### Target Disease

| Transient retinal ischemia by selective ligature of the ophthalmic vessels |

### Species

| Rat (Rattus norvegicus) |

### Short Description

To investigate the pattern of RGC loss that follows transient ischemia of the retina induced by selective ligature of the ophthalmic vessels (SLOV).

Ischemia-induced RGC death is a progressive event that takes place in at least two phases: an early rapid and a later more protracted period of cell loss. The amount and duration of these periods of cell loss are determined by the duration of the period of ischemia.

In brief, the left ON head is exposed in the orbit, the superior aspect of its dural sheath is opened longitudinally, and a 10-0 nylon suture is introduced between the dural sheath and the ON and tied around the sheath, to interrupt blood flow through the ophthalmic vessels, which run in an inferior and nasal aspect within the sheath. Care is taken not to damage the ON. Interruption of retinal blood flow during ischemia is assessed by direct ophthalmoscopy of the eye fundus through the surgical microscope. The animals that do not show a complete interruption of retinal blood flow during the ischemic period are excluded. At the end of the ischemic period, the ligature is released, and retinal reperfusion is assessed through the surgical microscope. The animals that do not show a complete recovery of retinal blood flow within the first few minutes after the ligature is released are also excluded. Eye fundus inspection is facilitated, because most eyes appeared mydriatic after the induction of transient ischemia. When necessary, a drop of 1% tropicamide is applied topically to induce mydriasis.

### Scientific Publications

**The neuropeptide NAP provides neuroprotection against retinal ganglion cell damage after retinal ischemia and optic nerve crush.**

The growth factor response in ischemic rat retina and superior colliculus after brimonidine pre-treatment.
PMID: 17113948

Death and neuroprotection of retinal ganglion cells after different types of injury.
Vidal-Sanz M, Lafuente M, Sobrado-Calvo P, Selles-Navarro I, Rodríguez E, Mayor-Torroglosa S, Villegas-Perez MP.

Ischemia results 3 months later in altered ERG, degeneration of inner layers, and deafferented tectum: neuroprotection with brimonidine.
PMID: 16186370

Transient ischemia of the retina results in massive degeneration of the retinotectal projection: long-term neuroprotection with brimonidine.
Avilés-Trigueros M, Mayor-Torroglosa S, Garcia-Avilés A, Lafuente MP, Rodríguez ME, Miralles de Imperial J, Villegas-Pérez MP, Vidal-Sanz M.
PMID: 14769369

Transient ischemia of the retina results in altered retrograde axoplasmic transport: neuroprotection with brimonidine.
Lafuente López-Herrera MP, Mayor-Torroglosa S, Miralles de Imperial J, Villegas-Pérez MP, Vidal-Sanz M.
PMID: 12504883

Neuroprotective effects of brimonidine against transient ischemia-induced retinal ganglion cell death: a dose response in vivo study.
Lafuente MP, Villegas-Pérez MP, Mayor S, Aguilera ME, Miralles de Imperial J, Vidal-Sanz M.
PMID: 11950228

Retinal ganglion cell death after acute retinal ischemia is an ongoing process whose severity and duration depends on the duration of the insult.
Lafuente MP, Villegas-Pérez MP, Sellés-Navarro I, Mayor-Torroglosa S, Miralles de Imperial J, Vidal-Sanz M.
PMID: 11784707

Brimonidine’s neuroprotective effects against transient ischaemia-induced retinal ganglion cell death.
Vidal-Sanz M, Lafuente MP, Mayor-Torroglosa S, Aguilera ME, Miralles de Imperial J,
Neuroprotective effects of alpha(2)-selective adrenergic agonists against ischemia-induced retinal ganglion cell death.
Lafuente MP, Villegas-Pérez MP, Sobrado-Calvo P, García-Avilés A, Miralles de Imperial J, Vidal-Sanz M.
PMID: 11481275

Retinal ganglion cell death induced by retinal ischemia. Neuroprotective effects of two alpha-2 agonists.
Vidal-Sanz M, Lafuente MP, Mayor S, de Imperial JM, Villegas-Pérez MP.
PMID: 11377446
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<th><strong>Target Disease</strong></th>
<th>Transient retinal ischemia by increasing the intraocular pressure</th>
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<td><strong>Species</strong></td>
<td>Rat (Rattus norvegicus), Mouse (Mus musculus)</td>
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<td><strong>Short Description</strong></td>
<td>To investigated the pattern of RGC loss that follows transient ischemia of the retina induced by elevation of intraocular pressure (IOP). Ischemia-induced RGC death is a progressive event that takes place in at least two phases: an early rapid and a later more protracted period of cell loss. The amount and duration of these periods of cell loss are determined by the duration of the period of ischemia. To increase the IOP, two 6-0 silk sutures are placed on the bulbar conjunctiva of the eye just below and above the corneoscleral limbus and are used to pull tangentially in opposite directions until the blood flow to the retina is interrupted completely. Sutures were tied to a metal frame designed for these experiments to maintain the IOP above systolic arterial levels throughout the transient ischemic period. Interruption of the blood flow to the retina is assessed constantly by examining the fundus of the eye through the operating microscope, and the sutures are retightened when needed to maintain interruption of the intraretinal blood flow. To permit microscopic observation of the eye fundus and, thus, of retinal blood flow, the pupil is dilated with a drop of 1% tropicamide, and the corneal surface is covered with a drop of 2% hydroxipropilmethylcellulose and a coverslip. During the periods of retinal ischemia, interruption of the blood flow to the iris also was observed. At the end of the transient ischemic period, the conjunctival sutures were released slowly, and this is followed by the complete restoration of the blood flow to the retina and the iris.</td>
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<td>Information</td>
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<tr>
<td><strong>Target Disease</strong></td>
<td><em>Selective motoneuron paralysis or death by injection of substances in oculomotor muscles</em></td>
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<tr>
<td><strong>Species</strong></td>
<td><em>Rat (Rattus norvegicus), Mouse (Mus musculus)</em></td>
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<tr>
<td><strong>Short Description</strong></td>
<td><em>To investigate in vivo the survival of abducens motoneurons (AMNs) at different periods of time after a single intramuscular injection of the neurotoxin botulinum toxin A (BTxA) or doxorubicin (DXR) in the abducens muscle. The AMNs were labeled with fluorogold (FG) applied intramuscularly in the lateral rectus muscle. The numbers of labeled neurons were determined in adult control animals, in young animals that had received BTxA and in adult rats that had received DXR, at various survival times. The intramuscular injection of BTxA in young animals does not induce significant motoneuron death, while DXR injection causes variable amounts of motoneuron death that is related both to the survival period and to the amount of DXR injected.</em></td>
</tr>
<tr>
<td><strong>Scientific Publications</strong></td>
<td><em>Effects of intramuscular injection of botulinum toxin and doxorubicin on the survival of abducens motoneurons.\nGómez-Ramírez AM, Villegas-Pérez MP, Miralles de Imperial J, Salvador-Silva M, Vidal-Sanz M.\nInvest Ophthalmol Vis Sci. 1999 Feb;40(2):414-24.\nPMID: 9950601</em></td>
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<tr>
<td>Target Disease</td>
<td>Selective axotomy-induced RGC death by microcrush lesion of optic nerve</td>
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<td>Species</td>
<td>Rat (Rattus norvegicus), Mouse (Mus musculus)</td>
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<td>Short Description</td>
<td>Injury of the optic nerve has served as an important model for the study of cell death and axon regeneration in the CNS. Microcrush lesion of the optic nerve, which completely transects all RGC axons and minimizes the amount of secondary damage, creates a well-defined injury site. The microcrush lesion of the optic nerve is an excellent model to study strategies designed to promote axon regeneration in the inhibitory white matter environment. In brief, the left optic nerve is exposed through a superior temporal approach, and the dural sheath is slit longitudinally, taking care to avoid the ophthalmic artery. Microcrush lesions are made with 10-0 sutures used to completely constrict the optic nerve by holding a tight knot for 60 s and then releasing the suture. The fundus oculi is examined to verify the integrity of the retinal blood circulation. Animals whose retinas show ischemic damage are discarded.</td>
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<tr>
<td><strong>Target Disease</strong></td>
<td>Selective axotomy-induced RGC death by crush lesion of optic nerve</td>
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<tr>
<td><strong>Species</strong></td>
<td>Rat (Rattus norvegicus), Mouse (Mus musculus)</td>
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<td><strong>Short Description</strong></td>
<td>Intraorbital nerve crush (IONC) is a type of injury in which RGC axons are interrupted by pressure without a physical gap among the edges of the injury. However, there is a high variability among the results reported using this kind of lesion which are mainly due to the diversity of tools utilized, to the distance from the eye where the ON is injured and to the pressure exerted, which, if not enough, results in a partial lesion with a variable number of axons spared. In our IONC model, pressing the ON at 3 mm from the eye with watchmaker's forceps during 10 s, resulted in complete interruption of the retinofugal projection, as demonstrated by their failure to transport CTB orthogradely and FG retrogradely, even several months after the lesion. In brief, to perform Intraorbital nerve crush (IONC) injury an incision is made in the superior orbital rim, the superoexternal orbital contents are dissected, and the superior and external rectus muscles are removed, then the optic nerve (ON) is crushed during 10 s at 3 mm from the optic disc using watchmaker's forceps. Before and after the procedure, the eye fundus is observed through the operating microscope to assess the integrity of the retinal blood flow.</td>
</tr>
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</table>
Immediate upregulation of proteins belonging to different branches of the apoptotic cascade in the retina after optic nerve transection and optic nerve crush.
PMID: 18775855

Time course profiling of the retinal transcriptome after optic nerve transection and optic nerve crush.
Mol Vis. 2008 Jun 3;14:1050-63.
PMID: 18552980

The neuropeptide NAP provides neuroprotection against retinal ganglion cell damage after retinal ischemia and optic nerve crush.
Jehle T, Dimitriu C, Auer S, Knoth R, Vidal-Sanz M, Gozes I, Lagrèze WA.
PMID: 18414890
<table>
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<tr>
<th><strong>Target Disease</strong></th>
<th>Selective axotomy-induced RGC death by intraocular transection of the optic nerve</th>
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<tr>
<td><strong>Species</strong></td>
<td>Rat (<em>Rattus norvegicus</em>), Mouse (<em>Mus musculus</em>)</td>
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<tr>
<td><strong>Short Description</strong></td>
<td>Death of retinal ganglion cells (RGCs) is the cause of blindness in several diseases, including glaucoma. In recent years, much effort has gone into attempting to elucidate the causes and mechanisms of RGC loss so that strategies can be developed to counteract the process. Intraorbital optic nerve transection (IONT) results in the loss of the injured neurons, the RGCs and within the following two weeks post-lesion, approximately 80% of the RGC population is lost, only approximately 15% of the RGCs remain in the retina. A variety of substances have been shown to attenuate RGC death after ONT, with brain derived neurotrophic factor (BDNF) receiving particular attention. This model has allowed quantitative studies on the capacity of axotomized RGCs for axonal regeneration. Moreover, IONT is a valuable model not only for investigation into pathways that contribute to RGC death but also as a model for neuronal apoptosis in the CNS. In experimental animals, the left optic nerve (ON) is cut close to its origin in the optic disc to axotomize the entire population of RGCs. To access the ON at the back of the eye, an incision is made in the skin overlying the superior orbital rim, the supero-external orbital contents were dissected, and the superior and external rectus muscles are sectioned. The dura mater of the ON is opened longitudinally, and the ON is transected completely as close as possible to the eye. Care is taken not to damage the retinal blood supply, which enters the eye separately in the inferonasal aspect of the ON sheath.</td>
</tr>
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</table>
| **Scientific Publications** | Axotomy-induced retinal ganglion cell death in adult mice: quantitative and topographic time course analyses.  
PMID: 21354138  
  
Brain derived neurotrophic factor maintains Brn3a expression in axotomized rat retinal ganglion cells.  
PMID: 21315070  
  
Short and long term axotomy-induced ERG changes in albino and pigmented rats.  
PMID: 19936311 |
Measurement of retinal injury in the rat after optic nerve transection: an RT-PCR study.
Chidlow G, Casson R, Sobrado-Calvo P, Vidal-Sanz M, Osborne NN.
PMID: 15947739

The effect of retinal ganglion cell injury on light-induced photoreceptor degeneration.
Casson RJ, Chidlow G, Wood JP, Vidal-Sanz M, Osborne NN.
PMID: 14744915

Effects of axotomy and intraocular administration of NT-4, NT-3, and brain-derived neurotrophic factor on the survival of adult rat retinal ganglion cells. A quantitative in vivo study.
Peinado-Ramón P, Salvador M, Villegas-Pérez MP, Vidal-Sanz M.
PMID: 8595949

Rapid and protracted phases of retinal ganglion cell loss follow axotomy in the optic nerve of adult rats.
Villegas-Pérez MP, Vidal-Sanz M, Rasminsky M, Bray GM, Aguayo AJ.
PMID: 8419522

Selective impairment of slow axonal transport after optic nerve injury in adult rats.
McKerracher L, Vidal-Sanz M, Essagian C, Aguayo AJ.
PMID: 1696983

Regrowth and connectivity of injured central nervous system axons in adult rodents.
Aguayo AJ, Bray GM, Carter DA, Villegas-Perez MP, Vidal-Sanz M, Rasminsky M.
PMID: 2130656

Persistent retrograde labeling of adult rat retinal ganglion cells with the carbocyanine dye dil.
Vidal-Sanz M, Villegas-Pérez MP, Bray GM, Aguayo AJ.
PMID: 3181354

Influences of peripheral nerve grafts on the survival and regrowth of axotomized retinal ganglion cells in adult rats.
Villegas-Pérez MP, Vidal-Sanz M, Bray GM, Aguayo AJ.
PMID: 2448429
# Target Disease

*Regenerative responses of injured neurons in the central nervous system of adult mammals through peripheral nerve grafts*

## Species

**Rat** (*Rattus norvegicus*)

## Short Description

The pattern of axonal regeneration, specificity of reinnervation, and terminal arborization in the brainstem by axotomized retinal ganglion cell axons is studied in rats with peripheral nerve grafts linking the retina with ipsilateral regions of the brainstem, including dorsal and lateral aspects of the diencephalon and lateral aspect of the superior colliculus. Functional studies on the restoration of the pupillary light reflex by regenerating retinal ganglion cell axons have been carried out with this experimental model.

In brief, autologous segments of the left common peroneal nerve are used to link the retina with the brainstem. The optic nerve of the left eye is exposed intraorbitally, and after longitudinal incision of the meningeal sheath, the nerve is completely transected close to the sclera without affecting the retinal blood supply. One end of a 3-cm-long autologous common peroneal nerve segment is apposed to the ocular stump with three 10/0 monofilament sutures. At the same time, the distal end of the PN segment is inserted into the ipsilateral side of the brainstem.

## Scientific Publications

**Reinnervation of the pretectum in adult rats by regenerated retinal ganglion cell axons: anatomical and functional studies.**

Vidal-Sanz M, Avilés-Trigueros M, Whiteley SJ, Sauvé Y, Lund RD.

*Prog Brain Res.* 2002;137:443-52.

PMID: 12440386

**Selective innervation of retinorecipient brainstem nuclei by retinal ganglion cell axons regenerating through peripheral nerve grafts in adult rats.**

Avilés-Trigueros M, Sauvé Y, Lund RD, Vidal-Sanz M.


PMID: 10627613

**Extent and duration of recovered pupillary light reflex following retinal ganglion cell axon regeneration through peripheral nerve grafts directed to the pretectum in adult rats.**

Whiteley SJ, Sauvé Y, Avilés-Trigueros M, Vidal-Sanz M, Lund RD.


PMID: 9878191

**Regenerated synapses persist in the superior colliculus after the regrowth of retinal ganglion cell axons.**

Vidal-Sanz M, Bray GM, Aguayo AJ.


PMID: 1809272

**Degenerative and regenerative responses of injured neurons in the central nervous system of adult mammals.**


Neuronal and nonneuronal influences on retinal ganglion cell survival, axonal regrowth, and connectivity after axotomy.
Bray GM, Villegas-Pérez MP, Vidal-Sanz M, Carter DA, Aguayo AJ.
PMID: 1789549

Synaptic connections made by axons regenerating in the central nervous system of adult mammals.
Aguayo AJ, Bray GM, Rasminsky M, Zwimpfer T, Carter D, Vidal-Sanz M.
PMID: 2280221

Slow transport rates of cytoskeletal proteins change during regeneration of axotomized retinal neurons in adult rats.
McKerracher L, Vidal-Sanz M, Aguayo AJ.
PMID: 2106015

[Regeneration of the visual system in the rat. Essay rewarded by the Premio de la Academia Curso 1989].
Vidal Sanz M.
PMID: 2244680

Regrowth and connectivity of injured central nervous system axons in adult rodents.
Aguayo AJ, Bray GM, Carter DA, Villegas-Perez MP, Vidal-Sanz M, Rasminsky M.
PMID: 2130656

Electrophysiologic responses in hamster superior colliculus evoked by regenerating retinal axons.
PMID: 2799387

Influences of peripheral nerve grafts on the survival and regrowth of axotomized retinal ganglion cells in adult rats.
Villegas-Pérez MP, Vidal-Sanz M, Bray GM, Aguayo AJ.
PMID: 2448429

Axonal regeneration and synapse formation in the superior colliculus by retinal ganglion cells in the adult rat.
Vidal-Sanz M, Bray GM, Villegas-Pérez MP, Thanos S, Aguayo AJ.
PMID: 3625278
The use of peripheral nerve grafts to enhance neuronal survival, promote growth and permit terminal reconnections in the central nervous system of adult rats.
Bray GM, Villegas-Pérez MP, Vidal-Sanz M, Aguayo AJ.
PMID: 3323406

Regeneration of axons from the central nervous system of adult rats.
Bray GM, Vidal-Sanz M, Aguayo AJ.
PMID: 3588955

Growth and connectivity of axotomized retinal neurons in adult rats with optic nerves substituted by PNS grafts linking the eye and the midbrain.
Aguayo AJ, Vidal-Sanz M, Villegas-Pérez MP, Bray GM.
PMID: 3474936

Responses to light of retinal neurons regenerating axons into peripheral nerve grafts in the rat.
Keirstead SA, Vidal-Sanz M, Rasminsky M, Aguayo AJ, Levesque M, So KF.
PMID: 4075162

Functional activity of rat brainstem neurons regenerating axons along peripheral nerve grafts.
Munz M, Rasminsky M, Aguayo AJ, Vidal-Sanz M, Devor MG.
PMID: 4027637
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<th>Target Disease</th>
<th>Human degenerative retinal conditions by inherited retinal dystrophy in experimental animal model</th>
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<td>Species</td>
<td>Royal College of Surgeons (RCS) rat (<em>Rattus norvegicus</em>) or P23H-1 rats</td>
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<td>Short Description</td>
<td>The dystrophic Royal College of Surgeons (RCS) and the P23H-1 rats are two well-known animal models of photoreceptor inherited degenerative diseases. Both models suffer an almost complete photoreceptor degeneration due to a defect in the MERTK (RCS) or in the rhodopsin (P23H) gene. RCS-p- (dystrophic) and RCS-p+rdy- (nondystrophic) pigmented rats and P23H-1 albino rats are available. These animals may be used to study the events taking place during retinal degeneration or to test strategies to prevent this degeneration, such as light exposure prevention, neurotrophic factor administration or intraocular cell transplantation.</td>
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| Scientific Publications | Retinal ganglion cell numbers and delayed retinal ganglion cell death in the P23H rat retina.  
PMID: 20955700  
Phototoxic-induced photoreceptor degeneration causes retinal ganglion cell degeneration in pigmented rats.  
Marco-Gomariz MA, Hurtado-Montalbán N, Vidal-Sanz M, Lund RD, Villegas-Pérez MP.  
PMID: 16856141  
Evolving neurovascular relationships in the RCS rat with age.  
Wang S, Villegas-Pérez MP, Holmes T, Lawrence JM, Vidal-Sanz M, Hurtado-Montalbán N, Lund RD.  
PMID: 14562184  
Progressive optic axon dystrophy and vacuolar changes in rd mice.  
Wang S, Villegas-Pérez MP, Vidal-Sanz M, Lund RD.  
PMID: 10670486  
Ganglion cell loss in RCS rat retina: a result of compression of axons by contracting intraretinal vessels linked to the pigment epithelium.  
Villegas-Pérez MP, Lawrence JM, Vidal-Sanz M, Lavail MM, Lund RD.  
PMID: 9482233  
Mechanism of retinal ganglion cell loss in inherited retinal dystrophy.  
Villegas-Pérez MP, Vidal-Sanz M, Lund RD.  
PMID: 8905711 |
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<th>Target Disease</th>
<th>Ocular hypertension by laser-induced ocular hypertension in retina of adult rodent mammal</th>
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<td>Species</td>
<td>Rat (<em>Rattus norvegicus</em>), mouse (<em>Mus musculus</em>)</td>
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<td>Short Description</td>
<td>In adult rats and mice, perilimbar and episcleral vein photocauterization induces ocular hypertension, which in turn results in devastating damage of the RGC population. In wide triangular sectors, preferentially located in the dorsal retina, RGCs lose their retrograde axonal transport, first by a functional impairment and after by mechanical causes. This axonal damage affects up to 80% of the RGC population, and eventually causes their death, with somal and intra-retinal axonal degeneration that resembles that observed after optic nerve crush. Importantly, while ocular hypertension affects the RGC population, it spares non-RGC neurons located in the ganglion cell layer of the retina. In addition, functional and morphological studies show permanent alterations of the inner and outer retinal layers, indicating that further to a crush-like injury of axon bundles in the optic nerve head there may by additional insults to the retina, perhaps of ischemic nature. To induce ocular hypertension, the left eyes of anesthetized animal are treated during a single session with a series of diode laser (532 nm, Quantel Medical, Clermont-Ferrand, France) burns. The laser beam is delivered directly, without any lenses, aimed at the limbal and episcleral veins. The spot size, duration, and power are set up according to the experimental animal species used and pigmented condition (albino or pigmented).</td>
</tr>
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</table>
| Scientific Publications | Anatomical and functional damage in experimental glaucoma.  
Agudo-Barriuso M, Villegas-Pérez M, de Imperial JM, Vidal-Sanz M.  
PMID: 23041078  
IOP induces upregulation of GFAP and MHC-II and microglia reactivity in mice retina contralateral to experimental glaucoma.  
PMID: 22583833  
Understanding glaucomatous damage: anatomical and functional data from ocular hypertensive rodent retinas.  
PMID: 21946033  
Myelination transition zone astrocytes are constitutively phagocytic and have synuclein dependent reactivity in glaucoma.  
Changes in the inner and outer retinal layers after acute increase of the intraocular pressure in adult albino Swiss mice.

Quantification of the effect of different levels of IOP in the astroglia of the rat retina ipsilateral and contralateral to experimental glaucoma.

Functional and morphological effects of laser-induced ocular hypertension in retinas of adult albino Swiss mice.
Mol Vis. 2009 Dec 5;15:2578-98. PMID: 20011633

Ocular hypertension impairs optic nerve axonal transport leading to progressive retinal ganglion cell degeneration.
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<th>Target Disease</th>
<th>Phototoxic retinal degeneration induced by light</th>
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<td>Species</td>
<td>Rat (<em>Rattus norvegicus</em>), Mouse (<em>Mus musculus</em>)</td>
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<tr>
<td>Short Description</td>
<td>Light-induced retinal damage (phototoxicity) selectively brings about photoreceptor cell death. Thus, this model is useful for studying the potential mechanisms underlying photoreceptor death and the subsequent retinal degeneration processes, since it mimics the photoreceptor degeneration that forms the main characteristic of human diseases such as retinitis pigmentosa or age-related macular degeneration. Moreover, it is useful to investigate the anatomic and functional changes triggered by light exposure in the experimental animal retina.</td>
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