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Treatment of epilepsy: the GABA-transaminase inhibitor, vigabatrin, induces neuronal plasticity in the mouse retina

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Abstract

Vigabatrin was a major drug in the treatment of epilepsy until the discovery that it was associated with an irreversible constriction of the visual field. Nevertheless, the drug is still prescribed for infantile spasms and refractory epilepsy. Disorganization of the photoreceptor nuclear layer and cone photoreceptor damage have been described in albino rats. To investigate the vigabatrin-elicited retinal toxicity further, we examined the retinal tissue of albino mice treated with two vigabatrin doses. The higher dose did not always cause the photoreceptor layer disorganization after 1 month of treatment. However, it triggered a massive synaptic plasticity in retinal areas showing a normal layering of the retina. This plasticity was shown by the withdrawal of rod but not cone photoreceptor terminals from the outer plexiform layers towards their cell bodies. Furthermore, both rod bipolar cells and horizontal cells exhibited dendritic sprouting into the photoreceptor nuclear layer. Withdrawing rod photoreceptors appeared to form ectopic contacts with growing postsynaptic dendrites. Indeed, contacts between rods and bipolar cells, and between bipolar cells and horizontal cells were observed deep inside the outer nuclear layer. This neuronal plasticity is highly suggestive of an impaired glutamate release by photoreceptors because similar observations have been reported in different genetically modified mice with deficient synaptic transmission. Such a synaptic deficit is consistent with the decrease in glutamate concentration induced by vigabatrin. This description of the neuronal plasticity associated with vigabatrin provides new insights into its retinal toxicity in epileptic patients.

Keywords

bipolar cells; horizontal cells; photoreceptors; toxicity; vision

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Introduction

Vigabatrin, gamma-vinyl GABA (VGB), is a drug that used to be widely used in the treatment of partial epilepsy, pharmacoresistant epilepsy and infantile spasm (French *et al.*, 1996; Lortie *et al.*, 1997; Curatolo *et al.*, 2006). VGB treatment greatly reduced the seizure frequency and made many patients seizure-free (Livingston *et al.*, 1989). VGB is an irreversible inhibitor of GABA-transaminase and its antiepileptic action is a result of it increasing the concentration of GABA in the brain. Unfortunately, peripheral visual field loss in patients treated with VGB was reported during the late 1990s (Krauss *et al.*, 1998; Ruether *et al.*, 1998; Daneshvar *et al.*, 1999). This functional impairment of vision was associated with altered electro-retinogram (ERG) measurements, suggesting damage to the retina in the visual system. The most consistently altered measurements include increased latency and reduced amplitude of the photopic ERG b-wave and abnormalities of flicker responses (Krauss *et al.*, 1998; Ruether *et al.*, 1998; Daneshvar *et al.*, 1999; Miller *et al.*, 1999; Harding *et al.*, 2000; Coupland *et al.*, 2001; Westall *et al.*, 2002). All of these electrophysiological changes are indicative of a dysfunction in the cone photoreceptor pathway. However, other studies also reported decreases in amplitude of the scotopic ERG b-wave consistent with these patients also suffering an alteration in the rod phototransduction pathway (Daneshvar *et al.*, 1999). The reversible decrease in electro-oculogram amplitude suggested that the cellular interactions between photoreceptors and the retinal pigment epithelium were affected (Arndt *et al.*, 1999; Lawden *et al.*, 1999). Finally, atrophy of the retinal fibre layer was observed in both infants (Buncic *et al.*, 2004) and adults (Wild *et al.*, 2006) taking vigabatrin.

In the retina, GABA activates GABA_C receptors that are more sensitive than GABA_A receptors and do not desensitize (Feigenspan & Bormann, 1998; Lukasiewicz *et al.*, 2004). Furthermore, GABA can remain excitatory in the adult retina (Vardi *et al.*, 2000; Varela *et al.*, 2005; Duebel *et al.*, 2006). VGB was shown to increase the retinal GABA concentration by up to sevenfold (Cubells *et al.*, 1987; Neal *et al.*, 1989) and therefore to a level that could trigger GABA excitotoxicity. Retinal examination identified major disorganization of the outer nuclear layer (ONL) in VGB-treated animals (Butler *et al.*, 1987), long before visual field constriction in VGB-treated patients was reported. Retinal damage was further documented recently by descriptions of major glial reactions in rats and rabbits (Duboc *et al.*, 2004; Ponjavic *et al.*, 2004). In addition, cone photoreceptors showed disorganized or lost inner/outer segments in central areas with a normal retinal morphology (Duboc *et al.*, 2004). However, these findings did not explain the loss of transmission from rod photoreceptors to bipolar cells indicated by scotopic ERG measurements (Coupland *et al.*, 2001).

We report evidence of an expected plasticity at photoreceptor synapses during the VGB treatment in mice, which could explain these electrophysiological results in patients under scotopic conditions.

Materials and methods

Animal treatment

All animal experiments were performed in compliance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research and with the guidelines of the INSERM ethics committee. BALB/c mice (16 treated, 10 controls) were purchased from Janvier (Le Genest-St-Isle, France) at 6 weeks of age. VGB was dissolved in 0.9% NaCl at 100 mg/mL and injected intraperitoneally daily for 30 days at doses of 5 and 3 mg/day as described for treatment of animal epilepsy (Andre *et al.*, 2001). These doses (250 or 150 mg/kg) are in the same range or lower than those prescribed to children (100 mg/kg, Bialer *et al.*, 2001) or infants (250 mg/kg, Bialer *et al.*, 2001; 400 mg/kg, Aicardi *et al.*, 1996).

Histology

Animals were killed by CO₂ inhalation. Eye cups were fixed overnight at 4 °C in 4% (w/v) paraformaldehyde in 0.01 M phosphate-buffered saline, pH 7.4. The tissue was cryoprotected by immersion in phosphate-buffered saline containing, successively, 10, 20 and 30% sucrose at 4 °C and then embedded in OCT (Labonord, Villeneuve d'Ascq, France). Vertical sections (8–10 µm thickness) were permeabilized for 5 min in phosphate-buffered saline containing 0.1% Triton X-100 (Sigma, St Louis, MO, USA), rinsed and incubated in phosphate-buffered saline containing 1% bovine serum albumin (Eurobio, Les-ULis, France), 0.1% Tween 20 (Sigma) and 0.1% sodium azide (Merck, Fontenay-Sous-Bois, France) for 2 h at room temperature (20°C). The primary antibody was added to the solution and the samples incubated for 2 h at room temperature. The polyclonal antibodies used were directed against protein kinase C alpha (PKCα) (1: 2000, Sigma), vesicular glutamate transporter 1 (VGLUT1) (1: 2000, Millipore, Billerica, MA, USA), Goα (K-20; 1 : 100, Santa Cruz Biotechnology, Santa Cruz, CA, USA), calbindin D-28K (1 : 500, Millipore), mouse cone arrestin (1 : 20 000; Zhu *et al.*, 2002) or glial fibrillary acidic protein (GFAP) (1 : 200, Dako, Carpinteria, CA USA). The monoclonal antibodies were directed against Goα (1 : 2000, Millipore), bassoon (1 : 100, StressGen, Ann Arbor, MI, USA) or calbindin D-28K (1 : 500, Sigma). After rinses, sections were incubated with the secondary antibody, goat anti-rabbit IgG or rabbit anti-mouse IgG conjugated to either Alexa TM594 or Alexa TM488 (1 : 500, Molecular Probes, Eugene, OR, USA), for 2 h. The dye, diamidiphenyl-indole (Sigma), was added to the solutions for the last incubations. Sections were rinsed, mounted with Fluorsave reagent (Calbiochem, San Diego, CA, USA) and viewed with a DM 5000B microscope (Leica Microsystems SAS, Rueil Malmaison, France) equipped with a Photometrics cool SNAP TM FX camera (Roper Scientific, Tuscon, AZ, USA). For higher resolutions, preparations were observed with a confocal inverted microscope [Leica TCS Spectral (SP2) instrument] using 63 and 100 oil immersion objectives both with a numerical aperture of 1.4. A krypton/argon mixed-gas laser and two helium/neon mixed-gas lasers were used to generate the bands at 488, 543 and 633 nm, respectively.

Results

In mice treated daily with 5 mg/day, there was disorganization of the retinal architecture with photoreceptor nuclei moving into the layers of inner/outer segments toward the retinal pigment epithelium (see Fig. 1). However, this disorganization of the ONL was observed in only five of the 16 treated mice. Therefore, the photoreceptor layer can become disorganized in VGB-treated mice but this effect was not observed in all VGB-treated animals.

As retinal damage is often associated with retinal gliosis and an increase in GFAP expression, we stained retinal sections of VGB-treated and control animals with a GFAP antibody. In control albino mice, some glial Müller cells were intensely GFAP immunopositive (Fig. 2A); VGB treatment did not increase the intensity of this GFAP immunolabelling (Fig. 2B) except in highly disorganized areas (see below).

Cone photoreceptors in VGB-treated mice were then examined following their identification with the mouse cone arrestin antibody (Zhu *et al.*, 2002). Cone outer segments and their terminals were intensely labelled with the cone arrestin antibody (Fig. 2C and E). In VGB-treated mice, cone outer/inner segments were altered in particular areas, whereas their terminals remained intensely labelled in the outer plexiform layer (OPL) (Fig. 2D and F). These observations indicate that the VGB treatment led to cone damage.

To examine the reaction of inner retinal neurones to the VGB treatment, ON rod bipolar cells were stained with the antibody directed against the protein PKCα. In control animals, ON bipolar cells exhibited cell bodies with very short dendrites extending to the OPL and axons

reaching the inner part of the inner plexiform layer (Fig. 3A and B). By contrast, in VGB-treated animals, rod ON bipolar cells extended their dendrites deep into the ONL (Fig. 3C and D). Note that on the diamidiphenyl-indole-stained section (Fig. 3C) this rod bipolar cell plasticity was observed in areas with no disorganization of the ONL. Indeed, growing dendrites of postsynaptic neurones were present throughout the retinal sections, suggesting that these features of plasticity occurred in postsynaptic neurones independently of the ONL disorganization. To determine whether this plasticity of postsynaptic neurones was restricted to bipolar cells, we used a calbindin antibody to label horizontal cells. Horizontal cell bodies in controls were located at the distal part of the inner nuclear layer with their dendritic processes extending into the OPL (Fig. 3E and F). In VGB-treated animals, horizontal cell processes were not limited to the OPL but they extended deep into the ONL (Fig. 3G and H). Therefore, the VGB treatment induced plasticity in both bipolar and horizontal cells.

To examine if this plasticity of postsynaptic neurones to photoreceptors was related to changes in synaptic terminals of photoreceptors themselves, we used an antibody directed against VGLUT1, a protein of photoreceptor synaptic vesicles. On retinal sections of control animals, the VGLUT1 immunolabelling was restricted to the OPL (Fig. 3I and J), whereas it was observed deep within the ONL in VGB-treated animals (Fig. 3K and L). We then used an antibody directed against the protein bassoon to study the synaptic specialization of photoreceptors, the synaptic ribbon. In the control retina, bassoon-positive structures were observed in both the OPL and inner plexiform layer (Fig. 3M and N), whereas in VGB-treated animals some synaptic ribbons were clearly identified in the ONL (Fig. 3O and P). These observations indicate that the synaptic plasticity of postsynaptic neurones was associated with a withdrawal of photoreceptor synapses.

Labelling with the cone arrestin antibody surprisingly suggested that cone photoreceptor terminals remained in the OPL even in highly disorganized areas (see Fig. 2D). To confirm that the neuronal morphology of cone terminals was not affected by VGB treatment in areas with sprouting of postsynaptic neurones, retinal sections were immunolabelled with the cone arrestin antibody and ON bipolar cells were identified with the Go α antibody. Although the OPL structure in VGB-treated mice was not as linear as in control animals, cone terminals remained in the OPL, whereas Go α -positive dendrites of ON bipolar cells were detected very deep inside the ONL (Fig. 4A–C). These observations suggested that rod but not cone photoreceptors were withdrawing their terminals toward their cell bodies.

To determine if the plasticity of photoreceptor postsynaptic neurones also involved neurones participating in cone terminals, cone ON bipolar cells were visualized by double immunolabelling with the protein Go α antibody to stain both cone and rod ON bipolar cells, and the protein PKC α antibody specifically to identify rod ON bipolar cells. In the inner nuclear layer, rod bipolar cells were labelled by both the anti-Go α antibody and the anti-PKC α antibody (green in Fig. 5A and D and yellow in Fig. 5C and F), whereas cone ON bipolar cells were only labelled by the Go α antibody (red in Fig. 5B, C, E and F). The sections were examined at high resolution with the confocal microscope; only rod ON bipolar cells exhibited dendrites extending into the ONL. Indeed, all Go α -positive dendrites seen to extend into the ONL were labelled with the PKC α antibody (yellow in Fig. 5F); this suggests that cone ON bipolar cells do not extend their dendrites into the ONL. These observations demonstrated that only rod ON bipolar cells underwent plasticity following VGB treatment.

We then examined if the withdrawing rod terminals and the sprouting postsynaptic neurones were forming ectopic synapses. We stained photoreceptor terminals with the antibody against the synaptic protein, VGLUT1, and ON bipolar cells with the Go α antibody. Under confocal microscopy, many dendritic tips of bipolar cells that had grown into the ONL were observed to be in contact with withdrawing VGLUT1-positive photoreceptor terminals (Fig. 6A–C, inset

in C). Similarly, Go α -positive bipolar cell dendrites that had grown into the ONL were often associated with calbindin-positive sprouting dendrites of horizontal cells (Fig. 6D–F, inset in F). To confirm that these contacts may be ectopic synapses, photoreceptor synaptic ribbons were immunolabelled with the bassoon antibody. As described above, these ribbon synapses were all distributed within the OPL in the control animals (Fig. 7A) but they were found deep within the ONL among photoreceptor nuclei in VGB-treated animals (Fig. 7B). In this location, although few synaptic ribbons appeared to be isolated on the optical section observed by confocal microscopy (arrowhead in the upper part of Fig. 7B), many were seen to be in contact with PKC α -immunopositive sprouting dendrites of rod bipolar cells (Fig. 7B, inset). These observations were consistent with the formation of ectopic synapses by rod photoreceptors in the ONL.

To investigate the dose dependence of this neuronal plasticity and of the photoreceptor layer disorganization, mice were treated with a lower VGB dose (3 mg/day). Under these conditions, retinal areas with photoreceptor layer disorganization were observed in all animals ($n = 7$) (Fig. 8); GFAP expression was abnormally high in all of these disorganized retinal areas (Fig. 8A and D). Surprisingly, alterations of cell morphology with sprouting of rod bipolar cells in the ONL were mainly restricted to the areas of photoreceptor layer disorganization (Fig. 8E). These observations indicate that the different histological changes (photoreceptor layer disorganization and neuronal plasticity) may be associated with different VGB doses.

Discussion

The first report of VGB retinal toxicity described a disorganization of the ONL at the periphery in treated albino rats (Butler *et al.*, 1987). This disorganization of the ONL with photoreceptor nuclei abutting the retinal pigment epithelium was subsequently confirmed (Duboc *et al.*, 2004). Retinal damage was further substantiated by descriptions of cone photoreceptor damage (Duboc *et al.*, 2004) and major glial reactions revealed as an increase in GFAP immunolabelling intensity (Ponjavic & Andreasson, 2001; Duboc *et al.*, 2004). In the present study, we confirmed the disorganization of the photoreceptor layer and cone damage in VGB-treated albino mice with a higher incidence at the lowest VGB dose. In mice treated with the higher dose of VGB (5 mg/day) the most drastic change was the plasticity of postsynaptic bipolar and horizontal cells, involving withdrawal of presynaptic rod photoreceptor synapses. This plasticity of postsynaptic neurones was not observed in all animals and, as was the case in mice treated with a low dose of VGB, was restricted to highly damaged retinal areas in VGB-treated rats (data not shown). By contrast, mice treated with the higher VGB dose displayed neuronal plasticity in all retinal areas prior to any disorganization of the ONL. This study therefore suggests that VGB treatment may cause various different phenomena including neuronal plasticity, cone damage and ONL dysplasia.

Plasticity of retinal neurones has been reported in different animal models including ageing mice (Liets *et al.*, 2006). In retinitis pigmentosa, photoreceptor degeneration is associated with photoreceptor axon sprouting into the inner nuclear layer, whereas postsynaptic neurones show only minor plasticity (Li *et al.*, 1995). By contrast, plasticity of postsynaptic neurones has been reported in animal models of retinal detachment that involve photoreceptor apoptosis (Cook *et al.*, 1995). This plasticity of the postsynaptic neurones is associated with deep withdrawal of photoreceptor synaptic terminals into the ONL (Lewis *et al.*, 1998; Liets *et al.*, 2006). Such plasticity has also been observed in a number of genetically modified animals in which synaptic transmission from photoreceptor to postsynaptic neurones is impaired. Examples of this observed plasticity include: (i) the Nob2 mouse with a mutation on the *Cacna1f* gene encoding the $\alpha 1F$ subunit of photoreceptor voltage-dependent calcium channels (Chang *et al.*, 2006); (ii) the knockout mice for the Ca^{2+} -binding protein, *Cabp4*, specific to photoreceptors (Haeseleer *et al.*, 2004); and (iii) the knockout mice for bassoon, the photoreceptor synaptic

protein (Dick *et al.*, 2003). In contrast, no synaptic plasticity was observed when the defect originated in the postsynaptic cells as, for example, in ON bipolar cells in either mGluR, metabotropic glutamate receptor 6 (mGluR6) (Masu *et al.*, 1995) or Goa knockout mice (Dhingra *et al.*, 2000). These various findings are consistent with the idea that a defect in photoreceptor synaptic transmission triggers plasticity of postsynaptic neurones, whereas dysfunction of postsynaptic neurones concerning reception of the presynaptic signal does not alter their synaptic organization. The VGB-induced photoreceptor impairment is further indicated by presynaptic plasticity in rod photoreceptors withdrawing their terminals as observed in both retinal detachment (Lewis *et al.*, 1998) and in bassoon knockout mice with ectopic synapse formation in the ONL (Dick *et al.*, 2003). Therefore, the plasticity of retinal neurones at rod photoreceptor synapses provides evidence for a defect in synaptic transmission from these photoreceptors.

Photoreceptors are glutamatergic neurones continuously releasing glutamate in the dark; this release is modulated by light stimulation such that the glutamate concentration in the synaptic cleft is a linear function of light intensity. We propose that the observed defect in photoreceptor synaptic transmission is related to the reduction in glutamate and glutamine concentrations previously reported in various brain structures of VGB-treated animals (Bernasconi *et al.*, 1988; Halonen *et al.*, 1991; Loscher & Horstermann, 1994). This decrease in glutamate concentration is related to a decrease in glutamine synthetase activity in VGB-treated animals (Waniewski & Martin, 1995). This would explain the formation of ectopic synapses in the ONL as seen in animal models with deficient photoreceptor synapses. It could also explain the degeneration of cone photoreceptors, because cone degeneration has also been described in bassoon knockout mice (Specht *et al.*, 2007). However, it cannot explain the disorganization of the ONL seen in both VGB-treated mice and rats because such a disorganization of the photoreceptor layers has never been reported in any of the genetically modified animals described above. This decrease in glutamate concentration could therefore contribute significantly to the formation of ectopic synapses and cone degeneration but photoreceptor layer disorganization must presumably involve additional mechanisms. In particular, it is unclear if the VGB-induced GABA increase (Cubells *et al.*, 1987; Neal *et al.*, 1989) contributes to these events.

In humans, the measurements most consistently altered by VGB treatment include an increase in latency and a decrease in amplitude of the photopic ERG b-wave, and abnormalities of flicker responses (Krauss *et al.*, 1998; Ruether *et al.*, 1998; Daneshvar *et al.*, 1999; Miller *et al.*, 1999; Harding *et al.*, 2000; Coupland *et al.*, 2001; Westall *et al.*, 2002). All of these electrophysiological changes are indicative of a dysfunction in the cone photoreceptor pathway. Alterations in the cone pathway have been confirmed by analysis of colour vision, visual acuity and other visual tasks depending on cone visual perception (Miller *et al.*, 1999; Hilton *et al.*, 2002). The absence of a significant difference in the ERG a-wave amplitude does not mean that cone photoreceptors themselves were not affected because the photopic ERG a-wave is attributed to neurones postsynaptic to cone photoreceptors in the primate retina (Bush & Sieving, 1994). The cone damage observed at all VGB doses in mice is therefore consistent with the decrease in the photopic ERG in VGB-treated epileptic patients.

Considering the rod pathway, the scotopic ERG a-wave is directly related to the kinetics of the rod photoreceptor response whereas the scotopic ERG b-wave is representative of ON rod bipolar cell function. A significant number of VGB-treated epileptic patients also display a scotopic ERG b-wave reduction (Coupland *et al.*, 2001; Hardus *et al.*, 2003). The observed plasticity of neurones postsynaptic to rod photoreceptors and the correlated impairment of synaptic transmission could readily explain these ERG changes. An impairment of the synaptic transmission from rod to ON bipolar cells should not affect the scotopic ERG a-wave but may decrease the amplitude of the ERG b-wave as in mGluR6 knockout mice (Masu *et al.*, 1995).

The withdrawal of rod photoreceptor terminals associated with the growth of postsynaptic dendrites may allow contacts to be maintained as in the bassoon knockout mice, thereby limiting the reduction in the scotopic ERG b-wave amplitude. Impairment of rod synaptic transmission and the correlated retinal cell plasticity could therefore explain the scotopic ERG b-wave amplitude reduction without change in the scotopic ERG a-wave amplitude. Observation of the retinal plasticity only at higher doses in VGB-treated mice may explain the inconsistency of the reduction in the scotopic ERG b-wave amplitude in VGB-treated epileptic patients.

Recent estimates of VGB-associated visual field loss suggest that approximately 30% of epileptic patients receiving this anticonvulsant treatment at normal therapeutic levels suffer visual abnormalities (Daneshvar *et al.*, 1999; Lawden *et al.*, 1999). Although neuronal plasticity can explain the change in the scotopic ERG-b wave, the reversible change in electro-oculogram in VGB-treated epileptic patients (Arndt *et al.*, 1999; Lawden *et al.*, 1999) cannot be a consequence of synaptic plasticity. The electro-oculogram provides an indication of a dysfunction at the interface between photoreceptors and the retinal pigment epithelium. This interface dysfunction may in fact trigger the neuronal plasticity. Indeed, in models of retinal detachment where photoreceptors are physically disconnected from the retinal pigment epithelium, a similar neuronal plasticity has been observed with rod photoreceptors withdrawing their terminals, whereas ON bipolar cells and horizontal cells exhibit dendrites sprouting into the ONL (Lewis *et al.*, 1998). Cones, in contrast to rods, were not retracting their terminals. Cone photoreceptor damage in VGB-treated rats and the observed reactive retinal glial cells crossing the outer limiting membrane have already been suggested as showing similarities with retinal detachment (Duboc *et al.*, 2004). The plasticity of postsynaptic neurones that we describe is further consistent with features of retinal detachment (Lewis *et al.*, 1998). The VGB treatment may therefore cause reversible changes at the interface between photoreceptors and the retinal pigment epithelium, and these events may trigger the irreversible damage to the neuronal network like that observed following retinal detachment.

Conclusion

We report VGB-elicited neuronal plasticity that has not previously been described and gives insight into the observed electrophysiological alterations in VGB-treated epileptic patients. Treating mice with VGB should therefore provide an excellent animal model for investigating the molecular mechanisms of VGB toxicity in different strains of genetically modified mice.

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Abbreviations

ERG	electroretinogram
GFAP	glial fibrillary acidic protein
mGluR	metabotropic glutamate receptor
ONL	outer nuclear layer

OPL	outer plexiform layer
PKC α	protein kinase C alpha
VGB	vigabatrin
VGLUT1	vesicular glutamate transporter 1

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Fig. 1. Disorganization of the photoreceptor ONL in VGB-treated mice. Diamidiphenyl-indole-stained vertical sections of the retina showing the linear ONL in a control mouse (A) and the photoreceptor nuclei migrating toward the retinal pigment epithelium (RPE) in a VGB-treated animal (B). Scale bar, 25 μ m. INL, inner nuclear layer.

Fig. 2.

Cone cell damage without glial reaction in VGB-treated mice. Vertical retinal sections in control (A, C and E) and VGB-treated animals (B, D and F) stained with diamidiphenyl-indole to visualize cell bodies (blue in A–F) and immunolabelled with antibodies directed against the GFAP protein (red in A and B) and against cone arrestin (red in C–F). The GFAP immunostaining shows that glial Müller cells are immunopositive in an untreated albino mouse (A) with no enhancement in a VGB-treated animal (B). (C–F) Cone arrestin immunolabelling (CARRES in red) reveals that there are fewer cone outer segments (OSs) in the VGB-treated mouse (D and F) than in the control animal (C and E). Note the disorganization of the ONL close to the area showing cone damage in D. Scale bars, 25 μm. INL, inner nuclear layer.

Fig. 3.

Morphological changes in the outer retinal cells of VGB-treated animals. Vertical retinal sections from control (A, B, E, F, I, J, M and N) and VGB-treated (C, D, G, H, K, L, O and P) animals both stained with diamidiphenyl-indole (A, C, E, G, I, K, M and O) and immunolabelled with antibodies directed against one of the following: PKC α (B and D), calbindin (F and H), VGLUT1 (J and L) or bassoon (N and P). In the control animal, PKC α -positive ON bipolar cells (B) and calbindin-positive horizontal cells (F) have their dendritic tips in the OPL. In contrast, in the VGB-treated mouse, rod bipolar cells (D) and horizontal cells (H) have dendritic processes extending deep into the ONL. On the photoreceptor side, VGLUT1-positive structures (J) and bassoon-positive synaptic ribbons (N) are only found in the plexiform layers (F) of control animals, whereas they are observed deep within the ONL of the VGB-treated animals (H and P). Scale bar, 25 μ m. IPL, inner plexiform layer; INL, inner nuclear layer.

Fig. 4.

No detectable withdrawal of cone photoreceptor terminals in VGB-treated animals. Vertical retinal sections in a VGB-treated mouse stained with antibodies directed against the protein Go α (green in A and C) and against cone arrestin (CARRES in red in B and C). Note the presence of Go α -positive ON bipolar cell dendrites in the ONL of VGB-treated animals (green in A and C), whereas cone arrestin-immunopositive terminals remain localized in the OPL (red in B and C) as in the control animals. Scale bar, 25 μ m. OS, outer segment.

Fig. 5.

Dendritic sprouting from rod but not cone ON bipolar cells in VGB-treated mice. Vertical retinal sections in control (A–C) and VGB-treated (D–F) animals were immunolabelled with antibodies directed against the PKC α to reveal rod bipolar cells (green in A, C, D and F) and against the protein Go α to identify rod and cone bipolar cells (red in B, C, E and F). These sections were also stained with diamidiphenyl-indole to reveal the nuclear layers (blue in C and F). Rod ON bipolar cells (green in A and D) are stained by both the anti-PKC α and anti-Go α antibodies (yellow in C and F), whereas cone ON bipolar cells are labelled only by the protein Go α antibody (red in B, C, E and F). Note that in the control animal (A–C), all ON bipolar cells have their dendrites ramifying in the OPL, whereas rod ON bipolar cells extend their dendrites into the ONL of the VGB-treated mouse (yellow in F). Scale bar, 20 μ m.

Fig. 6.

Maintenance of cell contacts during cellular plasticity, induced by the VGB treatment, at the photoreceptor synapses. (A–C) Vertical retinal sections in VGB-treated animals stained with antibodies directed against the protein Go α (green in A, C, D and F), calbindin (red in B and C) or VGLUT1 (E and F). Both Go α -positive ON bipolar cells (A) and calbindin-positive horizontal cells (B) have dendrites growing into the ONL. The dendritic tips of these two cell populations are often seen in close apposition in the ONL (C, inset). Similarly, when photoreceptor terminals are labelled with VGLUT1, numerous tips of ON bipolar cell dendrites growing into the ONL can be seen in close contact with a VGLUT1-positive withdrawn photoreceptor terminal (inset in F). INL, inner nuclear layer. Scale bar, 25 μ m.

Fig. 7.

Formation of ectopic synapses by rod photoreceptors. Vertical retinal sections in a control (A) and a VGB-treated (B) animal immunolabelled with antibodies directed against the protein Go α (green) and against bassoon (red) and stained with diamidiphenyl-indole (blue). In control animals (A), bassoon-immunopositive synaptic ribbons (red) are located in the OPL below the layer of photoreceptor nuclei and above the inner nuclear layer containing the Go α -positive bipolar cell bodies (green). In the VGB-treated mouse, some bassoon-positive structures are found among photoreceptor nuclei in the ONL (arrowheads) where they are often in contact with sprouting bipolar cell dendrites, thereby forming ectopic synapses (B). The inset illustrates such ectopic synapses between bassoon-positive structures and growing bipolar cell dendrites. Scale bar, 20 μ m.

Fig. 8. Glial cell reaction and bipolar cell plasticity in disorganized retinal areas of VGB-treated mice. Vertical retinal sections in a control (A–C) and a VGB-treated (D–F) animal immunolabelled with antibodies directed against GFAP (green in A, C, D and F) and against PKC α (red in B, C, E and F) and stained with the diamidiphenyl-indole nuclear dye (blue in A–F). Note that the ONL is disorganized in the VGB-treated animal (D–F) with reference to the control animal (A–C). In this disorganized area, more GFAP-positive glial cell processes are observed (D). The dendrites of PKC α -positive bipolar cell dendrites, which normally contact photoreceptor terminals in the OPL (B), are found to have grown into the ONL at the site of the disorganization in the retina (E). Scale bar, 25 μ m.