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Transgenic models for investigating the nervous system: currently available neurofluorescent reporters and potential neuronal markers

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Abstract

Recombinant DNA technologies have enabled the development of transgenic animal models for use in studying a myriad of diseases and biological states. By placing fluorescent reporters under the direct regulation of the promoter region of specific marker proteins, these models can localize and characterize very specific cell types. One important application of transgenic species is the study of the cytoarchitecture of the nervous system. Neurofluorescent reporters can be used to study the structural patterns of nerves in the central or peripheral nervous system *in vivo*, as well as phenomena involving embryologic or adult neurogenesis, injury, degeneration, and recovery. Furthermore, crucial molecular factors can also be screened via the transgenic approach, which may eventually play a major role in the development of therapeutic strategies against diseases like Alzheimer's or Parkinson's. This review describes currently available reporters and their uses in the literature as well as potential neural markers that can be leveraged to create additional, robust transgenic models for future studies.

Introduction

Detailed analyses of the cytoarchitecture of the nervous system have been accomplished by various means in the past. In 1873, Camillo Golgi introduced the silver stain that enabled the labeling of entire neurons and established the foundation on which modern neuroscience was built [1]. Using this Golgi stain, Cajal was able to reveal the diversity of morphologies within the human nervous system [1], demonstrating that some neurons like select granule cells can have simple processes extending from a small cell body, while others such as

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Purkinje cells can have more complex projections. The characterization of the morphology and overall network architecture of neurons is a subject heavily covered in the scientific literature, perhaps due to its direct implications in revealing the cells' underlying functions and connectivity with surrounding tissue. The culmination of efforts to map the global neural connections in the brain ("connectomes") has greatly expanded our understanding of the organization of the central nervous system [2]. Immunohistochemistry has also been a common tool for identifying neuronal subtypes and distinguishing them based on biochemical charact eristics. Highlighting cells involved in the activity of a certain neurotransmitter and identifying neurons that express a certain protein are tasks enabled by this method. The discovery of neuron-specific enolase and non-neuronal enolase as markers for neuronal and glial cells, respectively [3], has paved the way for immunolabeling to become a widely used tool, with subsequent descriptions of other relevant markers (external glycoprotein, choline acetyltransferase, parvalbumin, neurofilament protein, etc.).

The advent of recombinant DNA technology soon permitted the growing use of transgenic species that express fluorescent reporters developed for use in imaging studies. The elegance of transgenic models can be seen not only in the vibrant images taken via fluorescent microscopic techniques like confocal microscopy but also in their ability to provide visual evidence for physiologic or pathologic phenomena in the nervous system. In 2019 alone, this method was used for research related to Huntington's disease [4], Alzheimer's disease (AD) [5, 6], Parkinson's disease [7], and amyotrophic lateral sclerosis (ALS) [6], and there has been significant effort to innovate novel transgenic species to understand other poorly understood disease states like schizophrenia [8]. Another unique area of study is the eye, which receives input from both the central (CNS) and peripheral nervous system (PNS) [9]. The cornea has become a popular tissue for the visualization and study of peripheral nervous structure, repair, and regeneration due to its accessibility, innate avascularity , and immune privilege [10]. Recently, Bouheraoua et al. compiled a collection of transgenic mice reporters for corneal nerve visualization, including CGRP:GFP BAC and Wnt1:Cre, TAG-1:Cre, En1-Cre, Islet1:Cre, and Ret:CreER, that can be crossed with lines expressing lox-P-containing fluorescent reporters to generate novel reporter lines [11]. Notably, several of the neurofluorescent reporters reviewed herein have been employed in studies of nerve structure and regeneration in the cornea [11].

Thy1-YFP (yellow fluorescent protein) and Nestin-GFP (green fluorescent protein) are two well-described examples of fluorescent genes coupled with neurally expressed genes to enable in vivo imaging of nervous tissue. Thy1 (CD90) is a cell surface glycoprotein that contributes to intercellular communication, particularly in the immune and nervous systems. Various studies have demonstrated that Thy1 expression occurs predominately in the late stages of postnatal development; hence, the Thy1-YFP reporter line can be a tool for studying non-embryonic neural development and response to injury [12]. Nestin, on the other hand, is an intermediate filament expressed in many cell types including nervous tissue. Nestin-GFP allows visualization of neural progenitor cells in the CNS, especially during embryonic development. This also becomes particularly relevant in recent neuroscience research, with attention recently given to describing the unclear nature and mechanism of adult neurogenesis [13]. Thus, in this review, we present an extensive

discussion of currently available neurofluorescent reporter mouse models and discuss their possible utilities for investigations of the nervous system.

Biochemical markers and neurofluorescence

The synthesis of fluorescent reporters is made possible by the identification of specific molecular biomarkers that can be engineered to fluoresce via insertion of the gene of a known fluorescent protein (e.g., GFP, YFP, DsRed, mCherry) into the transcriptional site of the biomarker DNA [14]. The identification of biomarkers that represent a specific tissue of interest is crucial for the development of a transgenic neurofluorescence reporter model. Table 1 reviews some key nerve markers and provides examples of their previous experimental use [15].

Thy1 and Nestin remain amongst the most popular markers used in the generation of transgenic neurofluorescent reporter animal models. Thy1 has been particularly useful in visualizing corneal nerves. On the other hand, Nestin has been useful in retinal imaging due to its strong expression in the CNS [22, 23]. The following sections expand upon the clinical utility of potential nervous tissue markers in more detail.

Thy1

Thymus cell antigen 1 (Thy1 or CD90) is a glycosylphosphatidylinositol (GPI)-anchored cell surface glycoprotein involved in various cellular processes that include cell adhesion and communication [24]. Thy1 is expressed in various cell types in mice, particularly those involved in the nervous and immune systems. In order to selectively label neuronal Thy1 with YFP, the YFP transgene is directed by modified regulatory elements of the Thy1 gene that has a targeted deletion of exon 3 and its flanking introns [25, 26].

Thy1-YFP reporter mice allow for *in vitro* and *in vivo* fluorescent imaging in both the CNS and PNS. Alic et al. found that at day E14.5 in Thy1-YFP-16 transgenic mice, 22% of CNS neurons are positive for Thy1- YFP, and during 1 month of post embryonic development, the percentage of Thy1-YFP-positive neurons in the cortex will more than double to 50% [27, 28]. Thy1-YFP expression thus increases within multiple tissues over the course of embryonic development. Expression of Thy1-YFP also persists in mature neurons, which are characterized by morphological maturation, completion of neuronal migration, and the initiation of dendritic growth [29, 30]. Therefore, Thy1-YFP reporter mice can be used to visualize nerve repair and regeneration following injury after neural development [31].

In Thy1-YFP reporter mice, YFP-labeled axons comprise a minority of the total neuronal population, allowing for clear visualization of individual neurons and their branching patterns. Yu and Rosenblatt first proposed Thy1-YFP-16 reporter mice as a robust model for *in vivo* nerve imaging in the cornea [10]. Most research in Thy1-YFP mice has utilized two mouse lines-, Thy1-YFP-H and Thy-1-YFP-16 (Table 2), which have variable expression patterns of YFP in nervous tissues [32, 33].

Many studies investigating peripheral nerve injury have utilized Thy1-YFP expression in a subset of sensory nerves that innervate the transparent cornea to monitor nerve changes and

evaluate putative neurotrophic factors [34]. Using Thy1-YFP-16 reporter mice, Namavari et al. demonstrated that surgically transected corneal nerves recover to normal density, albeit with abnormal arrangements, compared to uninjured mouse corneas (Figure 1) [35]. In another recent study, Sarkar et al. disrupted Thy1-YFP mouse stromal nerve trunks *in situ* using a far infrared laser, which allowed the assessment of nerve regeneration after point, rather than planar, transections [36]. This 2019 study reinforces the utility of a Thy1-YFP reporter line for studying corneal nerve repair and healing processes *in vivo* [36].

Other studies have demonstrated that neurotrophins such as brain-derived nerve factor (BDNF), small proline-rich repeat protein 1A (Sprr1a), semaphorin 3A (Sema3A), and vascular endothelial growth factors A and B (VEGFA and VEGFB) are expressed post injury and contribute to corneal nerve regeneration *in vivo* [37–39], while the immunosuppressant cyclosporine inhibits corneal nerve growth [40]. Another study found that in Thy1-YFP mice, YFP+ bone marrow cells (YFP+ BMCs) infiltrate the cornea after excimer laser annular keratectomy and have the ability to promote trigeminal ganglion neurite growth through secretion of growth factors, like such as neural growth factor (NGF) [22].

The Thy1-YFP reporter line has been particularly useful in studies of peripheral neuropathy caused by diabetes mellitus. Corneal nerves in Thy1-YFP-16 mice treated with streptozocin (STZ) were viewed by high-resolution *in vivo* imaging using two types of confocal microscopy: laser scanning microscopy (CLSM) and two-photon microscopy (TPM) (Figure 2). CLSM is a non-invasive method for assessing nerve fiber changes and, helping determine the extent of nerve damage in diabetic patients. TPM is a form of nonlinear microscopy possessing a high signal-to-noise ratio and capable of producing can produce multimodal *in vivo* images. Both CLSM and TPM support a statistically significant decrease in corneal nerve fiber density in STZ-diabetic mice compared to the control mice [23]. According to the wide variety of potential imaging applications for Thy1-YFP reporter mice, we expect such imaging approaches to be useful for visualizing nervous structures in tissues beyond the cornea [41].

Outside of the cornea, a number of studies have used the Thy1-YFP reporter model to characterize peripheral and central nerve morphology and response to injury. Bierowski et al. observed Wallerian degeneration of distal tibial nerve axons in Thy1-YFP-H mice following sciatic nerve axotomy and validated a delay in degeneration in WldS-overexpressing mutants [42]. In a seminal paper that assessed nerve injury and regeneration *in vivo*, Kerschensteiner et al. characterized nerves undergoing acute axonal degeneration in contrast to the relatively delayed Wallerian degeneration. This was achieved by utilizing time-lapse imaging of a Thy1-GFP-S fluorescent reporter mouse line after nerve transection of centrally-projecting dorsal root ganglia (DRG) neurons. This study demonstrated that although many transected central neurons attempt to regenerate within 24 hours of injury, they are unable to meet their original, pre-injury tissue targets [43]. Alternatively, Carter et al. used a thoracic dorsal column crush spinal cord injury model in Thy1-YFP-H mice to characterize CNS changes within the cortex layer V pyramidal neurons and demonstrate the protective effects of chondroitinase ABC intracerebroventircular infusions, demonstrated as reduction in cell atrophy, increased axonal sprouting, upregulation of ERK1 and other

kinases thought to be important to neuronal survival [44]. This study verifiedthat the responses of intrinsically YFP-labeled neurons were comparable with those of neurons labeled with retrograde tracing methods, such as Fast Blue cell soma labeling. Additionally, patterns of fragmentation in Thy1-YFP+ neurons paralleled patterns of Wallerian degeneration as assessed by classical light microscopy and electron microscopy techniques (Figure 3) [42]. Therefore, although Comley et al. raised valid concerns that the fluorescent proteins expressed in Thy1-YFP mice are not biologically inert in degeneration studies and may have diverse unpredictable effects on neurodegeneration [45], the aforementioned findings by Carter et al. and Bierowski et al. reinforce the appropriateness of this transgenic reporter for the study of neuronal responses to injury.

Beyond mice, recently developed transgenic Thy1-GFP rat reporters have allowed for the study of nerve injury in a larger and longer-living animal model. For example, facial nerve re-innervation of the zygomaticus muscle was assessed 4 weeks after crush injury via confocal microscopic imaging of Thy1GFP labeled neurons in rats [46, 47].

In summary, Thy1 reporter lines demonstrate fluorescence only in a subset of central and peripheral neurons. However, this limited expression has proven to be particularly useful for *in vivo* imaging studies that track individual neuronal responses to injury. Use of Thy1 reporter lines has led to a number of breakthroughs in our understanding of nerve injury, regeneration, and putative therapeutic targets for promoting nerve repair.

Nestin

Nestin is a class VI intermediate filament (IF) protein and marker of neural stem cells that is expressed in many cell types. Through co-expression with GFP, Nestin has been used to identify progenitor cells in the CNS. Neural stem cells express Nestin during early embryonic nervous system development, but the protein is downregulated as these cells differentiate into mature cells. The differentiated cells in turn express either glial fibrillary acidic protein (GFAP) or neurofilaments. Therefore, the expression of Nestin can be used to assess the undifferentiated state of neural progenitor cells, whereas downregulation of Nestin correlates with differentiation of these cells in the developing CNS (Figure 4) [27, 48]. Mignone et al. confirmed this finding by using Nestin-GFP to identify undifferentiated neural stem cells [49].

Nestin deficiency leads to reduced self-renewal ability in neural stem cells, but surprisingly, such deficiency has no measurable effect on cytoskeletal integrity [50]. Moon et al. found that Nestin is expressed in Müller cells (glial cells of the retina) following acute injury, such as in experimentally induced glaucoma and pharmaceutically induced retinal degeneration. Upon inducing retinal degeneration using N-methyl-N-nitrosourea (MNU), cellular localization and temporal patterns were studied. Nestin expression on days 3, 5, 7 and 21 following MNU treatment was analyzed by Western blotting (Figure 5). The Nestin expression levels sharply increased following MNU treatment before returning to baseline, suggesting that this protein could be used as an immediate marker of acute retinal injury [51].

Nestin-GFP models have been used to analyze neuronal and glial markers in various parts of the gastrointestinal tract of newborn and adult mice under homeostatic conditions. Grundmann et al. found that the myenteric plexus houses a specific Nestin-GFP–positive neuron population with both marker expression and neuronal morphology (nNGFP). It was concluded that Nestin-GFP can serve as a marker of neuronal plasticity in enteric neurons [52].

Nestin-GFP models have also been used *in vivo* to follow mouse mammary tumor (MMT) cells and the subsequent nuclear-cytoplasmic deformation and partition during cancer cell death following immune rejection. Nestin is found beyond the nervous system in tumors, and the protein localizes to the nucleus in tumor cells. MMT cells were dual stained with red fluorescent protein (RFP) in the cytoplasm and Nestin-GFP in the nucleus, and at 6 days after their transplantation, GFP-expressing nuclei partitioned from RFP-expressing cytoplasm [53].

Although Nestin may not be a useful marker for neurons of the PNS, it represents a particularly useful marker for tracking early CNS development and tumor progression.

βIII-tubulin

βIII-tubulin is a moderately sensitive and specific nerve marker. Liu et al. synthesized a transgenic βIII-tubulin-YFP mouse reporter and demonstrated that βIII-tubulin is expressed in the developing CNS and PNS from embryonic day 9 onwards, as well as postnatally in structures that exhibit adult neurogenesis, such as the subgranular layer and hilus of the dentate gyrus (Figure 6) [17].

Another study conducted by Hwang et al. found that βIII-tubulin also serves as a strongly predictive marker for tracking the response to chemotherapy in metastatic gastric cancer, suggesting a clinical use for βIII-tubulin that extends beyond the nervous system [54].

Neurofilament heavy chain (NF-H)

Neurofilament (NF) chains generally consist of three filament proteins: light (L), medium (M), and heavy (H). These proteins play a major role in the maintenance of neuronal caliber. NF-H (Neurofilament-200) is specifically involved in axonal phosphorylation and neurofilament transport processes in mature axons; the two smaller NF proteins (NF-L and NF-M) are not known to have these functions. Furthermore, NF-H is thought to be involved in abnormal neurofilament accumulation during neurodegenerative disease processes; for example, mutations in the C-terminal of NF-H have been discovered in patients with ALS [18]. Within the sensory system, NF-H has classically been used as a marker of larger myelinated sensory neurons [55].

In another study exploring the degeneration and regeneration of corneal nerves, C57BL/6J mice were infected with herpes simplex virus type 1 (HSV-1) [56]. Corneas were harvested at predetermined time points following infection, and their nerves were assessed for expression of different biomarkers such as βIII-tubulin and NF-H by immunohistochemistry [57]. Analysis of corneal whole mounts revealed a regression of sensory fibers (and NF-H+

and βIII-tubulin+ staining) by day 8 post-infection. Moreover, this was followed by a pathological response characterized by abnormal nerve regeneration (with an abnormal presence of NF-H+ nerves) [58].

S100

The S100 group of proteins consists of 24 members and is expressed only in vertebrates. These members are functionally categorized into three subgroups: those with intracellular regulatory effects, those with extracellular regulatory effects, and those with both. Their expression is linked to the refinement of cell-specific gene expression and responses to external stimuli [59].

Certain cancers have been shown to express S100, including schwannoma and melanoma. S100A1 is abundantly expressed in skeletal muscle fibers, cardiomyocytes, and certain neuronal populations. S100A2 expression is downregulated in many cancers, where a loss of nuclear expression is associated with poor prognosis [59]. Extracellular S100A4 and S100B interact with epidermal growth factor and basic fibroblast growth factor, respectively. These interactions enhance the ability of the growth factors to affect the downstream activity of their corresponding receptors. In certain cases, the increased expression of an S100 protein may be an indicator of the cell's response to a stressor. For example, S100B is not typically expressed in cardiomyocytes but has been observed after an infarct, as it functions to limit the hypertrophic response of cardiomyocytes [59]. By using a S100B-EGFP transgenic reporter, Vives et al. found that S100B is expressed in both CNS and PNS glial cells, as well as certain neuronal populations. However, the diffuse expression and unknown precise physiological function of S100B in nervous tissue reduce its utility in monitoring individual neuronal growth and repair patterns following injury [60].

Substance P (SP)

Substance P (SP) is an 11-amino acid neuropeptide involved in pain transmission, inflammation, and wound healing. SP exerts its actions by interacting with neurokinin receptors, like NK-1. SP is expressedin naïve corneal nerve fibers and mobilizes stem cells for corneal repair [61]. SP can also activate epidermal growth factor receptor (EGFR), mitogen-activated protein kinases (MAPKs), extracellular signal–regulated kinases (ERKs), and phosphoinositide 3-kinase–Akt (PI3K-Akt) signaling pathways. Yang et al. analyzed the mechanism of SP in corneal epithelial wound healing in type 1 diabetic mice. After induction of type 1 diabetes in adult male C57BL/6 mice, topical SP was administered to the eye [62]. The effects of SP on corneal sensitivity and epithelial wound healing were assessed in control, diabetic, and NK-1 receptor antagonist-injected mice. SP application attenuated corneal sensitivity and significantly improved corneal epithelial wound healing in diabetic mice. In mice injected with NK-1 antagonist, SP did not alter the activation of the Akt, EGFR, and Sirt1 signaling cascades, and thus, epithelial wound healing was not observed [19].

Neuron-specific enolase (NSE)

Neuron-specific enolase (NSE) is an isoenzyme of the glycolytic enzyme enolase that is found in terminally differentiated neurons and neuroendocrine cells. As CNS neurons increase in number, levels of NSE also increase. In adults, NSE is one of the most abundant brain-specific proteins [20]. The utility of NSE in imaging mouse neurons when paired to a fluorescent marker is relatively unknown. However, one study that utilized a rat gamma protein kinase C-GFP reporter (which was under the control of an NSE promoter) confirmed that GFP expression was present in the majority of neurons and at significantly higher levels in pups than in adult rats.

Piezo2

Piezo channels represent a novel class of mechanically sensitive channels. A study on Piezo2 expression in guinea pig corneal afferent neurons found that approximately 26% of trigeminal ganglion neurons (Figure 7) and 30% of corneal afferent neurons express Piezo2. These neurons are neurochemically distinct from corneal polymodal nociceptors or cold-sensing neurons, suggesting that Piezo2 may solely contribute to the transduction of noxious mechanical stimuli. Therefore, Piezo2 could be a useful marker for analyzing the effects of physical injury and trauma in the cornea [63].

Calcitonin gene-related peptide (CGRP)

Calcitonin gene-related peptide (CGRP) is a well-documented marker of nociceptive DRG neurons. A 2012 study that used CGRPα-GFP reporter mice and confocal microscopy imaging found that CGRPα(+) DRG neurons contribute to responses in a pathway that is distinct from nonpeptidergic and cool temperature nociceptive pathways (Figure 8). A wide range of nociceptive ligands induced mostly small-to medium-diameter CGRPα-GFP+ neurons; these neurons innervate various cutaneous and visceral tissues, such as the submucosal layer of the small intestine. CGRPα-GFP expression was additionally present in a small subset of motor neurons, neurons intrinsic to the dorsal spinal cord, and in regions of the brain (which includes the visual cortex) [21].

Transient Receptor Potential Ankyrin 1 (TRPA1)

Transient Receptor Potential Ankyrin 1 (TRPA1) is a member of the structurally related TRP family, which includes seven channel groups: TRPA (ankyrin), TRPC (canonical), TRPN (no mechanoreceptor potential C), TRPM (melastatin), TRPML (mucolipin), TRPP (polycystin), and TRPV (vanilloid). Rec ent research on TRPV1 and TRPA1 demonstrated a correlation between these two channels. A study conducted by Fernandes et al. showed that 97% of TRPA1-expressing sensory neurons concomitantly express TRPV1. On the other hand, 30% of TRPV1-expressing neurons also express TRPA1. TRPA1 was shown to play an important role in pain and neurogenic inflammation via sensory nerve activation, aiding in the integration and processing of noxious stimuli [64] . As such, there may be a role for TRPA1 in further understanding the pathology of disease states where sensation to noxious stimuli is attenuated or diminished.

Transient Receptor Potential Vanilloid type 1 (TRPV1)

Transient Receptor Potential Vanilloid type 1 (TRPV1) is a non-specific cation channel that reacts to noxious stimuli, making it a mediator of inflammation. The expression of TRPV1 in the nervous system has become a recent topic of discussion. Some studies have reported that TRPV1 expression is limited to extrinsic afferent fibers, while others have argued for a role of TRPV1 in intrinsic afferent fibers. In a study by Buckinx et al., the distribution pattern of TRPV1 expression was shown to be dependent on the type of antibody used in immunohistochemical staining. This study was carried out using two antibodies directed against different epitopes of TRPV1. One primarily stained neuronal fibers, while the other stained perikarya of enteric neurons [65]. This suggests that different modulated forms of TRPV1 exist, and that each displays a unique imaging distribution pattern based on the specific antibody used.

Cavanaugh et al. used two separate lines of TRPV1 reporter mice by crossing TRPV1-Cre mice with multiple Cre-dependent reporter lines to investigate primary afferent expression of TRPV1. They found that TRPV1 is transiently expressed in peptidergic and nonpeptidergic C-fibers as well as in some myelinated DRG neurons during development (Figure 9). This expression ultimately narrows over time to a specific group of peptidergic sensory neurons. The study also emphasized that TRPV1 is more extensively expressed in primary pain afferents than previously thought [66]. An additional study found that 45% of corneal afferent neurons expressed TRPV1 in a guinea pig model, of which most neurons serve as polymodal nociceptors. This supports the notion that TRPV1 serves as a potential modulator of corneal sensation processes [67].

Additional neurofluorescent reporter mouse lines

Table 3 and the figures that follow serve to reinforce and further add to the list of transgenic mouse reporter lines that have been previously used in the context of *in vivo* imaging studies.

Discussion

In the last decade alone, there has been rapid development in tissue biology research, which can be partially attributed to the advent of transgenic fluorescent reporter mice. Specifically, an extensive literature search demonstrates a handful of useful neurofluorescent reporters now available for nerve assay-related studies. Each mouse reporter described in this review has distinctive features that make it applicable to specific neuroscience investigations. Collectively, we can appreciate the advantages that fluorescent mice provide for the *in vivo* study of both sensory and motor nerves in the CNS and PNS.

These mice have demonstrated stable and robust fluorescence along their axons, often extending to their terminals.[248] With these advantages, neurofluorescent reporter mice have been used extensively in brain imaging, especially for neurofluorescence using the promoter region of Thy1. [27, 68, 71, 74–76, 80, 83, 86, 87, 93, 112, 249–252] Some transgenic neurofluorescent reporters can additionally express multiple colored fluorescent proteins in different subsets of neurons via the *Brainbow* system, which uniquely labels

neurons with distinct colors using Cre/lox recombination to create a random expression between multiple fluorescent proteins. Brainbow elevates the utilization of neurofluorescent reporter mice for strategies such as for neuronal mapping, cellular dynamics, lineage tracing, and in-depth genomic and genetic analysis.[253, 254]. Nestin, another robust promoter for neurofluorescent, along with co-expression of GFP, has also been extensively used for investigating the nervous system, specifically in early CNS embryonic development. Nestin expression correlates with the undifferentiated state of neural progenitor cells, and its downregulation correlates with the differentiation of these cells [27, 49, 50, 52, 131, 255–258]. To further enhance the capability for deep tissue imaging and to preserve the quality and fluorescence of the specimens, several techniques of tissue preparation have been introduced, such as using dibenzyl ether clearing medium and tetrahydrofuran dehydration medium or utilizing the CLARITY or uDISCO clearing protocol.[259–262]

The ability to combine fluorescence has also permitted discoveries relating to the physiological competition and elimination of neonatal synapses that occurs in neuromuscular junctions [263]. Recently, transgenic reporter mice have been used to assess nerve regeneration as well as the performance of bioengineered nerve guidance conduits [264]. Fluorescent reporters optimize the time and cost associated with *in vivo* imaging, and ultimately, serve as a very useful tool in the detailed study of nerve physiology and pathophysiology.

Given that research using transgenic mice is becoming increasingly popular, novel reporter subtypes beyond those discussed in this review are continuously being generated. Transgenic reporters have been valuable in studies of nervous system development [265], physiology, and poorly understood pathologies, such as neurodegenerative diseases like Alzheimer's [266] and prion disease [267], as well as ethanol-induced neurodegeneration during fetal development [268]. This approach has also shown promise in ophthalmology research that extends beyond the cornea; one recent study successfully implemented spatiotemporal mapping to quantified patterns of photoreceptor degeneration in retinitis pigmentosa [269], while another explored potential neuroprotective features of mitochondrial uncoupling protein 2 (UCP2) in a glaucoma mouse model [270].

Though transgenic animal models have fundamentally changed modern approaches to understanding nervous system function and disease states, the generation of appropriate visualization models is a challenging and time-consuming process. One of the limitations to this process occurs in the pronuclear promoter transgene assembly injection. For a gene that is expressed in many cell types, the regulatory mechanism for that gene within a specific system is difficult to ascertain from imaging studies alone. Additionally, cis-acting elements that serve as enhancers to promote the specificity of expression could be located too far from the gene's recorded start site and may not have been fully identified yet [271]. During this process, the expected repetition of specific gene patterns by a relatively short promoter is not guaranteed. To date, only a small number of such promoters have successfully driven cell-specific expression in transgenic mice neurons [272]. Moreover, the integration process of these transgenes is a random process, and thus, their expression can be vastly influenced by uncontrolled activation/inactivation effects. This results in an expression that is ectopic and impertinent to the promoter in question or suppressed in targeted cells.

Transgene synthesis may also fail due to incomplete, erratic, and sometimes variable expression across the targeted cell region of an individual animal. For example, in the Thy1.2 YFP-16 model, it was found that YFP is only expressed in a select portion of non-nociceptive sensory neurons, while a significant portion of large, NF200-positive sensory neurons fail to express YFP [273]. Inherent variability among animal subjects being treated is also a significant limitation. These methods require laborious validation and verification of the expression of the transgenes for each animal. Interpreting data variation within mice groups can thus be a difficult task. To overcome these limitations, it seems necessary to develop transgenic lines of mice that have genetically encoded actuators and indicators that can produce regulated and inducible expressions. Additionally, there are conflicting data regarding the biological inertness of fluorescent proteins *in vivo*. Feng et al. claimed that repeated imaging is neither toxic nor phototoxic [32]. However, Comley et al. claimed that neuronal cells expressing YFP exhibit cell stress at the RNA and protein levels, leading to diverse and unpredictable effects on neurodegeneration pathways [45]. Furthermore, it is difficult to predict the variance of transgene expression due to the effects of genomic integration sites and transgene copy numbers, as mice used to exhibit a high expression level of transgenes are usually infertile and not viable.

The purpose of this review was to provide an in-depth exploration and discussion of the major transgenic fluorescent reporters used in nerve tissue visualization, including important considerations of their limitations and local expressions. For instance, Nestin has been used in studies of neural progenitor cell development, while S100 expression has been used to assess neoplastic growth and longitudinal therapeutic responses. These markers can be further used in studies that aim to uncover the molecular regulators driving nerve development following injury. For example, studies investigating the effects of NGF on corneal nerve regeneration and patterning may utilize a Thy1 transgenic mouse model in order to track the changes in nerve structure over time. Alternatively, investigations of the interactions of nerve patterning with vasculature (or other structures) following injury could bolster our understanding of how nerve regeneration may involve signaling pathways that extend beyond the nervous system. Future studies may also directly compare the relative success of IVCM versus transgenic mouse reporters in studies of neuronal regeneration and repair.

The application of transgenic mouse reporters extends beyond studies of just the nervous system. For example, one recent study used a type 1 collagen promoter-driven GFP reporter to study osteoblastic activity during skeletal regeneration [274], while another used Rip1Tag2 transgenic mice to monitor the progression of pancreatic neuroendocrine tumors [275]. The application of Flt1-dsRed and Prox1-GFP transgenic reporters in our lab at the University of Illinois have further enabled visualization of blood and lymphatic vessels, respectively. Such reporters have been important when analyzing patterns of neovascularization following corneal injury and how knockout of selected growth factors and their receptors may reduce unwanted vascular proliferation that results from hypoxic disease states. These transgenic reporters have also been bred to generate a Prox1-GFP/Flt1-dsRed (PGFD) dual transgenic reporter that permits simultaneous visualization of both vessel types[276] [277]. Similarly, we have bred Thy1-YFP with Flt1-dsRed mice to establish a dual blood vessel and nerve reporter, useful in imaging neurovasculature in

tissues including spinal dorsal root ganglia and the nervous plexus of the gastrointestinal tract. The generation of dual transgenic reporters serves to improve knowledge of the mechanistic interactions shared between multiple organ systems (rather than one system in isolation), and how the pathophysiology of one system may influence and/or be influenced by the other.

Sümbül et al. has introduced a reproducible, objective approach to classify neuronal cell types, by using dendritic arbor distribution and density as well as molecular characteristics of the cells.[278, 279] This algorithm was tested using the retina as the study bed, which is known to contain diverse types of neuronal cells,[280, 281] specifically the retinal ganglion cells (RGCs).[278, 279] It would be very compelling to combine this classification technique with the available neurofluorescent reporter transgenic mice. This could create a more robust and accurate neuronal cell classification system that may further impact the development of therapies for many nervous system-related diseases, such as those in the cornea and retina.[282]

Beyond injury models, transgenic reporter mice enable us to observe the anatomical evolution of neurons and vascular structures that occur because of development or normal aging. This serves to improve knowledge of normal physiologic functioning and isolate age-related structural changes from potentially pathologic ones. Ultimately, the application of this experimental approach can extend across multiple lines of research that take advantage of *in vivo* tissue imaging techniques. With the emergence of advanced imaging approaches such as those discussed in this paper, a clearer concept of the interrelationship between structure and function, as well as the mechanisms that drive inflammation and healing may pave the way for the development of improved therapeutic interventions for relevant pathologies.

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List of Abbreviations

5-HT	5-Hydroxytryptamine (serotonin)
ACh	Acetylcholine
Akt	Protein kinase B (PKB)
Mapt$^{tm1(EGFP)kit}$	Transgenic mouse reporter for microtubule-associated protein tau
βIII-tubulin-YFP	Transgenic mouse reporter for βIII-tubulin neuronal marker
BAC	Bacterial artificial chromosome (DNA construct)
BGEM	Brain gene expression map

BMC	Bone marrow concentrate
CCK-RGC	Cholecystokinin-retinal ganglion cell
CFP	Cyan fluorescent protein
CGRP	Calcitonin gene-related peptide
CGX	Cingulate cortex
ChAT^{BAC}-EGFP	Transgenic mouse reporter for choline acetyltransferase expression
ChAT-ChR2-EYFP BAC	Channel rhodopsin-expressing fluorescent mouse reporter
CLSM	Confocal laser scanning microscopy
CNPase	2’,3’-Cyclic-nucleotide 3’-phosphodiesterase (myelin-associated enzyme)
CNPase-GFP	Transgenic mouse reporter
CNP-EGFP	Transgenic mouse reporter
CNS	Central nervous system
Cp	Choroid plexus
CPZ	Cuprizone (copper-chelating agent)
Cre	Protein used in site-specific recombinant technology
CRISPR-Cas9	Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9, a DNA editing technology
CST	Corticospinal tract
CTb-594	Cholera toxin beta subunit coupled to alexa 594 (axon tracer)
D1-MSN	Direct dopamine pathway medium spiny neuron
DAPI	4′,6-Diamidino-2-phenylindole (blue-fluorescent DNA stain)**dLGN**: Dorsolateral geniculate nucleus of the thalamus
Drd1a-EGFP	Transgenic mouse reporter for type 1a dopamine receptor gene
Drd1a-tdTomato	Transgenic mouse reporter for type 1a dopamine receptor gene

Drd2-EGFP	Transgenic mouse reporter for dopamine receptor D2 expression
DRG	Dorsal root ganglia
ED	Embryonic day
EAE	Experimental autoimmune encephalomyelitis (used in transgenic mouse models)
EDL	Extensor digitorum longus
EGFP	Enhanced green fluorescent protein
EGFR	Epidermal growth factor receptor
EGL	External granular layer of the developing cerebellum
En1-Cre	Knock-in Cre expression mouse reporter line for the engrailed gene
ENS	Enteric nervous system
EPC	Endothelial progenitor cell
ERCC1	Excision repair cross-complementation group 1
FRAP	Fluoride-resistant acid phosphatase
GCL	Ganglion cell layer
GFAP	Glial fibrillary acidic protein
GFP	Green fluorescent protein
GFP-Cre	Transgenic mice reporter construct where GFP fluorescence is coupled to Cre expression
GPe	Globus pallidus externa
HDB	Horizontal diagonal band of Broca
Hoxb4	Homeobox B4 gene
hPRPH1-G	Peripherin-EGFP genomic reporter construct
HSV-1	Herpes simplex virus type 1
IBA	Ionized calcium binding adaptor molecule
IF	Immunofluorescence
iGABASnFR	GABA-sensing fluorescence reporter
INL	Inner nuclear layer of the retina

IVCM	*In vivo* confocal microscopy
LASIK	Laser-assisted *in situ* keratomileusis
CLSM	Laser scanning microscopy
MAP2	Microtubule-associated protein 2
MAPK	Mitogen-activated protein kinases
Math1	Basic helix-loop-helix (bHLH) transcription factor (in neural progenitor cells)
Math1-GFP	Transgenic mouse reporter
MIP	Maximum intensity projection
ml	Molecular layer
MNU	N-methyl-N-nitrosourea
MOB	Main olfactory bulb
MP	Myenteric plexus of the gastrointestinal tract
mRNA	Messenger RNA
NE	Norepinephrine
Nestin-GFP	Transgenic mouse reporter
Neu-N	Neuronal nuclei specific marker
NF	Neurofilament
NF-H	Neurofilament heavy chain
NF-200	Neurofilament 200
NGF	Nerve growth factor
NGF-EGFP	Transgenic mouse reporter
NGFR	Mouse nerve growth factor receptor
NGFpr	Nerve growth factor promoter
NK	Neurotrophic keratitis
NK-1	Neurokinin-1 receptor that binds Substance P
NMPP1	Cell-permeable protein phosphatase 1 (PP1) analog
nNGFP	Neuronal Nestin-GFP
NPC	Neural precursor cell

NPY	Neuropeptide Y
NSE	Neuron-specific enolase
OCT	Optical coherence tomography
OEC	Olfactory ensheathing cell
ONL	Outer nuclear layer of the retina
OVA	Ovalbumin
p75NTR	p75 neurotrophin receptor
PACAP	Pituitary adenylate cyclase-activating peptide
PD	Postnatal day
Peripherin-EGFP	Transgenic mouse reporter
PI3K	Phosphoinositide 3-kinase
Piezo2	Piezo type mechanosensitive ion channel component 2
PKH26	Red fluorescent dye
PNS	Peripheral nervous system
PSD95	Postsynaptic density protein 95 (membrane associated guanylate kinase)
rAAV/EnvA	Recombinant adeno-associated virus/avian virus envelope protein
RAG	Recombination-activating gene
rAION	rodent Anterior ischemic optic neuropathy
Ret-Cre^{ER}	Transgenic mouse reporter
RFP	Red fluorescent protein
RT-PCR	Reverse transcriptase polymerase chain reaction
SC	Sympathetic chain
SCG	Sympathetic chain ganglia
Sirt1	NAD-dependent deacetylase sirtuin-1 protein
SMN	Spinal motor neurons
SNr	Substantia nigra pars reticulata
SOX2-EGFP	Transgenic mouse reporter

SP	Substance P or Submucosal plexus of the gastrointestinal tract
Sprr1a	Small proline rich protein 1A
Str	Striatum
STZ	Streptozotocin
TAG-1	Transient axonal glycoprotein-1 or contactin-2
TAG1-Cre	Transgenic mouse reporter
TG	Trigeminal ganglia
Thy1	Thy-1 cell surface antigen (Thy1 or CD90)
TPM	Two-photon microscopy
TrKA	Tropomyosin receptor kinase A
TRPA1	Transient receptor potential ankyrin 1
TRPC	Transient receptor potential channel
TRPM	Transient receptor potential melastatin channels
TRPM8	Transient receptor potential cation channel, subfamily M member 8
TRPML	Transient receptor potential cation channel, mucolipin subfamily
TRPP	Transient receptor potential polycystic
TRPV	Transient receptor potential cation channel, vanilloid subfamily
TRPV1	Transient receptor potential cation channel, subfamily V member 1
TPM	Two-photon microscopy
UCHL1-EGFP	Ubiquitin carboxy-terminal hydrolase isozyme L1 transgenic mouse reporter
VAChT	Vesicular acetylcholine transporter
VEGF	Vascular endothelial growth factor
VIP	Vasoactive intestinal peptide
vLGN	Ventral lateral geniculate nucleus
Wnt1	Wnt family member 1

WP	Whisker pad
XFP	Generic abbreviation for fluorescent proteins (i.e. CFP, GFP, YFP, RFP, etc.)
YFP	Yellow fluorescent protein

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Highlights

- In neurofluorescent reporter mice, nerve cells are viewable via fluorescence imaging.
- In these models, fluorescent proteins are co-expressed with specific marker proteins.
- This fluorescence allows for studies of the structural patterns of nerves *in vivo*.
- These mice are useful for examining nerve formation, injury, repair, and degeneration.
- Marker proteins Thy1 and Nestin are used to visualize different subsets of neurons.

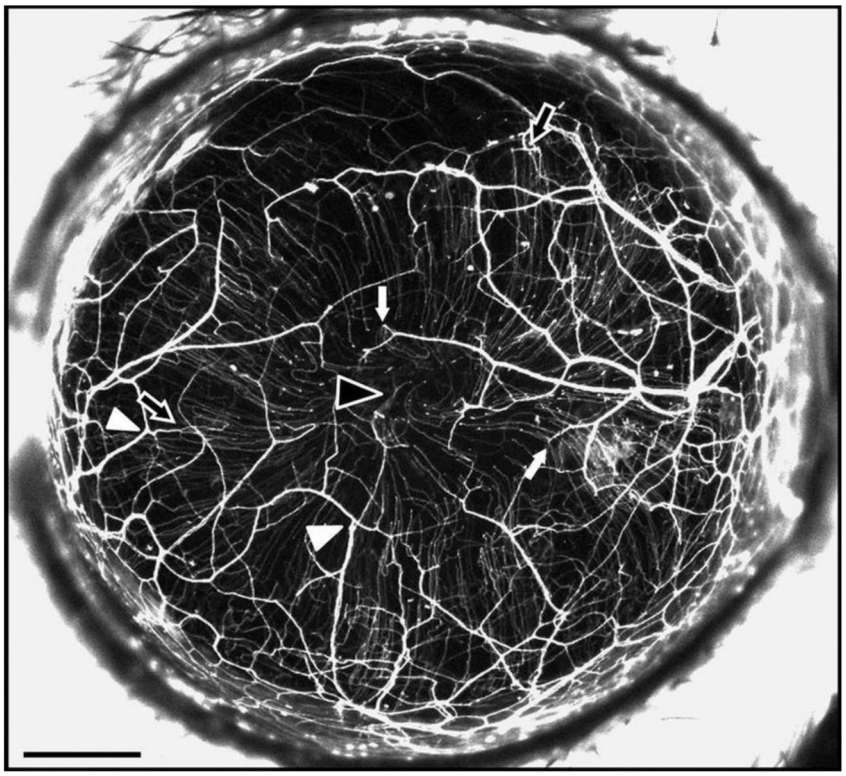

Figure 1.
In vivo image of fluorescent nerves in the healthy Thy1-YFP-16 mouse cornea focusing particularly on the stromal plexus. Following surgical transection, nerves display disorganized regenerated fibers Scale bar, 500 μm. Reproduced with permission from [35].

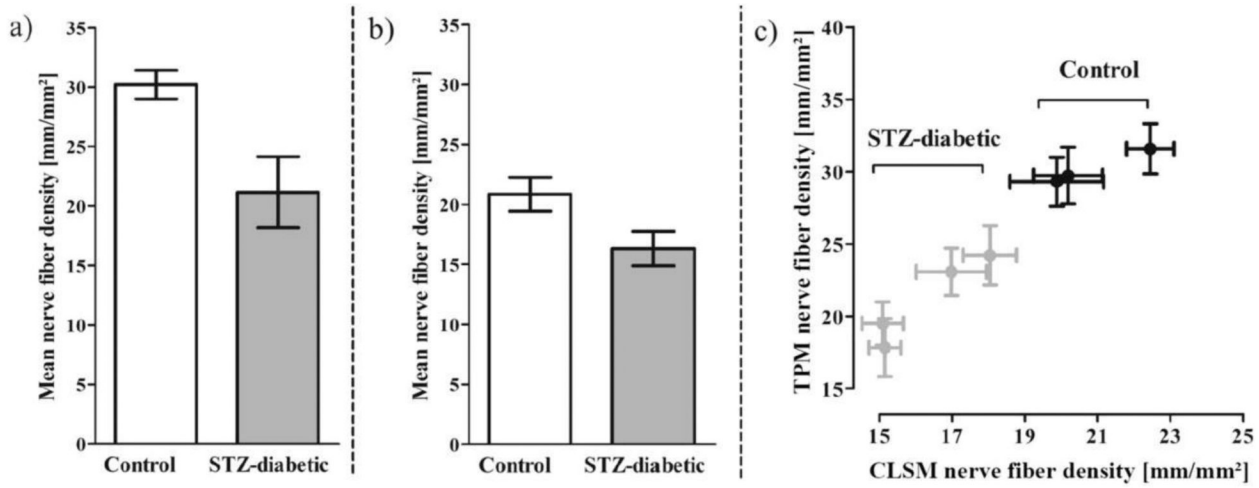

Figure 2.

Mean nerve corneal subbasal fiber density in four STZ-treated and three control Thy1-YFP mice. Based on quantitative results from confocal laser scanning microscopy (CLSM) and two-photon microscopy (TPM) (both of which are forms of confocal microscopy) of corneal nerves in vivo, STZ treatment caused significant reductions in corneal nerve fiber density. Reproduced with permission from [23].

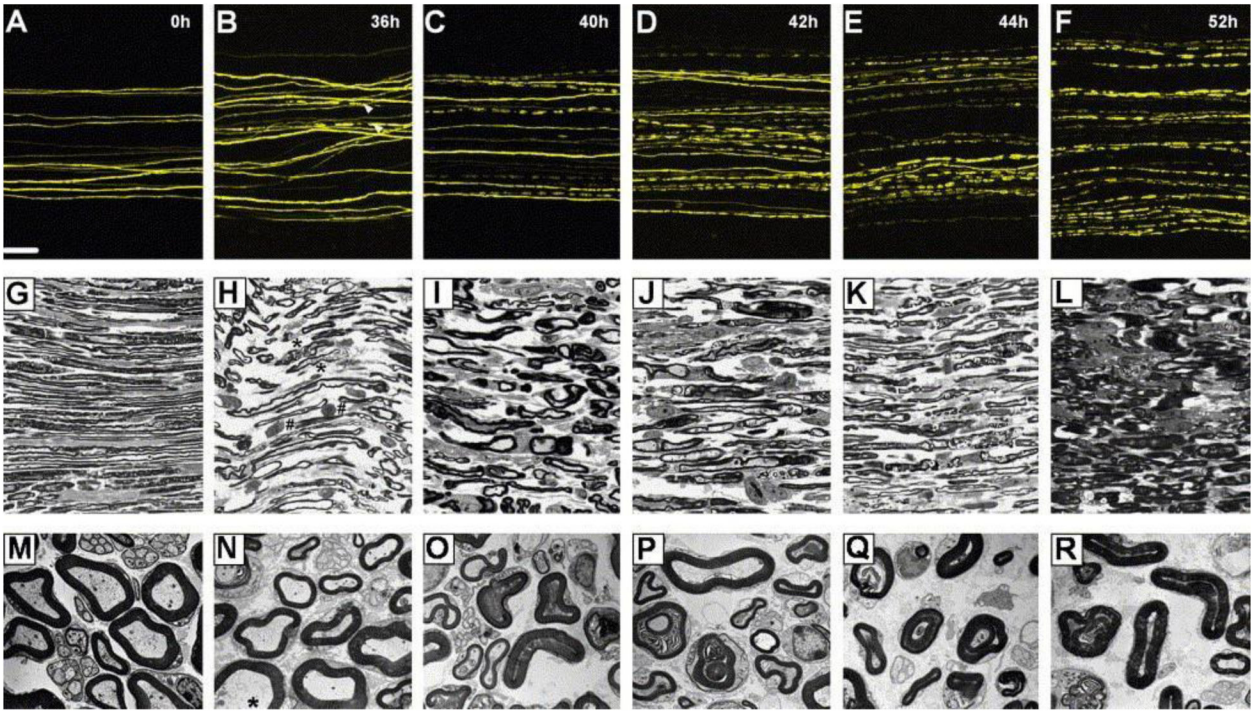

Figure 3.

Imaging of Thy1-YFP+ neurons revealed that YFP axons could be successfully used to display nerve changes following Wallerian degeneration. Such imaging was achieved through light and electron microscopy techniques. Scale bars, (A–F) 100 μm, magnification: (G–L) 1000×, magnification: (M–R) 4400×. Reproduced with permission from [42].

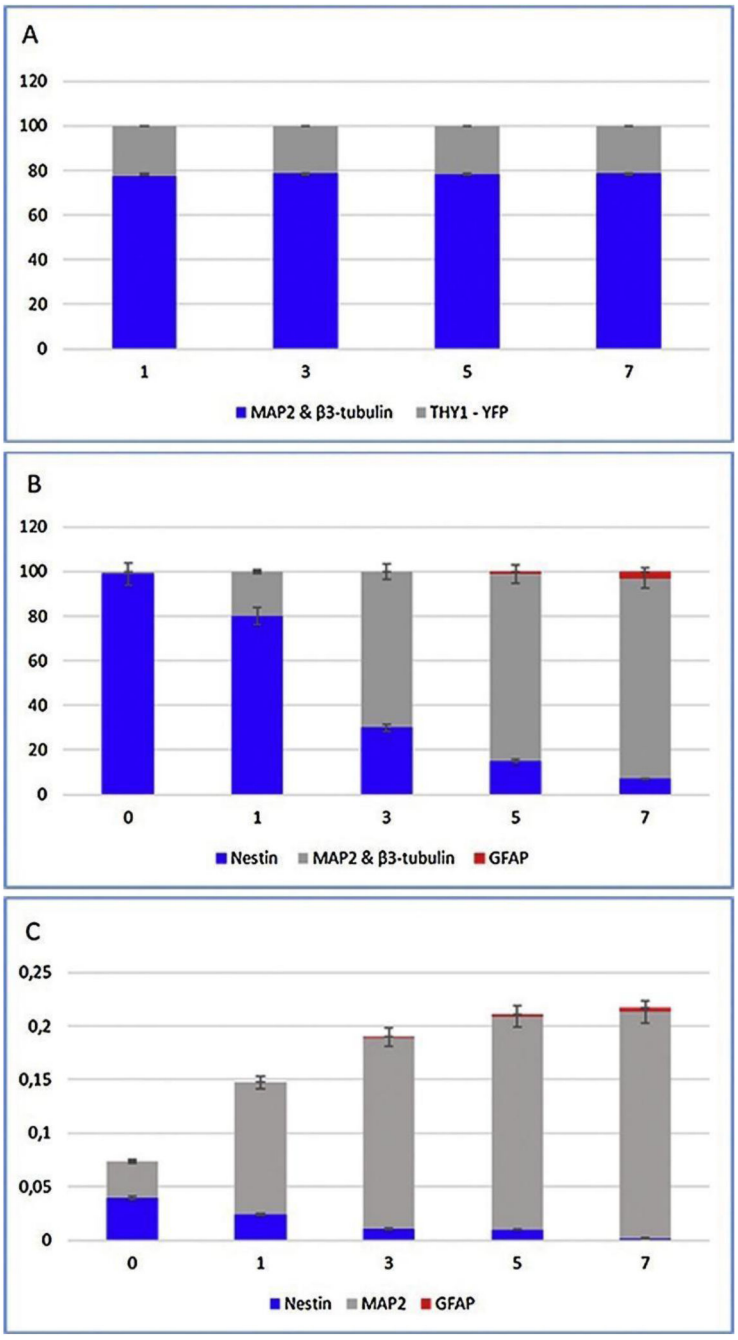

Figure 4.

Quantitative analyses of various cellular markers by immunocytochemistry (A–B) and RT-PCR (C). Notably, the percentage of cells expressing Nestin decreased from 99% to 7% during in vitro differentiation, because Nestin is preferentially expressed in undifferentiated neural progenitors. However, the expression of MAP2 and β3-tubulin conversely increased over time. Legend: x-axis – days in differentiation conditions; y-axis – number of counted cells (A), % of counted cells (B), and relative expression in RNA (C). Reproduced with permission from [27].

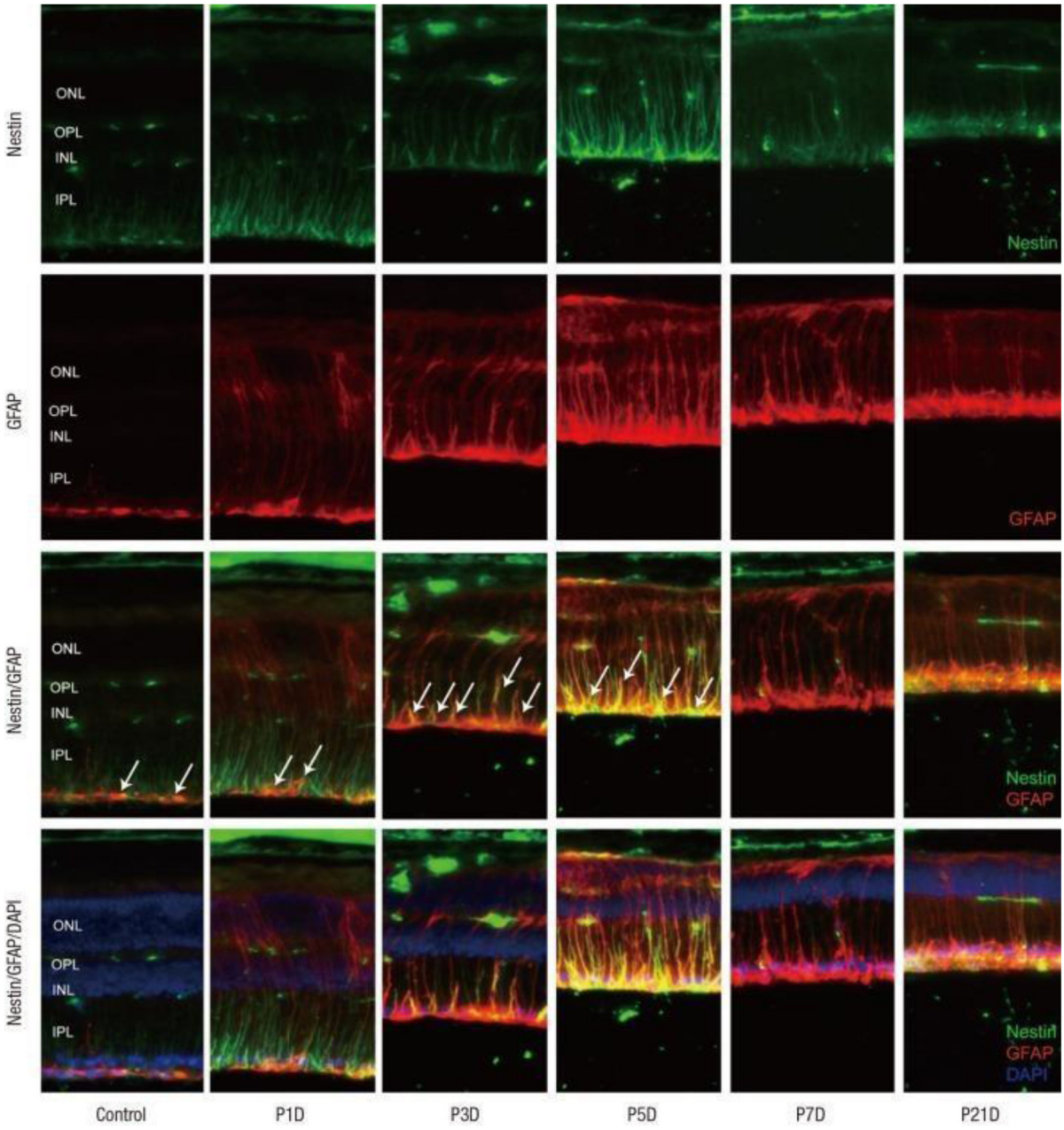

Figure 5.

Images showing Nestin, GFAP, and DAPI staining as green, red, and blue fluorescence, respectively. Following MNU-induced retinal degeneration, marker expression was assessed on days 1, 3, 5, 7, and 21 by Western blotting. Nestin expression in Müller cells progressively increased for 5 days and then decreased thereafter. Image labels: ONL – outer nuclear layer, OPL – outer plexiform layer, INL – inner nuclear layer, IPL – inner plexiform layer. Reproduced with permission from [51].

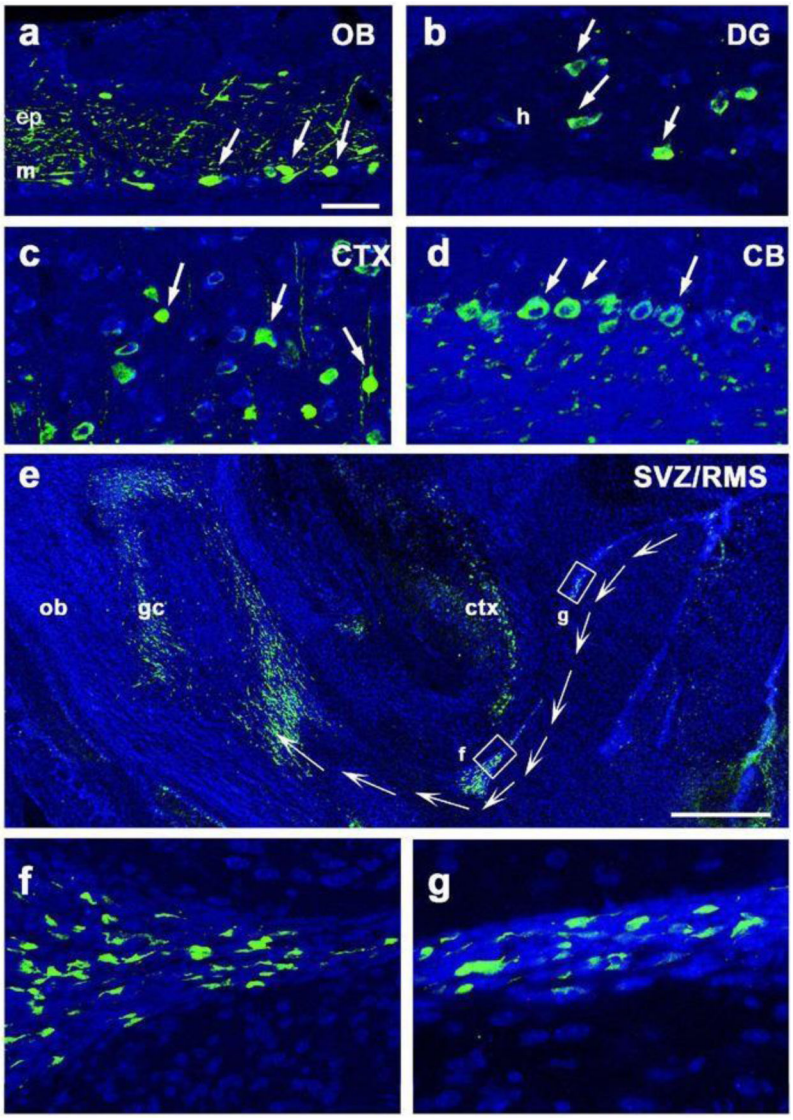

Figure 6.

Cellular and dendritic expression of YFP in a transgenic βIII-tubulin-YFP reporter. YFP+ cells were observed in the (A) mitral cell (m) and external plexiform (ep) layers of the olfactory bulb (OB), (B) hilus (h) and surrounding structures of the dentate gyrus (DG), (C) cortex (CTX), (D) cerebellum (CB), and (E-G) rostral migratory stream (RMS). Scale bars, 50 μm. Reproduced with permission from [17].

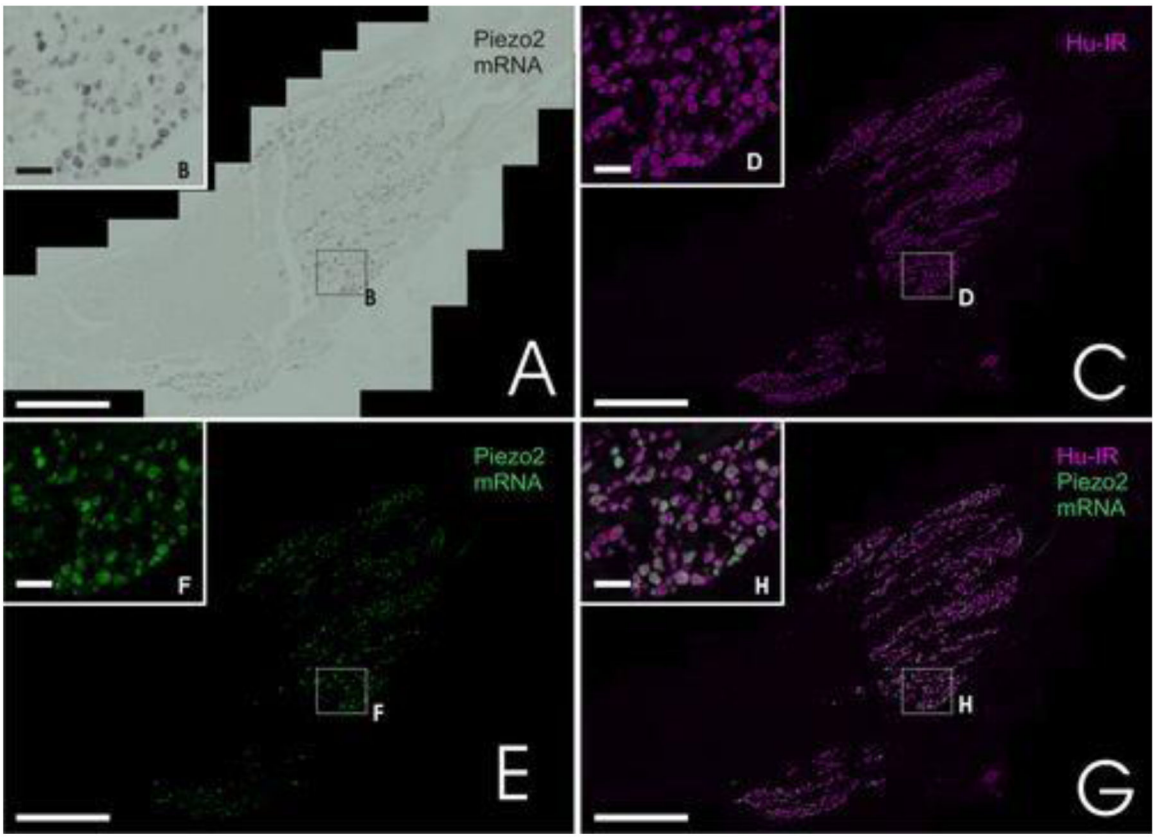

Figure 7.

Piezo2 expression in trigeminal ganglion neurons of guinea pigs. Approximately one-quarter of neurons were found to express Piezo2, as indicated by green fluorescence in image (G). Scale bars, 200 μm for main images, 20 μm for insets. Reproduced with permission from [63].

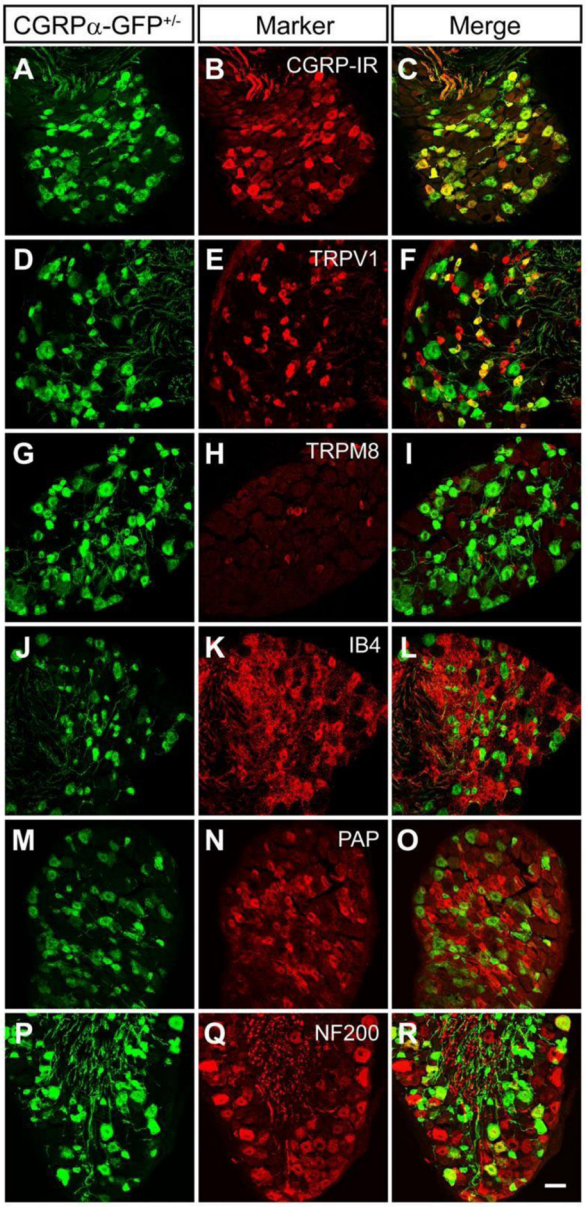

Figure 8.

Through confocal imaging of CGRPα-GFP reporter mice, sections of DRG from L4-L6 were stained with GFP-targeting antibodies to view CGRPα expression. CGRPα typically colocalized with peptidergic nociceptive neuronal markers. Scale bar, 50 μm. Reproduced with permission from [21].

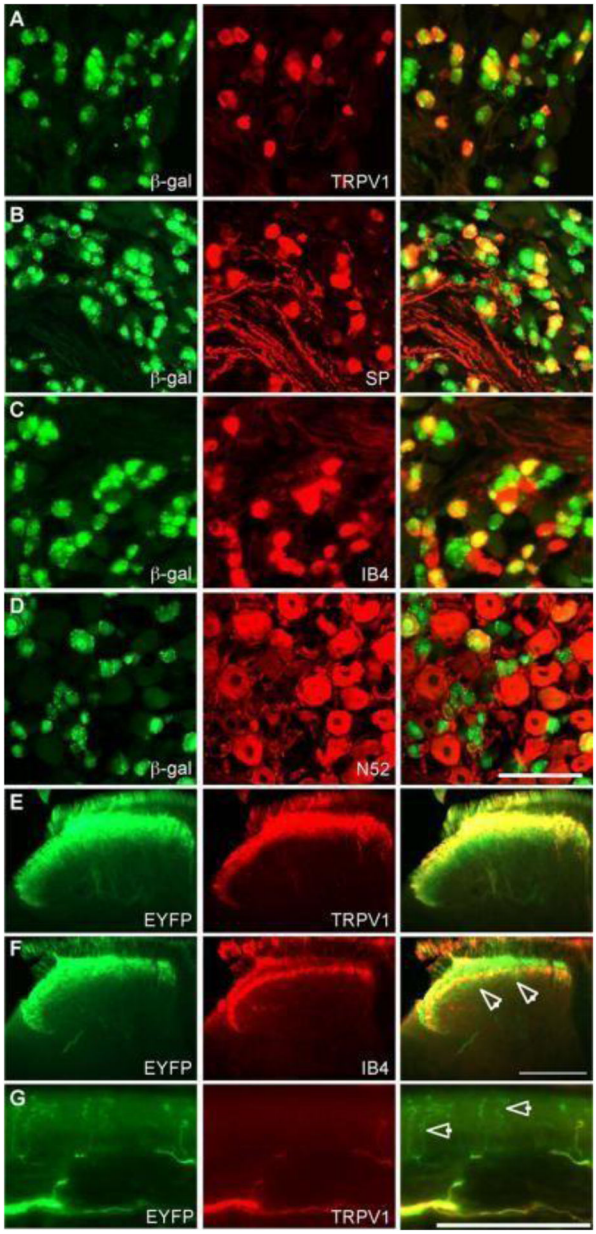

Figure 9.

Staining of DRG and spinal cord from TRPV1^{Cre} mice crossed with multiple Cre-dependent reporter lines. Immunohistochemical staining revealed TRPV1 expression in peptidergic and nonpeptidergic C-fibers, as well as myelinated DRG neurons during development. Scale bars, (D) and (G) 100 μm, (F) 200 μm. Reproduced with permission from [66].

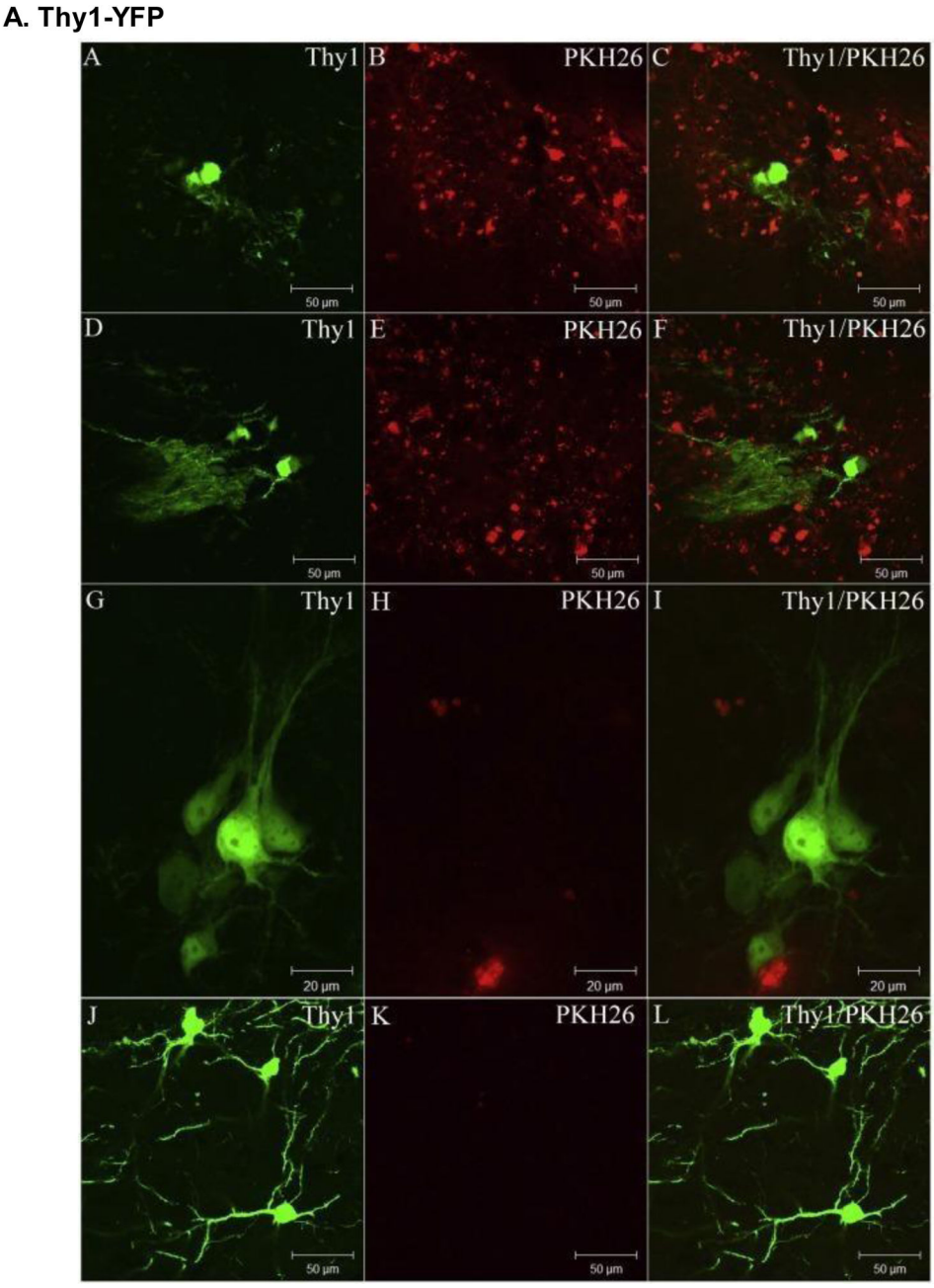

Figure 10.

Changes in Thy1-YFP expression in the mouse brain following PKH26-labeled cell transplantation over a 14-week period; (A-C) 2 weeks, (D-F) 4 weeks, (G-I) 8 weeks, and (J-L) 14 weeks. Thy1 expression consistently increased as time progressed, while PKH26 expression mostly regressed within 1 month of transplantation. Reproduced with permission from [27].

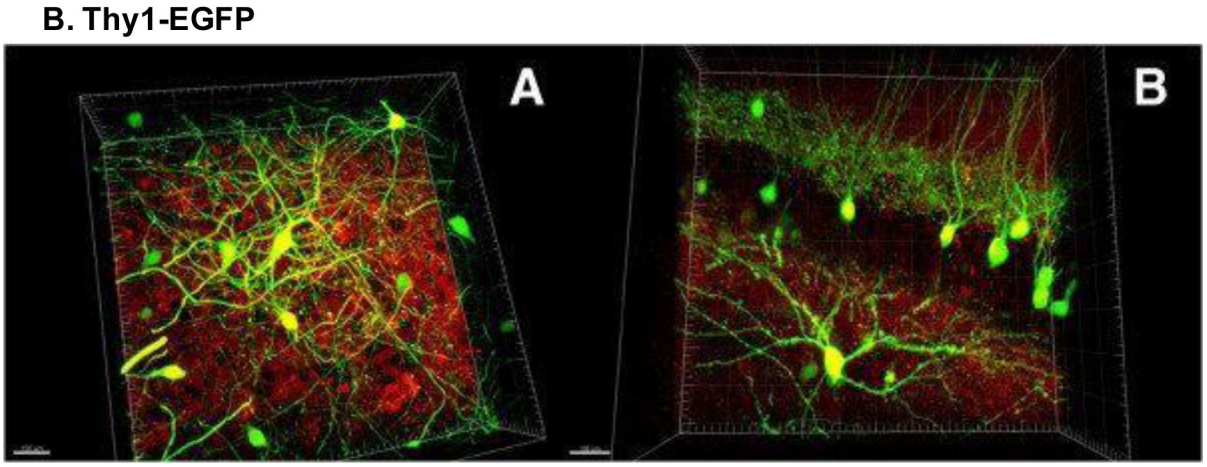

Figure 11.

Confocal images displaying Thy1 expression in striatal and hippocampal neurons of a Thy1-EGFP transgenic reporter mouse (GFP+ neurons in green, PSD95+ neurons in red). Scale bars, 100 μm. Reproduced with permission from [87].

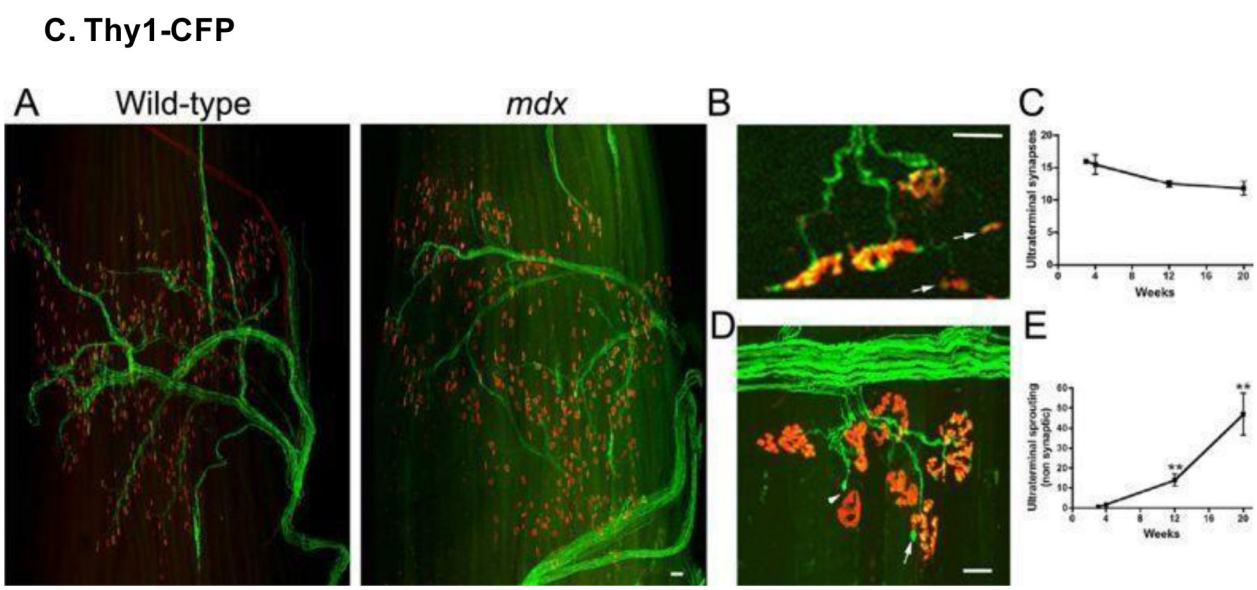

Figure 12.

Transgenic cyan fluorescence protein (CFP) expression in neurons of the third extensor digitorum longus (EDL) compartment in wild-type and mdx mice (for neuronal visualization in Duchenne muscular dystrophy mice); mdx muscles demonstrated profound infiltration of cells expressing acetylcholine receptors (AChR). Scale bars, (A) 100 μm, (B) and (D) 30 μm. Reproduced with permission from [110].

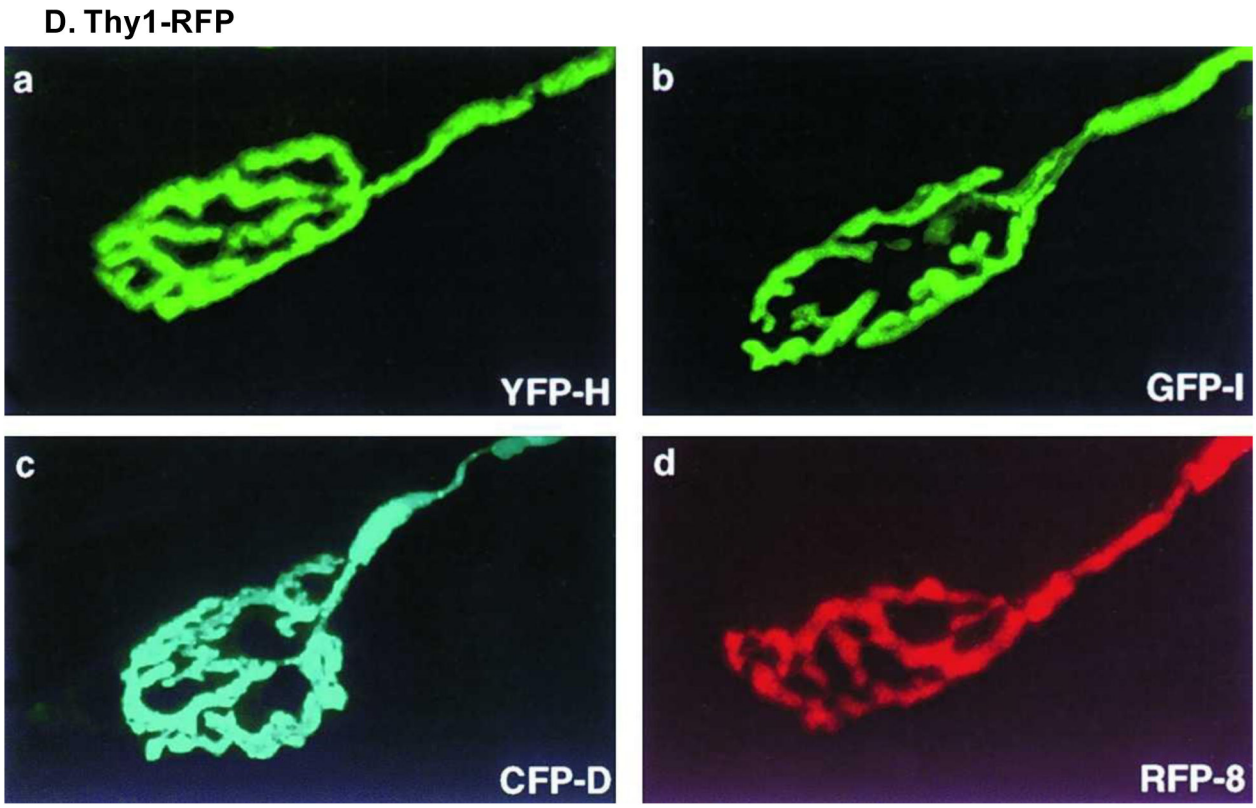

Figure 13.

Expression of four fluorescent proteins important for transgenic reporter staining. Muscles were labeled with bungarotoxin, and neuromuscular junctions were imaged in (A) Thy1-YFP line H, (B) Thy1-GFP line H, (C) Thy1-CFP line D, and (D) Thy1-RFP line 8 transgenic mice. Reproduced with permission from [32].

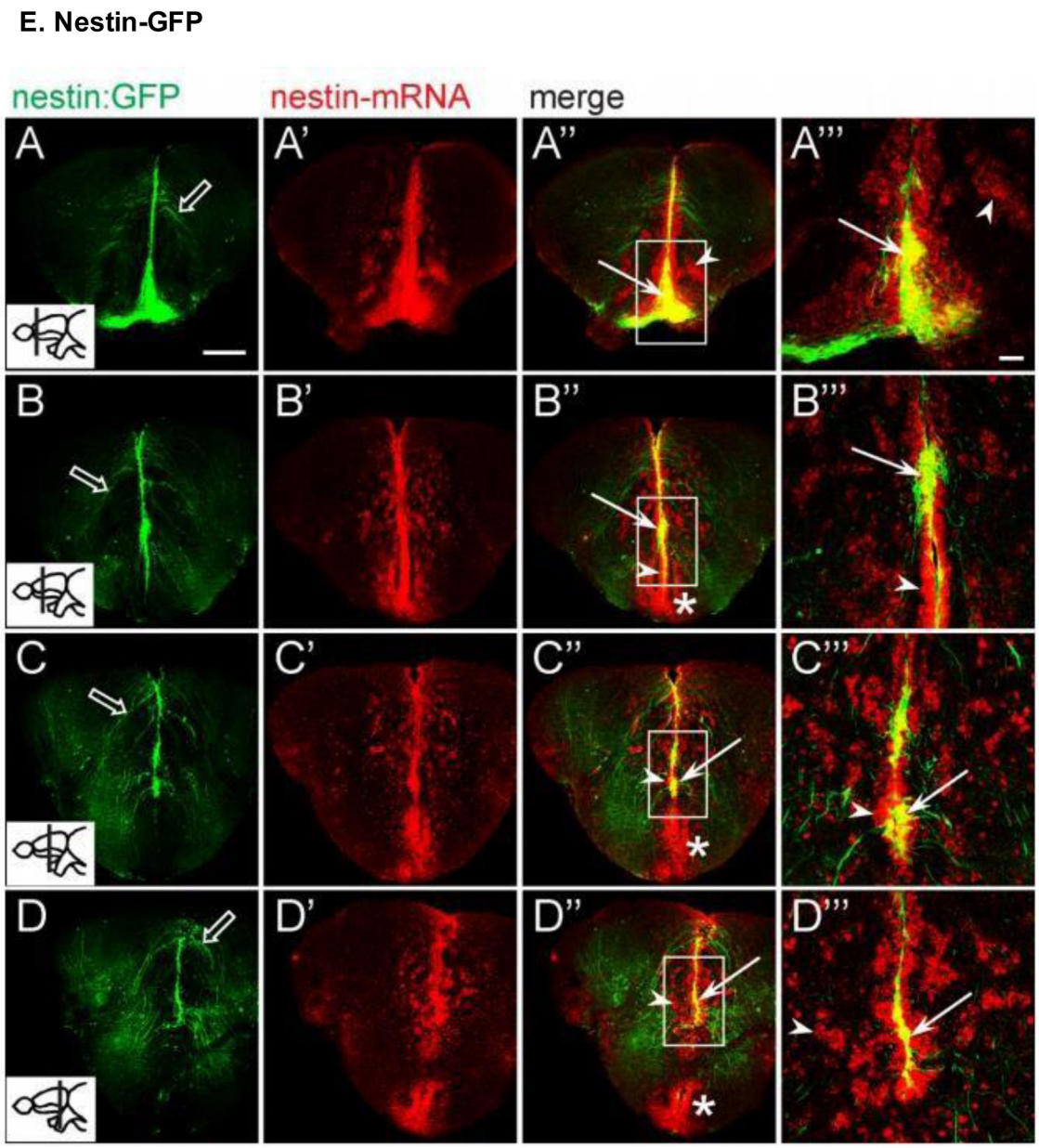

Figure 14.

Anterior to posterior expression of Nestin-GFP and endogenous Nestin mRNA in trigeminal neurons of the mouse cerebrum in confocal single optical sections (insets indicate the plane of section for each row). GFP+ cells emanated in projections from the ventricular zone; endogenous Nestin expression was most prominent in the ventricular zone as well as in scattered cells of the brain parenchyma. Scale bars, (A) 100 μm, (A') 50 μm. Reproduced with permission from [202].

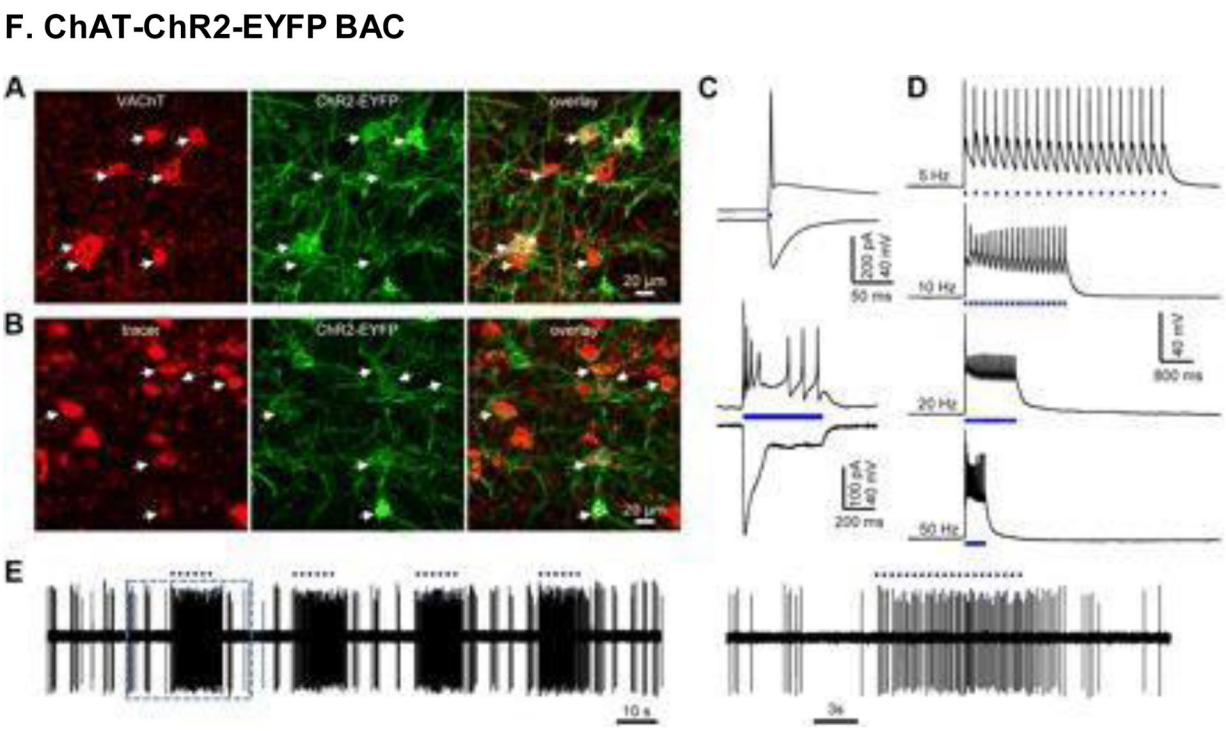

Figure 15.

Immunostaining against VAChT (red) revealed the expression of ChAT–ChR2–EYFP+ cells, with an affinity towards cholinergic transgenic mouse neurons. (A) Images taken from the horizontal diagonal band of Broca (HDB). EYFP expression was weak in somata, because ChR2 protein tends to localize to neuronal membranes. (B) Retrograde tracing confirmed that HDB neurons feed into the main olfactory bulb (MOB). Some neurons lacked EYFP expression, since the MOB is only partially innervated by cholinergic neurons. (C-D) Blue light stimulation up to 50 Hz led to activation and firing of ChR2– EYFP+ neurons in the HDB. (E) Light pulses produced vigorous subsequent firing of HDB neurons. Reproduced with permission from [204].

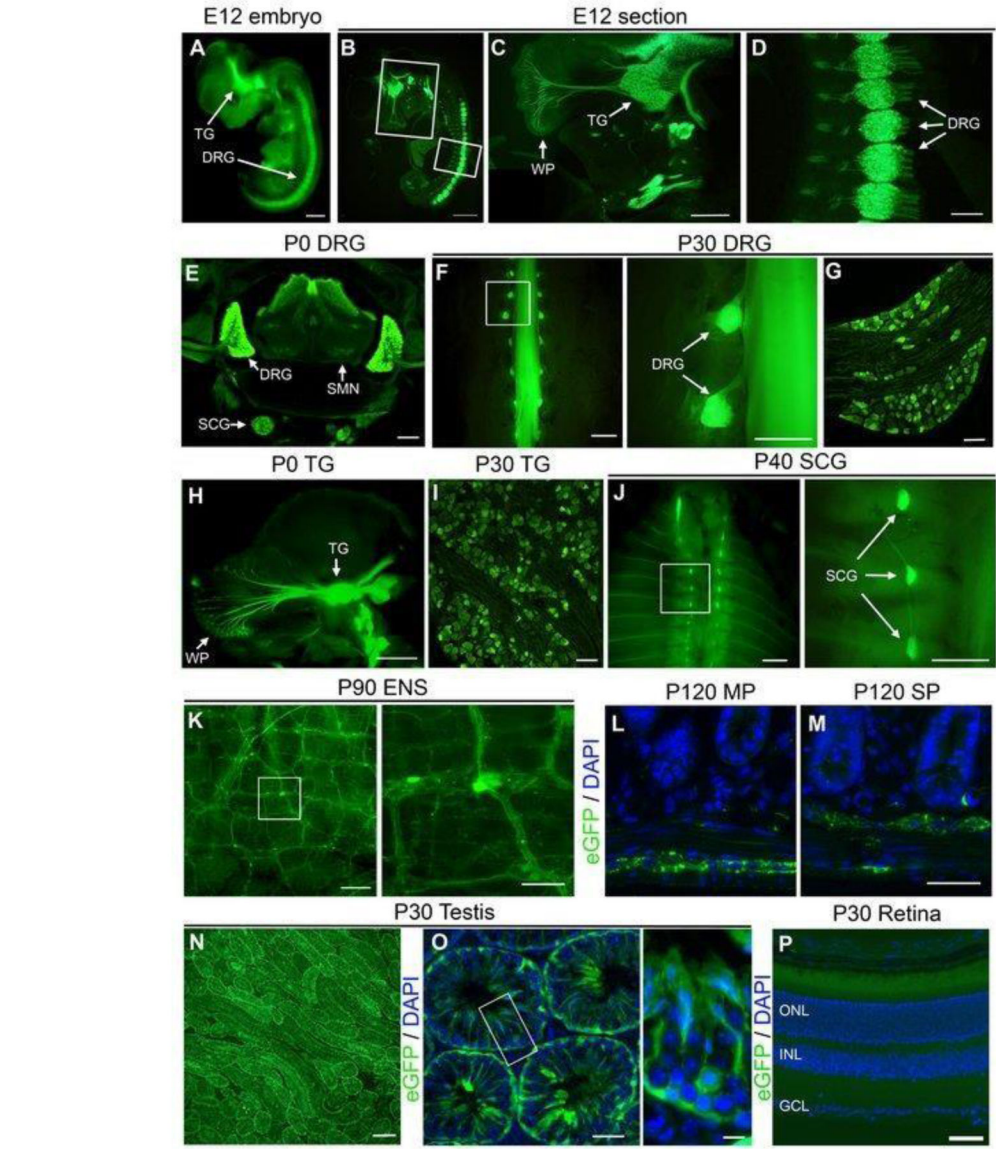

Figure 16.

The use of a UCHL1-EGFP transgenic reporter model enabled visualization of peripheral nerves in vivo starting from embryonic day (E) 12 (A). Levels of EGFP expression across various time points and tissues are displayed in this figure. (B-D) TG and DRG neurons at E12. (E) DRG neurons, sympathetic chain ganglia (SCG), and spinal motor neurons (SMN) of the ventral spinal cord on PD0. (F-G) Adult DRG neurons (with zoomed inset). (H) TG neurons on PD0. (I) Adult TG neurons. (J) SCG neurons (with zoomed inset). (K) Enteric nervous system (ENS) neurons (with zoomed inset). (L-M) Myenteric plexus (MP) and submucosal plexus (SP) of the gastrointestinal tract. (N-0) Adult testis neurons. (P) Lack of EGFP expression in the retina. Scale bars, (A, B, F inset and J inset) 1 mm; (C) 500 μm; (D, E, K, N) 200 μm; (F, H, J) 2 mm; (G, I, K inset, L, M, O) 50 μm; (O inset) 10 μm; (P) 100 μm. Reproduced with permission from [207].

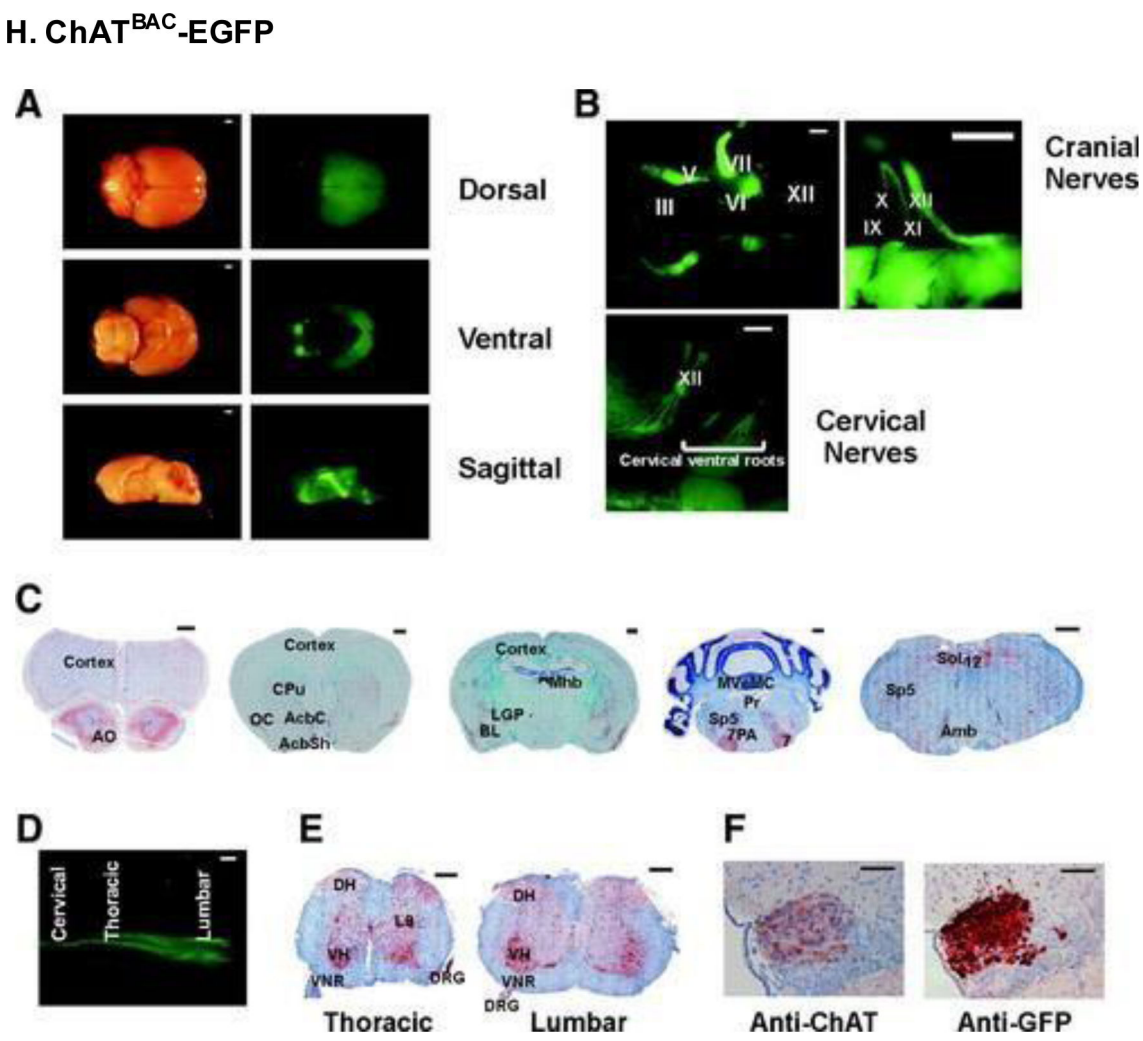

Figure 17.

ChAT^{BAC}-EGFP mice were used to visualize neurons of the central nervous system. (A) Light (left) and fluorescent (right) ex vivo microscopy images of adult mice. (B) Magnified in vivo images from the brainstem and rostral spinal cord. (C) Immunostaining for anti-EGFP antibody in various cross-sections of the brain. (D) Ventral spinal cord view on PD2. (E) Immunostaining for anti-EGFP antibody in various spinal cord segments. (F) Sections of the medial habenular nucleus of the brain showing expression of anti-ChAT (left) and anti-EGFP (right). Roman numerals III-XII correspond to their respective cranial nerves (e.g., III is oculomotor). Other abbreviations: AO, anterior olfactory nucleus; CPu, caudate putamen; AcbC, accumbens nucleus core; AcbSh, accumbens nucleus shell; OC, olfactory cortex; LGP, lateral globus pallidus; Mnb, medial habenular nucleus; BL, basolateral amygdaloid nucleus; 7, facial nucleus; Sp5, spinal 5 nucleus; MVeMC, medial vestibular nucleus; Pr, prepositus hypoglossal nucleus; 7PA, facial nucleus proximal axons; 12, hypoglossal nucleus; Sol, solitary tract nucleus; Amb, ambiguus nucleus; VH, ventral horn; L9, lamina 9; DH, dorsal horn; VNR, ventral nerve root. Scale bars, (A and B cranial left) 1 mm; (B cranial right) 500 μm; (B cervical nerves) 1 mm; (C) 500 μm; (D) 1 mm; (E) 250 μm; (F) 50 μm. Reproduced with permission from [208].

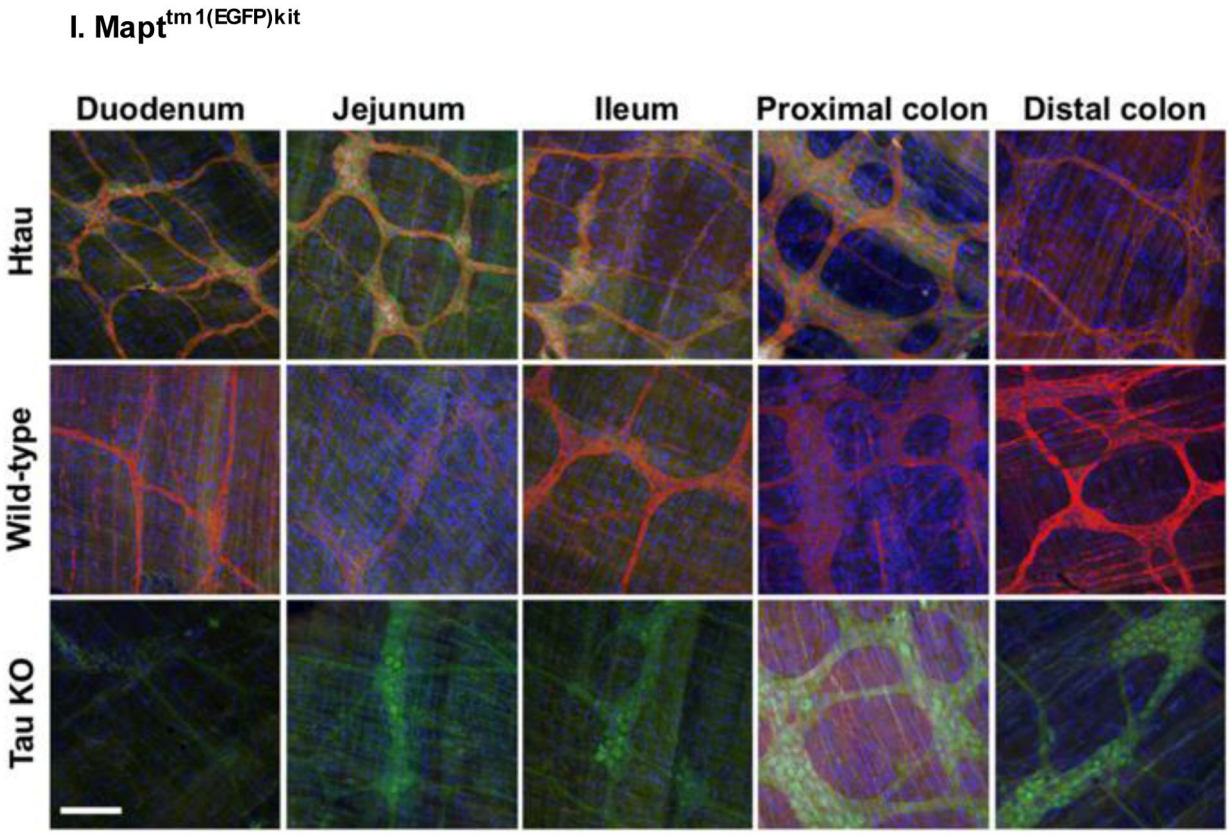

Figure 18.

Expression of tau in the myenteric plexus of various segments of the gastrointestinal system of 2-month-old htau ($Mapt^{tm1(EGFP)kit}$), wild-type (WT), and tau knockout (KO) mice. Images are merged showing tau in red, EGFP in green, and nucleic acid labels in blue. Scale bar, 100 μm. Reproduced with permission from [211].

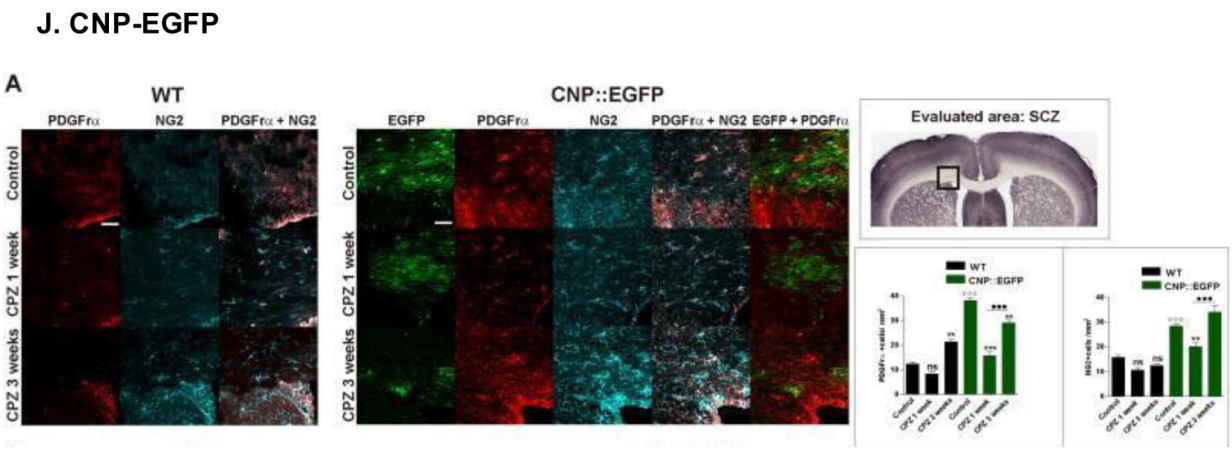

Figure 19.

(A) Confocal microscopy images of oligodendrocyte progenitors (OPCs) in CNP-EGFP and WT mice following 1 or 3 weeks of treatment with cuprizone (CPZ). OPC expression was markedly elevated in CNP-EGFP mice compared to WT mice, as confirmed by analysis of variance (ANOVA) and Bonferroni post-hoc statistical tests (ns = non-significant, **p<0.01, ***p<0.001). Scale bars, 50 μm. Reproduced with permission from [213].

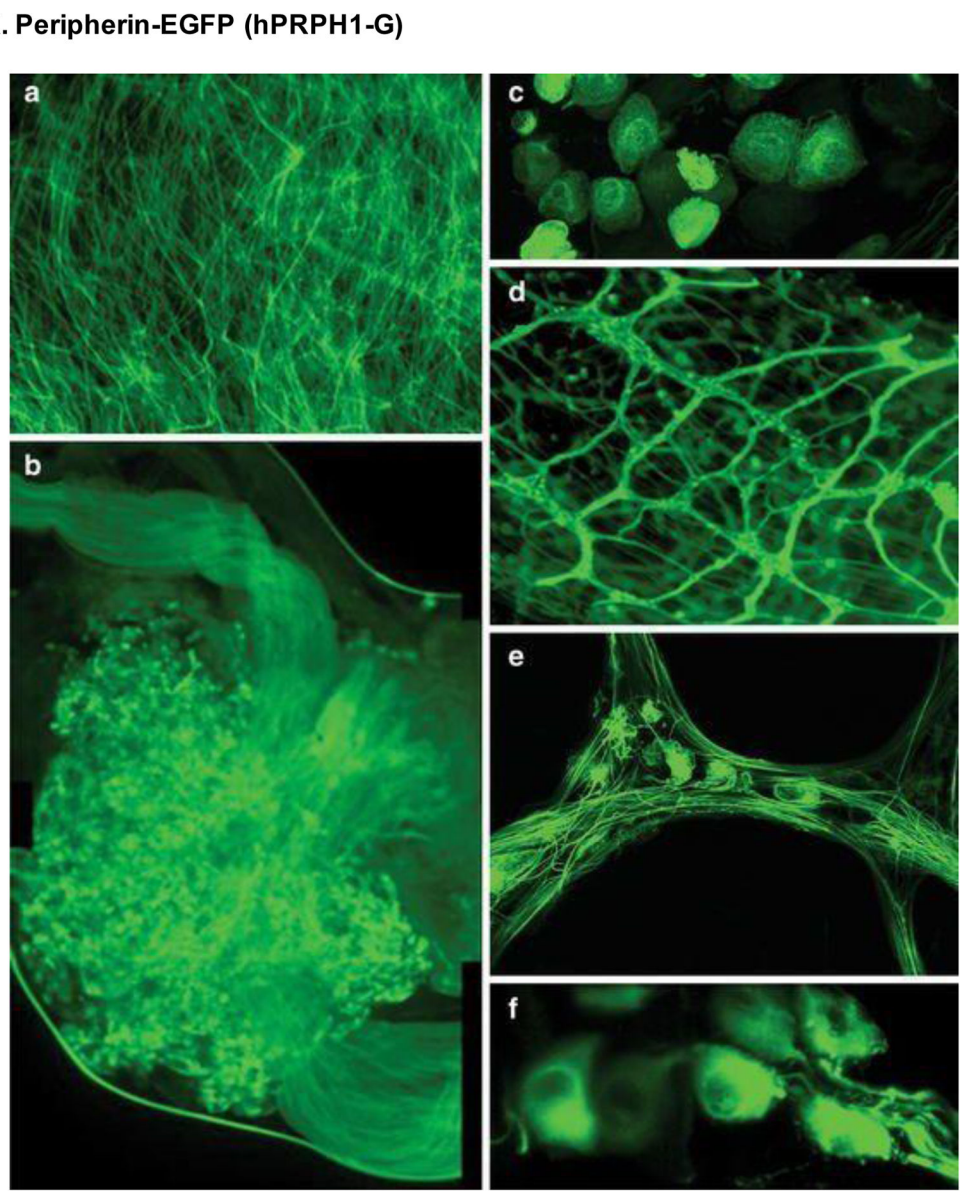

Figure 20.

Expression of peripherin-EGFP (hPRPH1-G) in unfixed tissues viewed by multiphoton and confocal microscopy. (A) Cervical spinal cord at 200× magnification. (B) Images combined to show the DRG. (C) DRG sensory neuron cell bodies. (D-E) Small intestine seen by fluorescence (D) and confocal (E) microscopy at 100× and 400× magnification, respectively. (F) Retinal images showing RGC bodies at 1000× magnification. Reproduced with permission from [220].

L. GFP-Cre

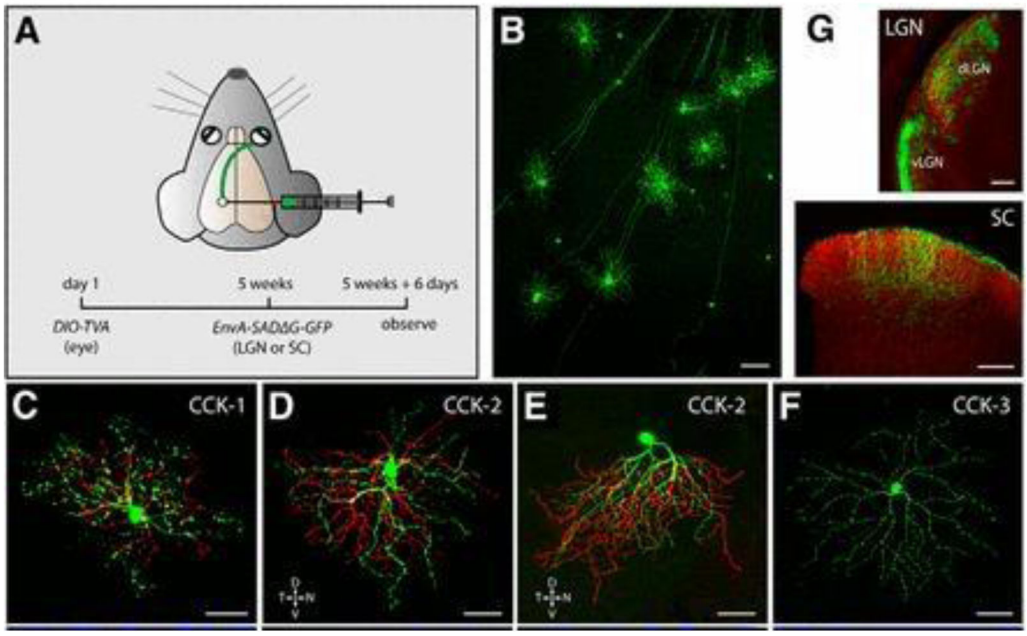

Figure 21.

GFP-Cre expression in RGCs. (A) Experimentation strategy for labeling CCK -RGCs at axon terminals. (B) GFP-labeled CCK-RGCs in the retina of CCKires-Cre mice. (C-F) Neuronal morphology of various CCK-RGCs. (G) CCK-RGC axon terminals in the spinal cord, dLGN, and vLGN (red corresponds to CTb-594 labeling of all RGC axons). Scale bars, (B) 100 μm; (C-F) 50 μm; (G) 200 μm. Reproduced with permission from [247].

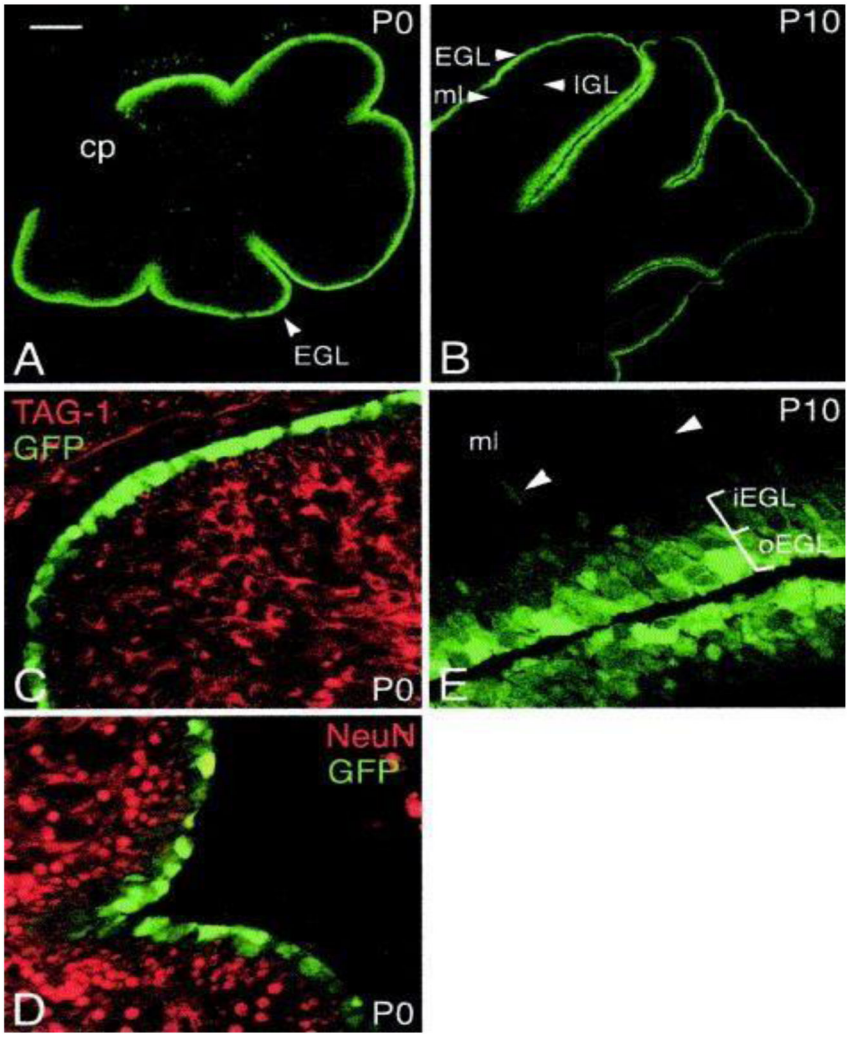

Figure 22.

Images of cerebellar granule cells on either P0 (A, C, D) or P10 (B, E). Math1-GFP expression was most prominent in the outer cerebellar EGL, which corresponds to normal expression of Math1. At P0, a lack of GFP expression overlap with markers of differentiating granule cells [TAG-1 and Neu-N in (C) and (D), respectively] was noted. (B) The majority of Math1-GFP–expressing cells were observed in the EGL, with minimal Math1-GFP expression in the IGL at P10. (E) Higher magnification images of the outer/inner EGL, with more intense Math1-GFP expression in the outer EGL; low Math1-GFP expression was also noted in the molecular layer (ml). Scale bars, (A) 150 μm; (B) 300 μm; (C-D) 25 μm; (E) 30 μm. Reproduced with permission from [222].

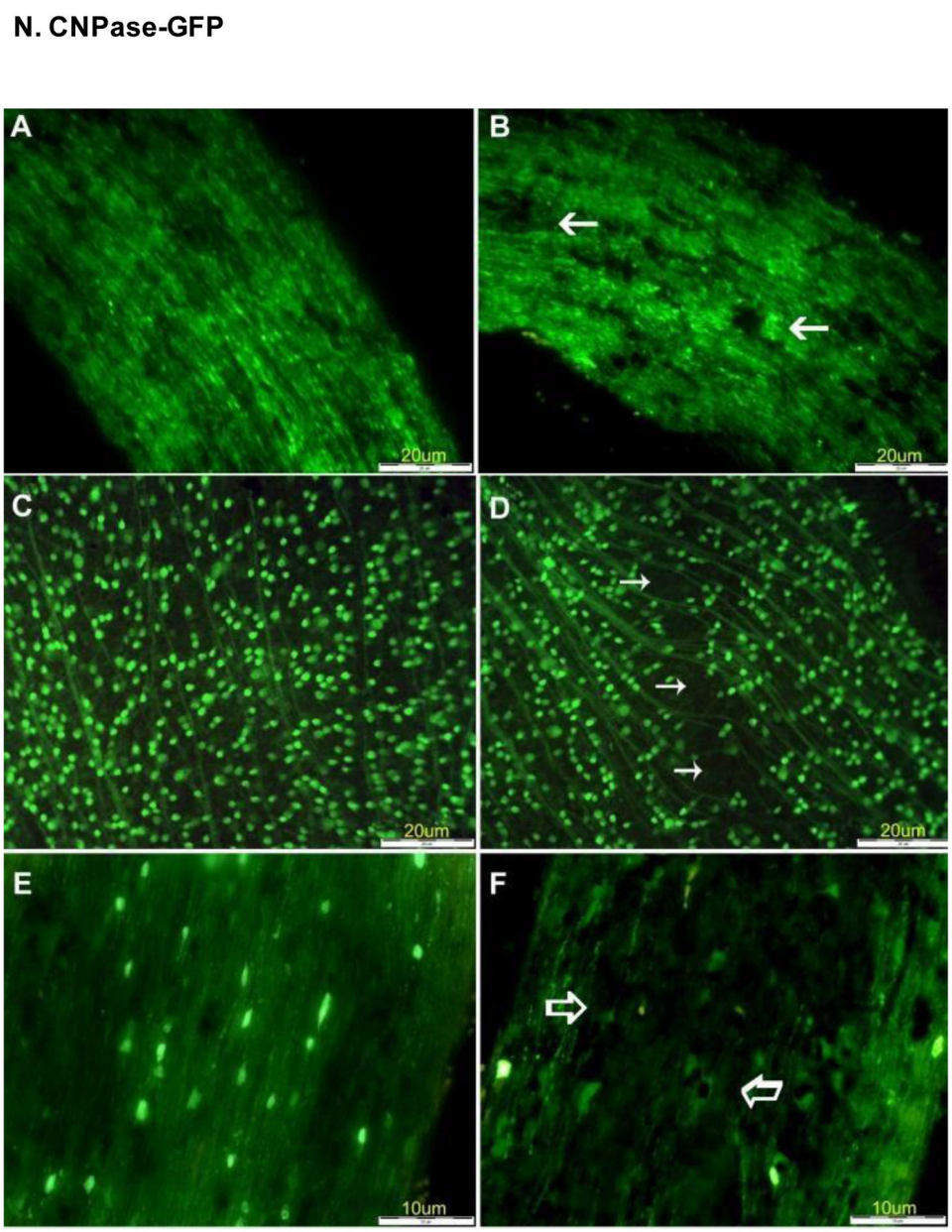

Figure 23.

Expression of Thy1-CFP and CNPase-GFP in the retina and optic nerve of a mouse model of experimentally-induced rodent anterior ischemic optic neuropathy (rAION). (A) Optic nerve images in control Thy1-CFP mice before induction of rAION. (B) View of optic nerve 21 days after induction of rAION, with arrows noting regions of significant axon loss. (C) Retina of control mouse before induction of rAION. (D) Optic nerve of CNPase-GFP mouse before induction of rAION. (F) Loss of oligodendrocyte expression 21 days after induction of rAION. Reproduced with permission from [105].

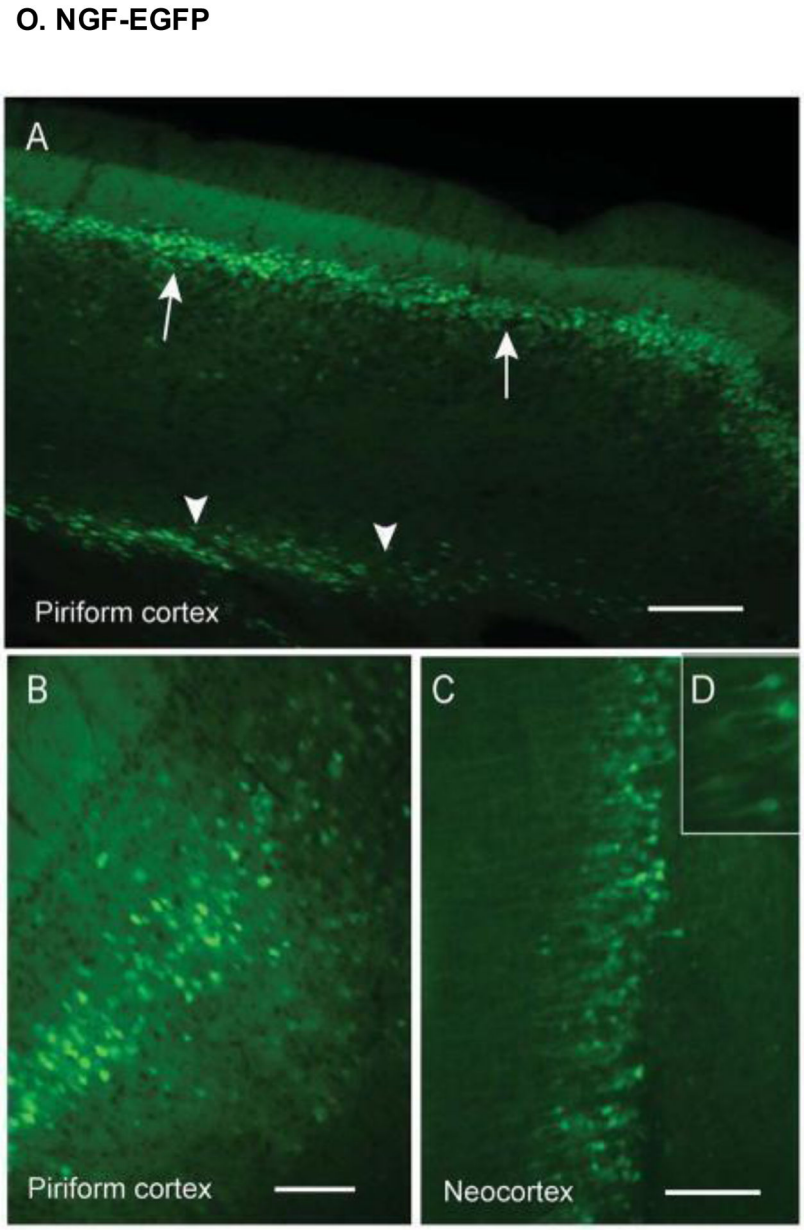

Figure 24.

EGFP expression in the cortex (axial sections) of NGF-EGFP transgenic mice. (A) The piriform cortex displayed prominent EGFP expression, especially in layer III. (B) Magnified image of the piriform cortex, showing both larger and smaller neurons. (C-D) The neocortex also displayed prominent EGFP expression, particularly in layer V. Scale bars, (A) 200 μm; (B & C) 100 μm. Reproduced with permission from [225].

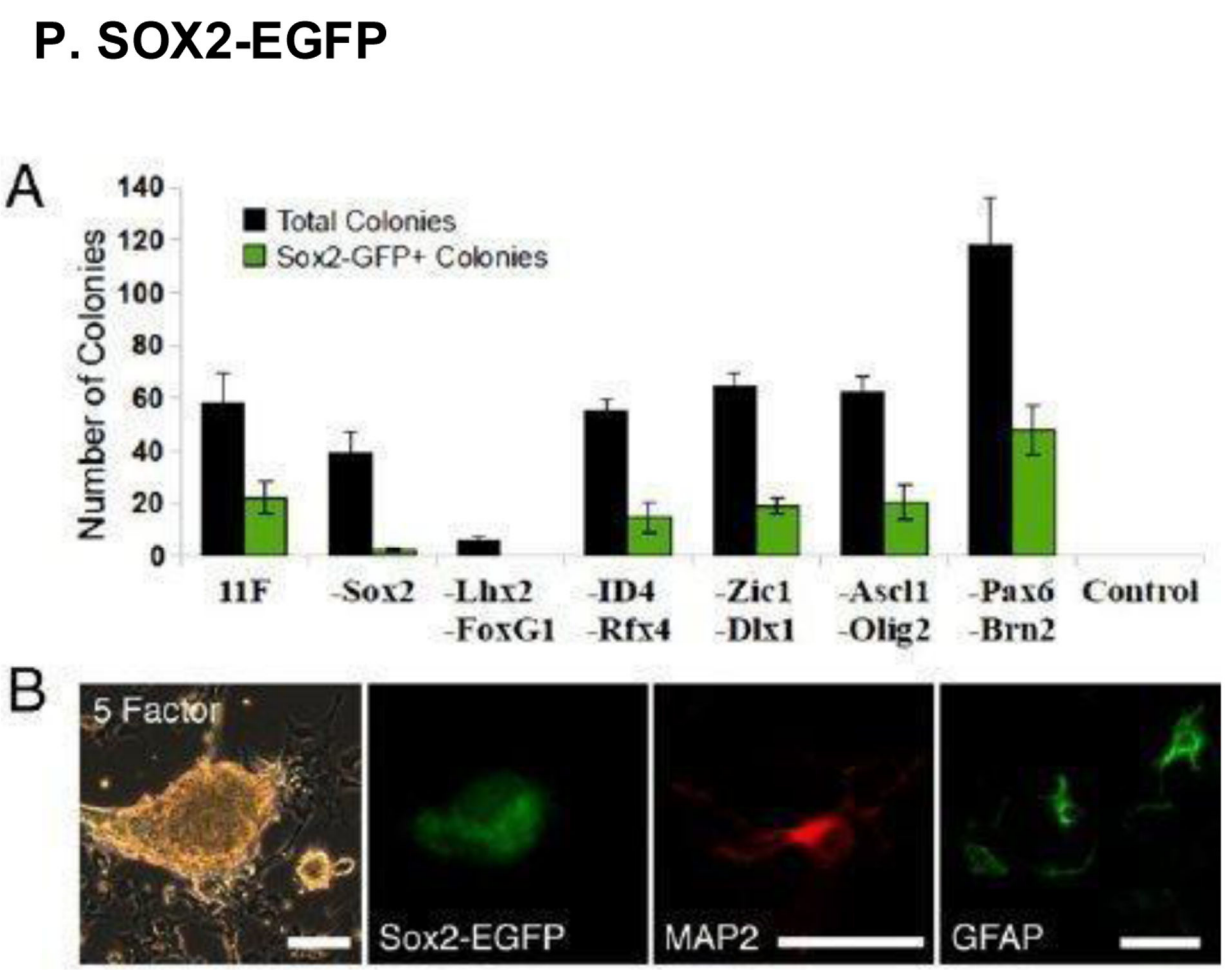

Figure 25.

(A) Comparison of total mouse colonies vs. colonies that express SOX2-EGFP 24 days after experimental transgene induction. (B) SOX2-EGFP expression in brain tissue, imaged 25 days after 5-Factor transgene induction, compared with MAP2+ (neuronal) and GFAP+ (astrocyte) cell expression. Scale bars, 50 μm. Reproduced with permission from [232].

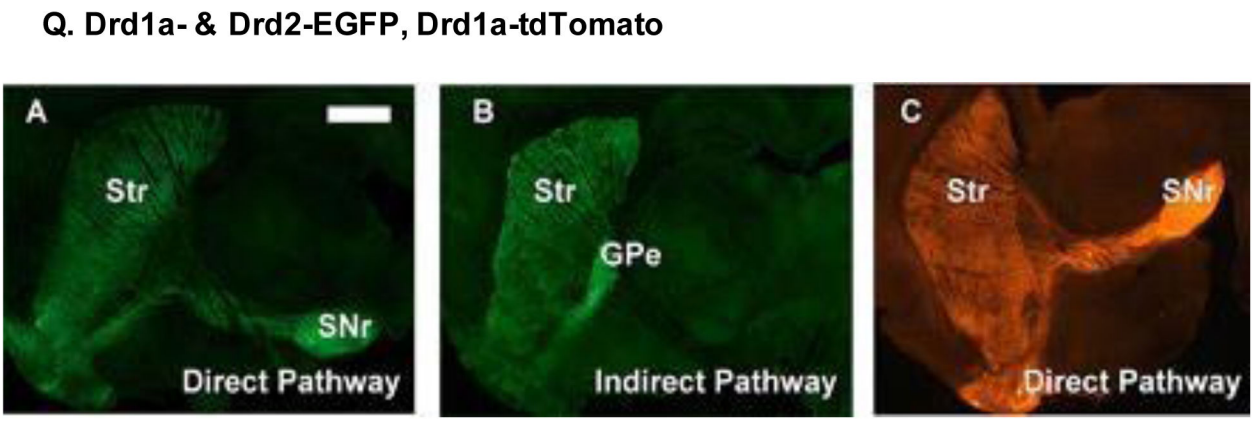

Figure 26.

Expression patterns in key basal ganglia structures of BAC transgenic mice. (A) Drd1a-EGFP, (B) Drd2-EGFP, and (C) Drd1a-tdTomato BAC lines. Fluorescence was most prominent in striatal soma and axonal projections. Scale bar, 1 mm. Other abbreviations: GPe, globus pallidus externa; SNr, substantia nigra pars reticulata; Str, striatum. Reproduced with permission from [243].

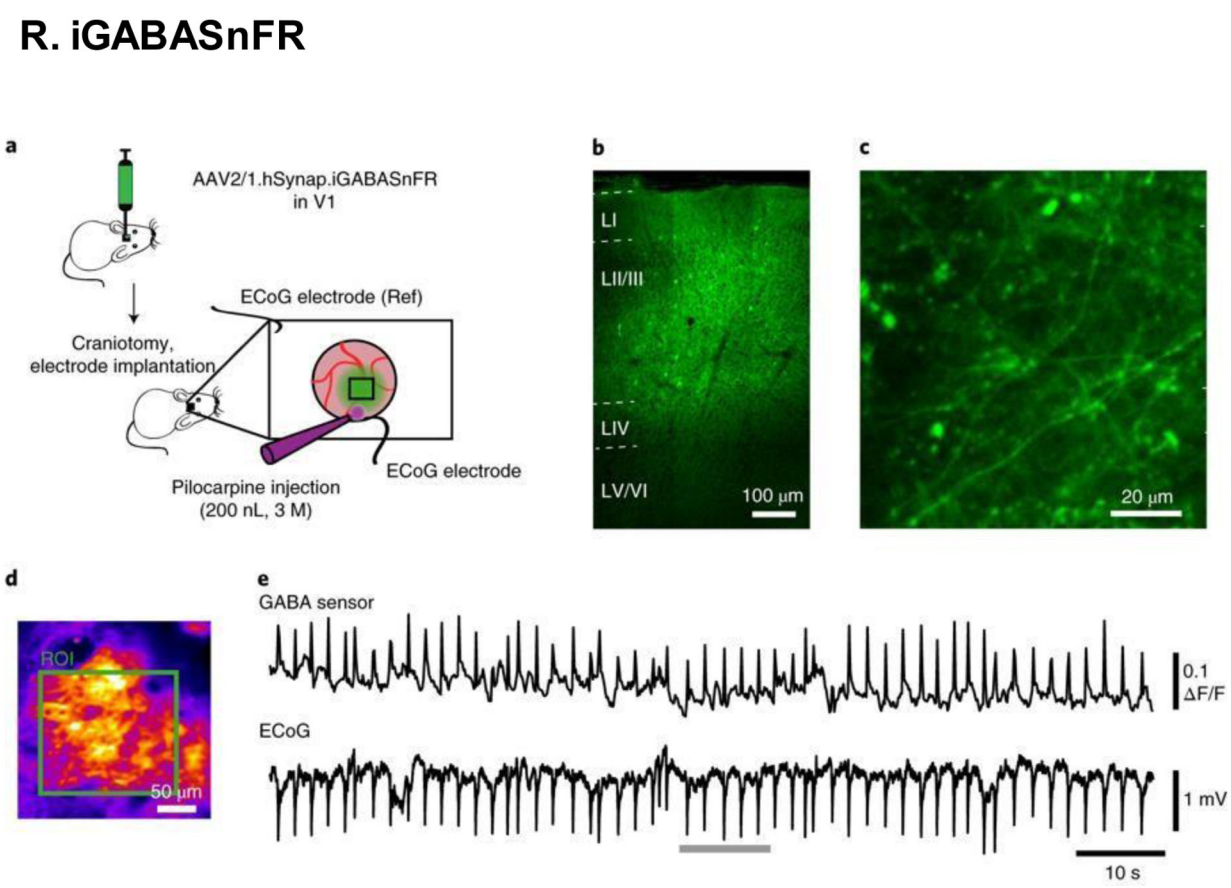

Figure 27.

Cortical iGABASnFR expression viewed under immunofluorescence microscopy. (A) Experiment diagram and design. (B) Images of cortical layers I-VI. (C) Images of cortical neurons proximal to the inferior pia. (D) Average intensity of interictal iGABASnFR fluorescence. (E) Interictal neuronal activity, as measured by electrocorticography. Scale bars, (B) 100 µm; (C) 20 µm; (D) 50 µm. Reproduced with permission from [245].

Table 1.

Common nerve markers and their locations, functions, and cited experimental application

Biomarker	Location	Function	Experimental Application
Thy-1 (CD90)	Subsets of neurons, thymocytes, peripheral T cells, myoblasts, epidermal cells, keratinocytes	Cell interactions	Visualization of nerve structure and regeneration in the cornea [10]
Nestin	Neuroepithelial cells, radial glia, ventricular zone progenitor cells, nascent ependyma/subependyma	Class VI Intermediate filament	Visualization of self-renewal and multipotency of CNS stem cells [16]
βIII tubulin	Neurons	Structural protein	Expression in the developing PNS and CNS using YFP [17]
Neurofilament heavy chain (NF-H)	Neurons (mature myelinated axons)	Axonal phosphorylation and neurofilament transport	Expression of high molecular weight neurofilaments in neurons and their transportation along axons [18]
Substance P	Subsets of sensory nerves	Pain transmission in CNS inflammation, wound healing	Understanding the contribution of Substance P to corneal epithelial w ound healing via mechanisms involving the Neurokinin-1 Receptor [19]
Neuron-specifice nolase (NSE)	Neurons, peripheral neuroendocrine cells	Neural differentiation and maturation	Expression of β-galactosidase in mature neurons under the control of a neuron-specific enolase promoter [20]
Calcitonin gene-related peptide (CGRP)	Subsets of sensory neurons	Vasodilation, smooth muscle relaxation, potentiates excitation caused by noxious stimuli and pronociceptive chemicals	Expression of CGRPα in sensory neurons to integrate pain and itch responses [21]

Table 2.

Thy1-YFP expression during embryonic development in Thy1-YFP-16 mice

Neural system	E12.5	E13.5	E14.5	E15.5	E16.5	E17.5	P0	W1	M1
CNS									
Brain									
Prosencephalon	+/−	+/−	+	+	++	++	++	+++	+++
Mesencephalon									
Rhombencephalon	+	+	++	++	+++	+++	+++	+++	+++
Spinal cord									
Ventral horn	+	++	++	+++	+++	+++	+++	+++	+++
Dorsal horn	+/−	+	+	++	+++	+++	+++	+++	+++
PNS									
Cranial nerves			+	++	++	++	++	+++	+++
Spinal nerves	+	+	++	++	++	++	++	+++	+++
Retina						+	++	+++	+++

E17.5 has been defined as the referral point and at that stage number of cells has been counted: “−” refers to no signal, “+/−” to signal present in less than 10% of cells, “ +” to signal present in 10–20% of cells, “++” to signal present in 20–35% of cells, “+++” to signal present in 35–50% of cells. Other stages were compared to the referral point and evaluated semi-quantitatively. “PNS” peripheral nervous system, “E” embryonic day, “P” newborn, “W1” 1-week-old pups, “M1” 1-month-old mice. Reproduced with permission from [27].

Table 3.

Previously used transgenic mouse reporters

Mouse Reporter Line	Other Associated References
Thy1-YFP	[68–84]
Thy1-EGFP	[85–87]
Thy1-CFP	[88–111]
Thy1-RFP	[112]
Nestin-GFP	[113–202]
ChAT-ChR2-EYFP BAC	[203–205]
UCHL1-EGFP	[206, 207]
ChAT^{BAC}-EGFP	[208, 209]
Mapt$^{tm1(EGFP)kit}$	[210–212]
CNP-EGFP	[213–219]
Peripherin-EGFP (hPRPH1-G)	[220]
Hoxb4-ENE-GFP-cre	[221]
Math1-GFP	[222]
CNPase-GFP	[105, 223]
NGF-EGFP	[224–226]
SOX2-EGFP	[227–240]
Drd1a- & Drd2-EGFP, Drd1a-tdTomato	[241–244]
GABASnFR	[245, 246]