. 3. Colocalization of classic PLP protein in oligodendroglial, but not neuronal, phenotype of neostriatal cells with activated *PLP* promoter transgene. **a–c** Localization of EGFP+ reporter and classic PLP protein. **a** Sparsely labeled EGFP+ cells contain no apparent PLP label at P5. Sparsely labeled medium spherical EGFP+ neurons also contain no apparent PLP label at P12 (white box). **b, c** Densely labeled small spherical EGFP+ oligodendroglia contain classic PLP label in cell bodies and proximal processes at P12 (arrows). **d** Histogram of average PLP label density. PLP density is significantly greater in small spherical cells with dense EGFP+ label at P12 [281 ± 34 AU (arbitrary fluorescence photometry units), $n = 21$] than in medium spherical cells with sparse EGFP+ label at P5 (15 ± 2 AU, $n = 30$) and P12 (29 ± 7 AU, $n = 27$; Kruskal-Wallis statistic = 44.31; reliable post-hoc comparisons for sparse P5 vs. dense P12 EGFP+ cells and sparse P12 vs. dense P12 EGFP+ cells; * $p_{\alpha} < 0.05$). Scale bar for cell images = 10 μ m.

and bipolar form had values <0.5 . When categorized in this fashion, EGFP+/NeuN+ cells were reliably associated with spherical form of medium size, while EGFP+/PDGFR α + and EGFP+/NG2+ cells were reliably associated with bipolar and spherical forms of small size (fig. 2d). The proportion of small spherical cells that colocalized EGFP+/PDGFR α + and EGFP+/NG2+ labels increased with OPC maturation, as shown by later-stage NG2 stage marker expression. The proportion of EGFP+/NeuN+ neurons in group I was reliably greater than proportions of EGFP+/PDGFR α + and/or EGFP+/NG2+ EGFP+ OPCs in groups II and III, in accord with the expected stability of settled, postmitotic neurons unlike the extended mitotic passage of and subsequent maturation of OPCs (fig. 2e).

Expression of Native PLP Proteins by Neostriatal Cells with Activated PLP Promoter

Neostriatal EGFP+ cells with activated *PLP* promoter transgene expressed distinct isoforms of native PLP proteins, as shown by immunohistochemistry.

At P5, prior to the onset of myelinogenesis, neither EGFP+ nor EGFP– cells localized classic PLP/DM20 isoforms (fig. 3a; detection of classic DM20, believed to show early expression, was uncertain in neostriatum with the employed monoclonal antibody directed against PLP; color plates for fig. 3, 4, 6, and 8 are shown in the online suppl. material; www.karger.com/doi/10.1159/000330321). EGFP label did not spread into processes in EGFP+ cells (0/30 examined cells). By and after P12, densely labeled EGFP+ cells (i.e. ‘bright’ fluorescence indicative of high cellular accumulations of the reporter) colocalized substantial PLP immunoreactivity (fig. 3b, c). Both EGFP and PLP labels spread into processes, which extended and branched up to 25 μ m from origins (16/17 examined cells). Densely labeled EGFP+/PLP+ cells had small spherical characteristics of group III cells. They were putative immature and mature interfascicular oligodendroglia, as signified by their PLP signature and structural correspondence to these lineage stages. In association with myelination during and after the 4th week of postnatal development, label density in group III cells was so great that it obscured detection of group I and II cells. Distinction between sparsely and densely labeled EGFP+ cells was objectively measured by photometry, and found to be statistically reliable (fig. 3d). Unlike neostriatum, sparsely and densely labeled bipolar OPCs and more mature small spherical oligodendroglia in groups II and III were the only EGFP+ cells in globus pallidus.

Frequent, sparsely labeled group I (medium spherical) EGFP+ neostriatal neurons persisted throughout the

Fig. 4. Colocalization of activated *PLP* promoter transgene and sr-PLP protein in neostriatal neuronal phenotype. **a** Low resolution ($\times 10$ objective) images of merged EGFP+ and sr-PLP+ labels at P5. Coronal section maps imaged region. **b** Higher resolution ($\times 40$ objective) images of merged sparse EGFP+ and robust sr-PLP labels at P12 in a similar site. **c** Detail from **b** (white

box) of colocalized EGFP+ and sr-PLP labels in medium spherical neurons. **d** Percentages of EGFP+/sr-PLP+ cells plotted as a function of age show no developmental differences with similar values at P5 ($97 \pm 1\%$, $n = 132$), P12 ($98 \pm 1\%$, $n = 130$) and P20 ($98 \pm 1\%$, $n = 110$; $p_{\alpha s} > 0.05$). Scale bars = $50 \mu\text{m}$ (**a**) and $10 \mu\text{m}$ (**b**, **c**).

study period. EGFP label did not spread into processes. In accord with a putative neuronal lineage, they did not express classic PLP (fig. 3b).

Both densely and sparsely labeled (groups I–III) neostriatal EGFP+ cells expressed sr-PLP/sr-DM20 isoforms (collectively termed sr-PLP cells; either or both sr isoforms might have been detected by the employed polyclonal sr-PLP antibodies). Regardless of intracellular accumulations of EGFP, EGFP+/sr-PLP+ cells were always found to be the most frequent cells in neostriatum from P4–P20 (fig. 4). The high degree of overlap between sparsely labeled EGFP+ cells and sr-PLP+ cells was stable across age, consistent with the persistent numerical predominance of sparsely labeled group I EGFP+ neostriatal neurons (contrast fig. 2d and 4d). This combination of double labeling, and consistently high frequencies of doubly labeled cells throughout early postnatal development, demonstrated that sparsely labeled group I EGFP+ neurons in neostriatum expressed sr-PLP in conjunction with activated *PLP* promoter.

Physiological Characteristics of Neostriatal Cells with Activated PLP Promoter

The different structural characteristics of neostriatal cells with activated *PLP* promoter provided a valuable opportunity to target patch clamp recording studies for de-

termination of viability and corresponding functional characteristics. We focused on sparsely labeled neostriatal EGFP+ cells, present from P4–P20 in transgenic mice, in order to compare the electrophysiological properties of nascent morphological groups.

During early postnatal development (P4–P7), the majority of sparsely labeled neostriatal EGFP+ cells exhibited high resistance membranes expected of immature cells, including neurons ($3.10 \pm 0.33 \text{ G}\Omega$, $n = 40$), but their other functional characteristics displayed categorical differences. Three functional classes (termed for ease of distinction from the three structural groups) of neostriatal EGFP+ cells were recognized based on their excitability and expression of voltage-gated K^+ currents (fig. 5a). Class I cells had moderate transient A-type (I_{KA}) currents, sustained delayed rectifier K^+ currents (I_{KDR}), moderate-to-large Na^+ currents (I_{Na}) and fired APs upon depolarization, which identified them as neurons [22]. Class I was subdivided into neurons firing single APs versus multiple APs in response to positive current injection. Class II cells did not fire regenerative APs, lacked a prominent I_{KA} , had a small I_{KDR} , and possessed small I_{Na} . Class III cells did not fire APs, exhibited large I_{KA} and sustained I_{KDR} , and had small I_{Na} .

Electrophysiological properties of these functional classes are summarized in table 1. Consistent with the

Fig. 5. Physiological characteristics of neostriatal neuronal and oligodendroglial phenotypes with *PLP* promoter transgene activation during the first postnatal week (P4–P7). **a** Current clamp (top traces) and voltage clamp (bottom traces) records from EGFP+ cells show three functional classes. Class I cells are a mature neuronal phenotype, subdivided into cells that fire single and multiple APs. Class II cells display physiological properties consistent with earliest stages of neuronal development. Class III cells exhibit physiological hallmarks of oligodendrocyte precursors. **b–d** Physiological maturation of class I EGFP+ neurons. Multiple AP neurons have a more mature functional profile than single AP neurons with greater AP slope ($t = 3.06$, d.f. = 23) and reduced AP half-width ($t = 3.53$, d.f. = 23; **b**), greater voltage-gated Na^+ currents ($t = 2.63$, d.f. = 23; **c**), and greater voltage-gated K^+ currents ($U = 27$; $* p_{\alpha} < 0.05$; **d**).

Table 1. Physiological characteristics of neostriatal cells with activated *PLP* promoter transgene

Excitability	Class III OPC	Class II NPC/migrating neuron	Class I neuron	
	no/graded spike	no/graded spike	single AP	multiple AP
RMP, mV	-72 ± 7.2	-60 ± 4.5	-61 ± 4.1	-65 ± 2.8
R_{in} , $\text{G}\Omega$	1.6 ± 0.2	5.3 ± 1.2	3 ± 0.6	3 ± 0.3
Transient $-I_{\text{KA}}$, pA	$1,271 \pm 105$	333 ± 26	440 ± 32	570 ± 38
Steady-state I_{KDR} , pA	534 ± 79	303 ± 39	404 ± 33	520 ± 38
Ratio $I_{\text{KA}}/I_{\text{KDR}}$	$2.6 \pm 0.3^*$	1.2 ± 0.1	1.1 ± 0.0	1.1 ± 0.0
Max I_{Na} , pA	-209 ± 51	-131 ± 35	-498 ± 74	-924 ± 112

RMP = Resting membrane potentials. $* p_{\alpha} < 0.05$, pairwise contrasts between classes III vs. II, I-single AP and I-multiple AP.

predominance of EGFP+/NeuN+ neurons, class I cells capable of firing APs formed the majority of the targeted sample (25 of 40 recorded cells). Among class I cells, AP waveforms were sharper in the multiple AP than the single AP subdivision, which reflected a range of maturational states (fig. 5b). For class I neurons in the multiple AP subdivision, AP slope was reliably increased while AP

half-width was reliably reduced, indicative of enhanced AP kinetics (fig. 5b). AP rise and decay times were significantly reduced in these cells, while peak I_{Na} and I_{KDR} were reliably increased (fig. 5c, d). Class II cells (8 of 40 recorded cells) had the low degree of excitability, apparent lack of I_{KA} , and moderate I_{KDR} typical of early NPCs or pre-settlement stages of development for neurons [23].

Fig. 6. Correlation of morphological groups and electrophysiological classes of neostriatal cells with *PLP* promoter transgene activation during the first postnatal week (P4–P7). **a–c** Examples of voltage clamp (upper traces) and current clamp (lower traces) records (physiology column; **insets** show boxed regions on an expanded time base) from EGFP+ cells (arrows, patch EGFP column) for small bipolar cells (**a**, **b**) and medium spherical cells (**c**). Putative OPCs (**a**) and putative NPCs and migrating neurons (**b**)

generate graded spikes, while putative neurons (**c**) display all-or-nothing APs. Filled with biocytin (biocytin column), these cells are then labeled for OPC and neuronal lineage markers (immuno column). Class III oligodendroglial cell is NG2+ (P7), class II early neuron is NG2– (P4), and class I neuron is Map2+ (P5; a multiple AP cell). Scale bars for physiology traces: voltage clamp = 200 pA/50 ms, current clamp = 20 mV/50 ms, **inset** traces = 20 mV/10 ms. Scale bar for cell images = 10 μ m.

Class II cells were distinguished from class III OPCs by absence of a pronounced I_{KA} , which was reflected in a transient/steady-state I_K ratio of ~ 1 . Class III cells (7 of 40 recorded cells) had the prominent I_{KA} and I_{KDR} , and lack of a regenerative AP typical of OPCs [17, 24]. The large amplitude of I_{KA} in putative OPCs increased the ratio of I_{KA}/I_{KDR} (transient/steady-state I_K), not apparent in other classes of EGFP+ cells.

In all cases, class I cells were selected as representatives of medium spherical EGFP+ (structural group I) neostriatal cells. Double labeling revealed positive examples of class I cells that were EGFP+/Map2+ neurons, a cell lineage and developmental status consistent with their substantial Na^+ current and regenerative APs (fig. 6c). Also in all cases, class II and III cells were selected as representatives of small bipolar EGFP+ (structural group II) neostriatal cells. Double labeling revealed no examples of class II EGFP+ neostriatal cells that expressed NG2, a negative outcome suggestive but not conclusive of their putative neuronal lineage (fig. 6b). These cells also escaped detection with neuronal lineage markers NeuN and Map2, probably due to their very early developmental

status (fig. 2d); however, they regularly generated a graded low amplitude spike consistent with an early stage of neuronal differentiation (fig. 6b). Double labeling revealed positive examples of class III cells that were EGFP+/NG2+ OPCs (fig. 6a). In some cases, depolarization of putative OPCs produced a small spike whose amplitude increased in a graded fashion with increasing current injection (fig. 6a inset); nevertheless, kinetics, amplitude and lack of regenerative response distinguished these spikes from neuronal APs (fig. 6a, c).

Ontogeny of Neurotransmission in Neostriatal Neurons with Activated PLP Promoter

The increasing excitability of neostriatal EGFP+ neurons during early postnatal development was due, at least in part, to progressive maturation of synaptic afferents and/or receptive substrates. The appearance of sEPSCs was contrasted between early and later postnatal periods in sparsely labeled EGFP+ neostriatal neurons identified by their morphological properties, pattern of membrane currents, and excitability (group I/class I; fig. 7). During the first postnatal week (P4–P5), few EGFP+ neurons had

Fig. 7. Acquisition of synaptic connectivity by the neuronal phenotype of neostriatal cells with *PLP* promoter transgene activation. **a** Sample sEPSC records from EGFP+ neurons during 1st (grey trace, P4–P7) and 2nd (black trace, P8–P14) postnatal weeks. Lower trace is an example of a spontaneous synaptic current on an expanded time base (asterisk denotes location of **inset**). **b** Histogram of average frequencies for sEPSC records during 1st and 2nd weeks. sEPSC frequency is reliably greater in EGFP+ neu-

rons in 2nd postnatal week (1st week = 0.003 ± 0.002 Hz, $n = 14$; 2nd week = 0.13 ± 0.04 Hz, $n = 14$; $U = 20$). **c** sEPSC frequency as a function of input resistance. Input resistance is reliably and negatively correlated with sEPSC frequency ($r = -0.65$). In **inset**, average input resistance for EGFP+ neurons decreases significantly with age (1st week = 3.14 ± 0.31 GΩ, $n = 14$; 2nd week = 0.59 ± 0.07 GΩ, $n = 14$; $t = 8.03$, d.f. = 26, $*p_{\alpha} < 0.05$). **a** Scale bars for **inset** = 50 pA/10 ms.

spontaneous membrane currents (2/14 recorded cells; fig. 7a), and when present, these currents had small amplitudes (~ 5 pA). During the second postnatal week (P9–P15), most EGFP+ neurons had numerous spontaneous currents (12/14 recorded cells; fig. 7a), and these currents had increased amplitudes (range = 5–50 pA). In these more mature neurons, current frequency was attenuated by the AMPA-type glutamate receptor antagonist CNQX (pre-CNQX: 13 ± 3 Hz, $n = 3$; post-CNQX: 2 ± 2 Hz, $n = 3$), and thus represented glutamatergic sEPSCs. The average frequency of sEPSCs increased between the first and second postnatal weeks (fig. 7b). EGFP+ neurons also had a reliable decrease in input resistance during this period (fig. 7c inset), while input resistance was always negatively correlated with sEPSC frequency (fig. 7c). Progressive, parallel decreases in input resistance and increases in synaptic input were consistent with dendritic growth and/or spine generation.

Identification of Type(s) of Neostriatal Neurons with Activated PLP Promoter

The collective structural and functional characteristics of EGFP+ neostriatal neurons with activated *PLP* promoter identified them as medium spiny neurons. Medium spiny cells (with two minor variants that both emit projection and local axonal collaterals) constitute approximately 95% of neostriatal neurons [18, 25], a value that closely approximated the prevalence of EGFP+/NeuN+ neostriatal neurons observed during and after

the first postnatal week in transgenic mice. Between P4–P20, frequent medium EGFP+ neurons and infrequent large EGFP– cells, likely in the latter case to have represented large ‘aspiny’ neurons that usually emit only local axons, were found in neostriatum. This observation indicated that *PLP* promoter activation was widespread but restricted to particular type(s) of neostriatal neurons. This categorization also afforded a valuable opportunity to target patch clamp recordings to these two contrasting types of cells for comparisons of their development in the early postnatal period.

Medium spherical EGFP+ neostriatal neurons (group I/class I) displayed a striking electrophysiological uniformity. They showed little or no spontaneous AP generation at normal resting potentials (fig. 8a) and significant spike accommodation during current injection (fig. 8b). Their mean input resistance (642 ± 0.09 MΩ) was comparable to values obtained previously for immature medium spiny neurons [26, 27] and was significantly greater than the value obtained from large EGFP– cells (380 ± 1.49 MΩ; $U = 11.5$, $p < 0.05$). Similar to an earlier report [22], EGFP+ neurons had a pronounced inward rectification of the I–V relationship during hyperpolarizing and depolarizing current injection, responses that were absent in large EGFP– cells (fig. 8c). EGFP+ neurons also had a positive expression of DARPP-32, a dopamine receptor-associated protein sometimes used to identify putative medium spiny neurons in intact forebrain (fig. 8d) [28]. When recovered after intracellular biocytin injection

Fig. 8. Physiological characteristics of medium spiny neostriatal neurons with *PLP* promoter transgene activation (P10–P16). **a** Infrequent or absent spontaneous APs in EGFP+ neurons. Examples from EGFP- (grey trace) and EGFP+ (black trace) neurons [left; numerals = resting membrane potentials (mV); scale bar = 20 mV/5 s]. Average spontaneous firing frequency in EGFP- (0.63 ± 0.15 Hz, $n = 9$) and EGFP+ neurons (0 ± 0 Hz, $n = 10$; right). **b** Pronounced AP accommodation in EGFP+ neurons. Examples of APs evoked by injection of 0.12 nA (left; scale bar = 20 mV/50 ms). Average threshold (no AP failure) is significantly lower in EGFP+ neurons (right; EGFP- = 0.27 ± 0.04 nA, $n = 9$, vs. EGFP+ = 0.08 ± 0.01 nA, $n = 9$; $U = 8.5$; $*p_{\alpha} < 0.05$). **c** Pronounced rectification of I-V response in EGFP+ neurons. Examples of voltage responses from EGFP- (grey) and EGFP+ (black) neurons in presence of tetrodotoxin (left; scale bars = 20mV/50 ms). Average I-V curves from EGFP+ ($n = 10$) and EGFP- neurons ($n = 9$; right). **d** Postrecording DARPP-32 localization in an EGFP+ neuron (P7). Fluorescent image of biocytin loaded into EGFP+ cell during patch clamp recording (left) and immunolabel of the same site with anti-DARPP-32 (middle). This cell shows significant AP accommodation, a usual feature of EGFP+ neostriatal neurons (right). Scale bar for cell images = 10 μ m; for physiology records = 20 mV/50 ms.

tions, all EGFP+ neurons had medium-sized and spherical cell bodies of 10- to 15- μ m diameter (fig. 9a). Their dendrites (4–7 primary dendrites/neuron) had thin shafts and numerous varicosities, which decreased with age. Their distal dendritic segments, usually >3rd branch order, emitted characteristic spines, which became more frequent and uniformly shaped with age (fig. 9). Thus, sparsely labeled EGFP+ neurons were directly identified as developing medium spiny neostriatal neurons consistent with previous functional and structural descriptions obtained from young rats [26, 27].

Large EGFP- cells were also neurons, as shown by spontaneous generation of APs at normal resting potentials (fig. 8a). In contrast to EGFP+ neurons, they did not exhibit spike accommodation during current injection (fig. 8b). Their post-AP AHPs were reliably prolonged when compared to EGFP+ neurons (21.6 ± 3.89 vs. 2.48 ± 0.54 ms). These properties resembled those found for aspiny neurons of comparable size in young rats [29]. There was also evidence of additional diversity among EGFP- neurons, with one example (1/20 cells) of a fast-spiking cell with a high firing frequency (114.3 Hz) and brief AP/AHP kinetics [30]. When recovered after intracellular biocytin injections, all EGFP- neurons had large-sized multipolar cell bodies of 20- to 30- μ m maximum diameter, and were reliably bigger than EGFP+ neurons obtained at the same age. Their proximal dendritic segments tended to be thick, dendritic varicosities were uncommon, and dendritic spines were rarely observed regardless of age.

Discussion

Novel evidence obtained in this investigation demonstrates beyond a reasonable doubt that *PLP* gene expression occurs in both neuronal and oligodendroglial phenotypes in the developing neostriatum of mice that bear the *PLP* promoter transgene. This conclusion is based on three fundamental observations. First, an EGFP reporter of *PLP* promoter activation in transgenic mice is certainly localized in two distinct populations of cells, which exhibit collective, emergent differences of morphological and physiological characteristics in accord with the neuronal and oligodendroglial phenotypes of neostriatal cells found during early postnatal development in both mutant and wild-type mice [17, 24, 31, 32]. The present assessment of characteristics that comprise the phenotypes is not exhaustive, but it is sufficient to demonstrate the age-related divergence of viable neuronal and oligodendroglial cells

Fig. 9. Identified medium spiny neostriatal neurons with *PLP* promoter transgene activation (P6–P14). **a** Camera lucida drawings of representative EGFP+ and EGFP– neurons filled with biocytin during patch clamp recording. EGFP+ cell (P10) is an immature medium spiny neuron with thin primary dendrites, numerous varicosities (grey arrows) and distal dendrites decorated with spines (black arrows). EGFP– cell (P16) is an immature large aspiny neuron with thick primary dendrites, infrequent varicosities and few dendritic spines. Somatic axes of medium spiny EGFP+ neurons (long axis = $12 \pm 1 \mu\text{m}$, short axis = $8 \pm 1 \mu\text{m}$, $n = 9$) are reliably less than in large aspiny EGFP– neurons (long axis = $26 \pm 2 \mu\text{m}$, short axis = $11 \pm 1 \mu\text{m}$, $n = 14$; long axis: $U = 0.5$; short axis: $U = 12.5$; $p_{\alpha s} < 0.05$; scale bar = $50 \mu\text{m}$). Sample records from these cells show characteristic firing responses of EGFP+ and EGFP– neurons. Note pronounced AP accommodation by EGFP+ cell, and extended AHP duration by EGFP– cell (dashed lines). **b** Photomicrographs of representative EGFP+ and EGFP– neurons. Lower images are enlargements of boxed areas. EGFP+ neurons have thin spiny dendrites (*), while EGFP– neurons have thick, smooth aspiny dendrites. Scale bars for cell images = $10 \mu\text{m}$.

that activate the *PLP* promoter transgene, and to resolve, particularly by electrophysiological means, several key morphological characteristics shared by these two phenotypes during their early, primitive stages of development. Second, the EGFP reporter of *PLP* promoter activation in transgenic mice is appropriately positioned to serve as a regulator of *PLP* gene expression. It is colocalized with native *PLP* proteins in both neuronal and oligodendroglial phenotypes; however, only soma-restricted(sr) *PLP* protein isoforms are localized in the neuronal phenotype, while classic and sr-*PLP* isoforms are localized in the oligodendroglial phenotype [8, 10]. Third, the EGFP reporter of *PLP* promoter activation in transgenic mice is placed to regulate *PLP* gene expression in only one neuronal phe-

notype among the several that constitute neostriatum. It is localized in medium spiny neurons, but not large aspiny neurons [26, 27]. Taken singly or together, these positive results necessarily lead to rejection of the hypothesis that oligodendroglia, but not neurons, exhibit *PLP* gene promoter activation in neostriatum of mice that bear the *PLP* promoter transgene.

The new observations are a modest, incremental advance in the elucidation of *PLP* gene expression in the oligodendroglial phenotype. As expected, morphological and electrophysiological patterns of development for the oligodendroglial phenotype in fiber fascicles of internal capsule that pass through neostriatum closely resemble patterns obtained in corpus callosum and subcortical

white matter [17]. The former is rarely studied, while the latter is the subject of many prior investigations. Of greater significance, immature and mature oligodendroglia in transgenic and wild-type mice contain the molecular machinery required for the production of PLP proteins, as indicated by colocalizations of interrelated mRNA, promoter and proteins [10, 33–35]. With age, the oligodendroglial phenotype shifts from a sparse to a dense pattern of intracellular EGFP+ labeling, an upregulation likely to reflect a high level of activation (and consequent reporter accumulation) of native and transgene *PLP* promoter elements triggered only within the oligodendroglial phenotype by events within the context of normal development. This shift coincides with previously described up-regulations of PLP mRNA and classic PLP levels that accompany myelinogenesis. The sparse pattern of intracellular EGFP+ label precedes the shift, and may reflect a low, steady-state activation of native and transgene promoter elements that accompanies expression of sr-PLP, but not classic PLP, during early development (OPCs) and throughout myelinogenesis (immature and mature interfascicular oligodendroglia) [10, 12].

The new observations are a significant advance in the elucidation of *PLP* gene expression in the neuronal phenotype. Due to technical limitations, previous *in situ* hybridization and immunohistochemical studies do not recount localization of substantial levels of mRNA or protein markers of *PLP* gene expression in neostriatal neurons in wild-type mice. However, the development of the neostriatal neuronal phenotype with *PLP* promoter transgene activation resembles, and likely precedes, the sparse label pattern observed in the oligodendroglial phenotype. This pattern of sparse EGFP+ label is maintained throughout early postnatal development and the establishment of synaptic connectivity. It may reflect a low, steady-state activation of native and transgene promoter elements that accompanies expression of sr-PLP, but not classic PLP. It is not upregulated during myelinogenesis, which is not an autonomous function of the neuronal phenotype. To a reasonable certainty, colocalization of EGFP and sr-PLP in neostriatal neurons of transgenic mice is in accord with marginal observations of sparsely labeled neostriatal neurons that localize sr-PLP in wild-type mice [pers. obs., 10]. Taken together, these results suggest that *PLP* gene expression, insofar as it pertains to production of sr-PLP, is a shared characteristic of neuronal and oligodendroglial phenotypes in neostriatum in mice, perhaps derived from a common precursor within their respective cell lineages.

PLP promoter transgene activation in a neuronal phenotype in neostriatum is consistent with and extends previous reports of EGFP reporter localization in brainstem and hypothalamic neurons [2, 11]. These earlier studies, which do recognize the colocalization relationship between *PLP* promoter transgene and sr-PLP expression in neuronal and oligodendroglial phenotypes, do not localize these markers in neostriatum. They provide no systematic account of emergent morphological and electrophysiological characteristics of neurons with *PLP* promoter transgene activation as they progress through distinct developmental stages, important features of the present study. A recent report demonstrates conditional activation of a *PLP* promoter transgene in forebrain neurons [35]. Focused on cerebral cortex, it also shows derivation of activated neostriatal neurons from a pre-existent neuronal lineage, similar to our findings of immature EGFP+ NPCs and neurons, and consistent with infrequent but persistent postnatal neurogenesis of mouse neostriatum. In neostriatal neurons, tamoxifen triggered, over a permissive background of native development, a late, rapid onset of low, steady-state transgene activation accompanied by accumulation of PLP mRNA. We show that the native course of development is adequate to trigger and maintain an early, gradual onset of low, steady-state transgene activation in neostriatal neurons.

There is probable cause to suspect that *PLP* promoter transgene activation leads to upregulation of PLP proteins in mutant vs. wild-type mice, an issue complicated by inclusion of lineage-specific enhancers in constructs used to generate these transgenes [36]. In young neostriatal neurons, sr-PLP is clearly shown in transgenic mice by fluorescence immunohistochemistry, but less certainly shown in wild-type mice by peroxidase immunohistochemistry, a more efficient detection method. Enhanced levels of *PLP* gene expression in transgenic mice do not appear to impair the viability of neuronal or oligodendroglial phenotypes, which resemble intact neostriatum in wild-type rodents studied at similar ages [26, 27, 29]. Such negative functional outcomes, drawn from indirect comparisons of transgenic and wild-type mice, are suggestive but not conclusive; however, they weigh against proposals that augmented levels of *PLP* gene products, particularly classic PLP isoforms, reduce cell survival via programmed cell death [6, 8].

The new evidence for *PLP* promoter transgene activation and sr-PLP expression in a single neuronal phenotype in developing neostriatum, medium spiny neurons, has several significant implications: (1) It lends credibility to recognition of sparse sr-PLP accumulation in this

largest subpopulation of mature neostriatal neurons, which indicates that *PLP* gene expression has both maintenance and developmental roles in restricted neuronal phenotypes. Within neostriatum, contrasts between medium spiny neurons (with sr-PLP expression) and large aspiny neurons (without sr-PLP expression) may help to resolve non-canonical roles of PLP isoforms in endocytotic trafficking/recycling, ion exchange, and/or process outgrowth [7]. (2) Medium spiny neostriatal neurons synthesize γ -aminobutyric acid as their signature inhibitory neurotransmitter [37], a characteristic not associated with neocortical, brainstem and hypothalamic neurons previously shown to have *PLP* gene expression [10, 11, 15, 30]. This suggests that *PLP* gene expression is unrestricted in regard to excitatory vs. inhibitory neurotransmitter signatures. (3) Medium spiny neostriatal neurons emit myelinated projection and unmyelinated local collateral axons [38, 39]. Myelination of projection axons through interactions with interfascicular oligodendroglia is unlikely to be a direct consequence of their autonomous *PLP* gene expression, initiated before the onset of myelinogenesis and with different outcomes in projection vs. local axonal domains.

The sr-PLP products of the *PLP* gene arise from alternative splicing of mouse exon 1.1 [8]. This exon is not conserved in humans, but two novel exons found within intron 1 of the human *PLP1* gene [40] may have similar functions to those performed by the mouse sr-PLP tran-

script. Their alternative splicing generates four novel mRNAs. Three show neuronal expression in human cerebral cortex, cerebellum and neostriatum, and one leads to a neuronal PLP protein product. These human PLP transcripts and proteins show patterns of expression that resemble mouse sr-PLP [10, 11], and neostriatal expression of *PLP* gene products may be a conserved feature of mammalian evolution. Neurons may also serve as a pathologic locus, and perhaps a primary pathologic cause, in human neurological disorders associated with mutations in the *PLP* gene [15, 16]. Accordingly, postmortem tissue shows significant neuronal loss in PMD cases with duplications of human *PLP1* [13]. Many sites of neuronal loss in this disorder correspond to brain regions in transgenic mice, including neostriatum, that contain neuronal phenotypes capable of *PLP* promoter activation and sr-PLP expression [40]. Similar forms of degeneration of neostriatal projection neurons occur in spastic paraplegia type 2 [41] in association with mutations in the human *PLP1* gene.

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