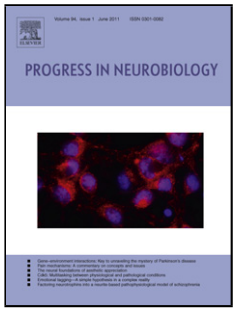

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Title: Emerging novel roles of Neuropeptide Y in the retina: from neuromodulation to neuroprotection.

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Abstract

Neuropeptide Y (NPY) and NPY receptors are widely expressed in the central nervous system, including the retina. Retinal cells, in particular neurons, astrocytes, and Müller, microglial and endothelial cells express this peptide and its receptors (Y_1, Y_2, Y_4 and/or Y_5). Several studies have shown that NPY is expressed in the retina of various mammalian and non-mammalian species. However, studies analyzing the distribution of NPY receptors in the retina are still scarce. Although the physiological roles of NPY in the retina have not been completely elucidated, its early expression strongly suggests that NPY may be involved in the development of retinal circuitry. NPY inhibits the increase in $[Ca^{2+}]_i$ triggered by elevated KCl in retinal neurons, protects retinal neural cells against toxic insults and induces the proliferation of retinal progenitor cells. In this review, we will focus on the roles of NPY in the retina, specifically proliferation, neuromodulation and neuroprotection. Alterations in the NPY system in the retina might contribute to the pathogenesis of retinal degenerative diseases, such as diabetic retinopathy and glaucoma, and NPY and its receptors might be viewed as potentially novel therapeutic targets.

Key words

Neuropeptide Y; NPY receptors; Retina; Retinal neural cells; Neuroprotection; Neuromodulation.

Contents

1. The Retina

1.1. Visual pathways in the retina

The vertebrate retina, like other regions of the central nervous system (CNS), is nervous tissue derived embryologically from the neural tube (Yang, 2004). The retina is composed of four main groups of cells: neurons, glial cells (astrocytes, Müller and microglial cells), epithelial cells (retinal pigment epithelium) and vascular cells. There are five basic types of neurons in the retina: photoreceptors, bipolar cells, horizontal cells, amacrine cells and ganglion cells. These cells are organized into clearly distinct layers, namely three layers of nerve cell bodies: outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL), and two layers of synapses: the outer plexiform layer (OPL) and the inner plexiform layer (IPL). The ONL contains cell bodies of rods and cones, the INL contains cell bodies of bipolar, horizontal and amacrine cells, while the GCL contains cell bodies of ganglion cells and displaced amacrine cells. In the OPL there are connections between rods and cones, with vertically running bipolar, and horizontally oriented horizontal cells. The second synaptic area is the IPL. It works simultaneously as a relay station for the vertical-information-carrying nerve cells, the bipolar cells, to connect to ganglion cells, and as a station for information processing which is mainly carried out by amacrine cells. It is at the end of all this neural processing in the IPL that the message concerning the visual image is transmitted to the brain along the optic nerve (Figure 1).

1.2. Neurotransmitters in the retina

Chemical transmission mediated by neurotransmitters is predominant in the neural circuitry of the retina. Although the retina contains a variety of neurotransmitters, glutamate and γ-

aminobutyric acid (GABA) are the major excitatory and inhibitory neurotransmitters, respectively. Glutamate is responsible for the radial flow of the visual signal in the retina, and both photoreceptors (rods and cones) and bipolar cells release glutamate, which induces and/or alters the activity of the post-synaptic neurons (horizontal and bipolar cells for photoreceptors in the outer retina; amacrine and ganglion cells for bipolar cells in the inner retina) by directly changing membrane permeability to ions or by activating intracellular systems through ionotropic and metabotropic glutamate receptors (iGluRs and mGluRs) (Yang, 2004). There is also a lateral or indirect pathway in the retina. This pathway is mainly mediated by GABA, which is used by numerous horizontal and amacrine cells, modulating synaptic transmission in both synaptic layers. In the OPL, horizontal cells receive direct input from photoreceptors and reply with a negative feedback to cone photoreceptors. Horizontal cells mediate the responses of the surrounding receptive field of bipolar cells. The inputs to bipolar cells are from both photoreceptors and horizontal cells. In the IPL, reciprocal synapses connect bipolar and amacrine cells, and both types of cells send input to ganglion cells. Amacrine cells are involved in spatial and temporal integration of visual signals in the IPL (Yang, 2004).

Although glutamate and GABA are the main neurotransmitters in the retina, other neurotransmitters are present, such as glycine, acetylcholine (Lindeman, 1947), dopamine (Haeggendal and Malmfors, 1963), serotonin (Kojima et al., 1961), ATP and adenosine (De Berardinis and Auricchio, 1951). Retina has also several neuropeptides, which we describe below.

1.3. Neuropeptides in the retina

Neuropeptides are widely distributed, both in the central and peripheral nervous systems. Functionally, neuropeptides act as neurotransmitters and/or neuromodulators through the activation of specific receptors to modulate the functional properties of neurons, such as their

membrane excitability or their signal transduction pathways (Bagnoli et al., 2003). Over the last decades, several neuropeptides, which were highly conserved during evolution, have been discovered in the eye. Substance P was the first peptide described in the retina and is also present in peripherally innervated tissues of the eye (Duner et al., 1954; Stone et al., 1987). The interest was extended to investigate the presence and distribution of other neuropeptides including calcitonin gene-related peptide (CGRP) (Kiyama et al., 1985), vasoactive intestinal polypeptide (VIP) (Loren et al., 1980), pituitary adenylate cyclase-activating polypeptide (PACAP) (Onali and Olianas, 1994), cholecystokinin (CCK) (Yamada et al., 1981), somatostatin (Rorstad et al., 1979), galanin (Hokfelt et al., 1992), neurokinin A and B (Schmid et al., 2006), corticotrophin-releasing factor (CRF) (Kiyama et al., 1984), angiotensin II (Senanayake et al., 2007), secretoneurin (Overdick et al., 1996), and neuropeptide Y (NPY) (Bruun et al., 1984). In this review we will focus on the NPY system and its role in the retina.

2. Neuropeptide Y (NPY) and NPY receptors in the retina

NPY is a member of a peptide family named NPY family or "PP-fold" family that also includes peptide YY (PYY) and pancreatic polypeptide (PP) (Michel et al., 1998). NPY is a 36-amino acid peptide that possesses an amidated C-terminal residue and a large number of tyrosine residues (which are abbreviated by the letter Y) included in both ends of the molecule. NPY was first isolated from the pig brain in 1982 by Tatemoto (Tatemoto et al., 1982). NPY is one of the neuropeptides with the highest degree of phylogenetic preservation, while the PP differs considerably between species (Larhammar et al., 1992). The NPY gene is located on human chromosome 7 at the locus 7p15.1 (Cerda-Reverter and Larhammar, 2000). In mouse, it is located in chromosome 6, locus 6 B3; 6 26.0 cM while in rat it is localized in chromosome 4, locus 4q24 (Pruitt et al., 2012). The prepro-NPY generated after translation is directed into the endoplasmic reticulum, where a 28 amino acid peptide is removed and Pro-NPY produced.

This NPY precursor, Pro-NPY, is a 69 amino acid peptide formed by NPY_{1-39} where the carboxylic group is flanked by a group of 33 amino acids called the C-flanking peptide of NPY (CPON). The following processing step is the cleavage of the precursor Pro-NPY at a dibasic site by prohormone convertases, which generates NPY_{1-39} and CPON. Then, a truncation at the C-terminal end by a carboxypeptidase B (CPB) generates NPY_{1-37}, which is a substrate for the enzyme peptidylglycine alpha-amidating monooxygenase (PAM) and leads to the biologically active amidated NPY_{1-36} (NPY) (Medeiros Mdos and Turner, 1996). NPY can be further cleaved by two enzymes, dipeptidyl peptidase IV (DPP-IV) and aminopeptidase P (AmP) (Medeiros and Turner, 1994; Medeiros Mdos and Turner, 1996).

All known NPY receptors belong to the large super-family of G-protein-coupled, heptahelical receptors (Michel et al., 1998). The NPY family receptors are the same for all members of the NPY family (NPY, PP, PYY) and are comprised of the receptor subtypes NPY Y_1, Y_2, Y_4, Y_5 and y_6 (Silva et al., 2005; Xapelli et al., 2008).

Generally, NPY receptors use similar signal transduction pathways, acting via pertussis toxin-sensitive G-proteins, i.e., members of the Gi and Go family. Thus, inhibition of adenylyl cyclase upon NPY receptor activation is found in almost every tissue and cell type investigated (Michel, 1991; Olasmaa and Terenius, 1986). However, the inhibition of adenylyl cyclase cannot probably explain all functional responses observed upon stimulation of NPY receptors (Michel et al., 1998). Additional signaling responses that are restricted to certain cell types include modulation of the Ca^{2+} or K^+ channels conductance (Gammon et al., 1990; Michel and Rascher, 1995; Millar et al., 1991; Xiong and Cheung, 1995). Moreover, there is also evidence to suggest that NPY may be associated with the activation of phospholipase A2 (Martin and Patterson, 1989), mitogen-activated protein kinases (MAPK) (Alvaro et al., 2008a; Keffel et al., 1999; Rosmaninho-Salgado et al., 2009; Thiriet et al., 2011), protein Kinase C (PKC) (Chen et al., 2008; Pons et al., 2008; Rosmaninho-Salgado et al., 2007; Rosmaninho-Salgado et al.,

2009), phosphatidylinositol 3-kinase (PI3K) (Zhou et al., 2008), guanylyl cyclase (Rosmaninho-Salgado et al., 2007), nitric oxide (NO) synthesis (Ferreira et al., 2010; Hodges et al., 2009; Rosmaninho-Salgado et al., 2009), or protein kinase A (PKA) (Pons et al., 2008; Rosmaninho-Salgado et al., 2009).

2.1. Localization of NPY and NPY receptors in the retina

The presence of NPY in the retina was first described in guinea pig in the early eighties (Bruun et al., 1984). Later studies demonstrated that NPY is also present in the retina of several non-mammalian vertebrates, as described in Table 1, and NPY-IR localizations in the retina of several mammalian species are depicted in Figure 2.

In rat and mouse retinas, NPY-IR is present in the inner retina being localized in cell bodies in INL and GCL, mainly in amacrine cells and displaced amacrine cells, respectively, and also in processes located in the IPL (Oh et al., 2002; Sinclair and Nirenberg, 2001). All NPY-immunoreactive amacrine cells are also GABAergic, containing GABA or the GABA synthesizing enzyme, glutamic acid decarboxylase 65 (GAD65), or even GAT-1, a vesicular GABA transporter (VGAT) (Kang et al., 2001; Oh et al., 2002; Sinclair and Nirenberg, 2001). Additionally, in rat retinal cells in culture, NPY-IR is present not only in retinal neurons, but also in Müller and microglial cells (Alvaro et al., 2007; Santos-Carvalho et al., 2012). The NPY-IR is also present in processes that ramify in the IPL in species such as pigeon, chicken, pig and baboon (Bruun et al., 1986; Oh et al., 2002; Sinclair and Nirenberg, 2001). A study in dolphin and dog retina shows that NPY-IR appears in polygonal and oval medium to large ganglion cells in the GCL which processes extended to IPL but only a few cells in the INL, IPL, OPL or ONL are NPY-immunoreactive (Chen et al, 1999). Furthermore, in cat retina the NPY-IR is localized in processes in the IPL, amacrine cells at INL and in ganglion cells in GCL (Hutsler and Chalupa, 1994, 1995), while only small amounts of NPY-IR are found in rabbit retina (Osborne et al., 1985). Moreover, immunoreactivity to NPY is also detected in

bovine retinal pigment epithelium (RPE) (Ammar et al., 1998). Finally NPY-IR is detected in RPE, amacrine cells at INL and in ganglion cells in GCL (Ammar et al., 1998; Jen et al., 1994; Straznicky and Hiscock, 1989)."In the case of non-mammalian retinas, in trout, carp, goldfish and killifish retinas, NPY-IR is found in cell bodies of amacrine cells (middle and innermost INL) and its processes constituting distinct sub-layers in the IPL (Bruun et al., 1986; Osborne et al., 1985; Subhedar et al., 1996). Goldfish retina also has dense fiber plexus with NPY-IR in layers 1, 3 and 5 of IPL (Muske et al., 1987). Zebrafish and gilthead seabream (*Sparus aurata L.)* retina presents NPY-IR in amacrine cells (Mathieu et al., 2002; Pirone et al., 2008). In skates (*Raja clavata*, *Raja radiate* and *Raja oscellata*), NPY-IR is localized in amacrine cells in the innermost part of INL while NPY-immunoreactive fibers are found in IPL (Bruun et al., 1985). In river lamprey (*Lampetra japonica*), NPY-IR is present in a subclass of pyriform amacrine cells (Negishi et al., 1986). No NPY-IR is found in squid retina (Osborne et al., 1986).

Retina of anuran species, such as frogs (ex. *Bufo marinus* and *Xenopus laevis*), has the highest NPY-IR levels among other species (such as pigs, cats, rabbits, chickens) and is characterized by seasonal variations (Bruun et al., 1991; Hiscock and Straznicky, 1989). NPY-IR is located in a small population of amacrine cell bodies in INL co-localizing with GABA (Bruun et al., 1986; Hiscock and Straznicky, 1990; Osborne et al., 1985; Zhu and Gibbins, 1995, 1996), in bipolar-like cell bodies sparsely in the middle of INL, in GCL with ovoid shape, in Müller cells within the INL (Zhu and Gibbins, 1996) and in IPL processes (Bruun et al., 1986).

In reptiles, such as lizards (*Pogona vitticeps* and *Varanus gouldii*), NPY-IR is present in the retina in two classes of amacrine cells: type A (large cell body) and type B (small cell body) located in INL and, occasionally, in displaced amacrine cell bodies at GCL (Straznicky and Hiscock, 1994). In contrast, in turtles the retina presents NPY-IR in bipolar cells and in three types of amacrine cells: type A, with large cell body located at INL and occasionally at GCL,

and processes at IPL and; type B, with smaller cell body at INL and processes at IPL; type C, amacrine cells located at the periphery of retina (Isayama and Eldred, 1988). NPY-IR is also located in cytoplasm and within large vesicles of amacrine and bipolar cells in turtle retina (Isayama et al., 1988; Wetzel and Eldred, 1997).

With NPY receptors, there are only a few studies showing their presence and localization in the retina (Table 2). In rat retina, we and others have detected the presence of mRNAs for NPY Y_1, Y_2, Y_4 and Y_5 receptors (Alvaro et al., 2007; D'Angelo and Brecha, 2004). The NPY Y_1 receptor-IR was found to be localized in horizontal and amacrine cell bodies and processes (D'Angelo et al., 2002).

Others have suggested that NPY Y_1 receptors are present in retinal neurons and responsible for the modulation of glutamate release and consequent inhibition of osmotic swelling of Müller cells (Uckermann et al., 2006). A more recent study has revealed that NPY inhibits the swelling of freshly isolated rat Müller cells (Linnertz et al., 2011), suggesting that rat Müller cells express the NPY Y_1 receptor. In fact, the presence of NPY Y_1 receptor in Müller cells has recently been observed both in rat retinal neural cell cultures and rat purified Müller cell cultures (Santos-Carvalho et al., 2012). Milenkovic and collaborators also suggest the presence of NPY Y_1 receptor in guinea pig Müller cells (Milenkovic et al., 2004). Additionally, NPY Y_1 and Y_2 receptors immunoreactivities are found in photoreceptors (rhodopsin-positive cells), bipolar (PKC α-positive cells), horizontal (calbindin-positive cells), amacrine (parvalbumin- or calretinin-positive cells) and ganglion cells (Brn3a-positive cells), as well as macroglial (GFAP- and vimentin-positive cells) and microglial (CD11b and ED1/CD68-positive cells) cells in rat retinal cultures (Santos-Carvalho et al., 2012). NPY Y_1 and Y_2 mRNAs were also detected in mouse retina (Sinclair and Nirenberg, 2001; Yoon et al., 2002). In human retinal pigment epithelium (RPE), NPY Y_1, Y_2 and Y_5 receptor mRNAs were detected, while in the bovine RPE only the NPY Y_1 and Y_2 receptors were detected (Ammar et al., 1998).

2.2. NPY and retinal development

Several studies indicate that NPY plays a role in the beginning of and during retinal development. Chicken NPY-immunoreactive retinal neuroblasts appear at embryonic day 13 in INL. Between that day and day 15 they migrate to their positions in innermost layers. At day 17, these immature cells start to differentiate in amacrine cells establishing connections with ganglion cells. At day 19, NPY-IR appears in few cell bodies of amacrine cells and large cells in GCL (Prada Oliveira et al., 2003). In zebrafish, NPY-IR appears in amacrine cells at embryonic day 15, suggesting its involvement in retinal synaptogenesis during ontogeny (Mathieu et al., 2002).

In *Xenopus laevis* retina, NPY-IR appears early in larval life. The dendritic maturation of NPY–IR amacrine cells occurs later during larval development than in cell bodies, and just before metamorphosis. In the adult retina of this frog, NPY-immunoreactivity (IR) is present in a wide field of amacrine cells in the INL and GCL (Hiscock and Straznicky, 1990). In the retina of blue acara (*Aequidens pulcher*), NPY-immunoreactive amacrine cells appear in IPL around hatching, at day 3-4 (Negishi and Wagner, 1995).

During cat retina development, NPY-IR is detected in central retina within the GCL at embryonic day 46 and amacrine cells within INL at embryonic day 50. Cat NPY-IR in amacrine population reaches adult levels at P7, while NPY-IR in ganglion cell population shows an extended development, with new cells expressing NPY until the third post-natal week (Hutsler and Chalupa, 1995).

Regarding the developing human retina, NPY-immunoreactive amacrine cells are found around 14 weeks of gestation (Jotwani et al., 1994). Another study indicates the presence of round and pear-shaped NPY-immunoreactive amacrine cells in INL after 15 weeks of gestation. NPY positive-ganglion cells were only detected at 17 weeks of gestation. NPY-immunoreactive amacrine and ganglion cells are located in INL and GCL, respectively, at 26-28 weeks of

gestation. Later, by 38-40 weeks of gestation, NPY-immunoreactive cells are present in INL, GCL and IPL (Jen et al., 1994).

In rats, NPY-IR appears in the retina in small quantities in GCL only at E18, and increases over pre- and postnatal development. Subsequently, at eye opening (P13) NPY-IR markedly increases in INL and GCL, but falls during maturation until adult levels forming two subpopulations in INL and GCL. This transient increase at eye opening may have a role in modulating the developing retina circuitry (Ferriero and Sagar, 1989).

In conclusion, several studies report the presence of NPY in undifferentiated retinal cells indicating a putative role of NPY in retinal development (Ferriero and Sagar, 1989; Hiscock and Straznicky, 1990; Hutsler and Chalupa, 1995; Jen et al., 1994; Jotwani et al., 1994; Mathieu et al., 2002; Negishi and Wagner, 1995; Prada Oliveira et al., 2003).

3. Modulatory effects of NPY in the retina

A fine tuning neuromodulator has the capacity to exert subtle influence on synapse activity by changing receptor activation of other neurotransmitters or neuromodulators as well as its own receptors. NPY co-localizes with other neurotransmitters in different areas of the CNS (Allen et al., 1983; Hendry et al., 1984; McDonald, 1996; Silva et al., 2005) and modulates the release of several neurotransmitters (Silva et al., 2005), inhibiting the release of glutamate, aspartate, growth hormone, epinephrine and acetylcholine (Bitran et al., 1999; Bleakman et al., 1992; Greber et al., 1994; Gu et al., 1983; Hastings et al., 2004; Martire et al., 1995; Potter, 1987; Rettori et al., 1990b; Rodi et al., 2003; Schwertfeger et al., 2004; Silva et al., 2001; Silva et al., 2003; Tsuda et al., 1995), and enhancing the release of somatostatin and dopamine and the production of nitric oxide (Ault and Werling, 1999; Bitran et al., 1999; Rettori et al., 1990a). Therefore, NPY may play a fine-tuning modulator in the nervous system (Grandt et al., 1996;

Magni, 2003; Mazzocchi et al., 1996; Prod'homme et al., 2006). Some studies have also suggested that NPY may be a neuromodulator in the retina. NPY modulates the intracellular calcium concentration (!Ca^{2+}!$_i$) in rat retinal neurons. NPY inhibits the depolarization-evoked Ca^{2+} influx into rod bipolar cells through the activation of NPY Y_2 receptors (D'Angelo and Brecha, 2004). NPY also inhibits the KCl-evoked increase in $[Ca^{2+}]_i$ in cultured rat retinal neurons through the activation of NPY Y_1, Y_4 and Y_5 receptors (Alvaro et al., 2009). On the other hand, when applied exogenously, NPY stimulates the release of [^{3}H]-glycine, [^{3}H]-dopamine, [^{3}H]-5-hydroxytryptamine and [^{3}H]-choline chloride-derived radioactivity in the rabbit retina and of [^{3}H]-GABA, [^{3}H]-5-hydroxytryptamine and [^{3}H]-choline chloride-derived radioactivity in chicken retina (Bruun and Ehinger, 1993). These results and the presence of NPY in amacrine cells (in the INL) and displaced amacrine cells (in the GCL), which may connect with other amacrine cell subtypes and ganglion cells that are not immunoreactive for NPY, suggest that NPY may also play a role as a neuromodulator in the inner retinal layers (D'Angelo et al., 2002; Oh et al., 2002)".

The ablation of NPY-immunoreactive amacrine cells causes alteration of receptive field surround size of ganglion cells, suggesting that NPY-immunoreactive amacrine cells are involved in tuning ganglion cells to low spatial frequencies/large spatial patterns (Sinclair et al., 2004).

In conclusion, NPY may affect neurotransmission between different retinal neurons (photoreceptors, and bipolar, ganglion, horizontal and amacrine cells), which depends on $[Ca^{2+}]_i$ regulation, and therefore NPY may exert a relevant fine tuning neuromodulatory role in retinal cells.

4. Potential role of NPY in cell proliferation, differentiation and neuroprotection in the retina

In vitro and *in vivo* studies suggest that NPY has pro-neurogenic properties in the olfactory epithelium, subventricular zone (SVZ) and subgranular zone (SGZ) of dentate gyrus (Agasse et al., 2008; Decressac et al., 2011; Hansel et al., 2001b; Howell et al., 2005; Howell et al., 2007; Rodrigo et al., 2010). In addition, in the CNS, NPY induces alterations in the rostral migratory stream, differentiation of progenitor cells into distinct interneuronal subsets in the olfactory bulb (Stanic et al., 2008), migration of newly generated neurons to the striatum and the olfatory bulb and also increases the number of cells in the rostral migratory stream, olfactory bulb and striatum (Decressac et al., 2009). These NPY effects on neural cell proliferation and differentiation are mediated by the NPY Y_1-receptor activation (Agasse et al., 2008; Decressac et al., 2011; Hansel et al., 2001b; Howell et al., 2003; Rodrigo et al., 2010; Stanic et al., 2008). The involvement of Y_2 receptor in these NPY effects is controversial (Decressac et al., 2011; Stanic et al., 2008). The neurogenic effect of NPY requires ERK1/2 activation (Agasse et al., 2008; Hansel et al., 2001a; Howell et al., 2005) while NPY promoting effect on neuronal differentiation and axonal sprouting is mediated through the activation of the SAPK/JNK pathway (Agasse et al., 2008). These studies suggest that secreted NPY may act locally in an autocrine/paracrine manner, at least in the hippocampus, to stimulate proliferation or neuronal differentiation, either at an equal or even greater level than other trophic/growth factors, such as ciliary neurotrophic factor, vascular endothelial growth factor, and transforming growth factor (Decressac et al., 2011; Emsley and Hagg, 2003; Jin et al., 2002).

In the retina, it has been shown that NPY induces proliferation of retinal glial (Müller) cells mediated by NPY Y_1 receptor activation, through ERK 1/2, and partially, p38 pathways (Milenkovic et al., 2004). However, this proliferative effect on Müller cells is biphasic: at lower

concentrations (0.1 ng/mL and 1 ng/mL) NPY decreases the cell proliferation rate, while at higher concentration (100 ng/mL) increases Müller cell proliferation (Milenkovic et al., 2004). It was accepted that the mature mammalian retina lacked regenerative capacity (Tropepe et al., 2000). However, many studies in fish, amphibians, birds, rodents and humans have identified neural progenitors in the adult eye with capacity to generate all retinal cell types (Ahmad, 2001; Cepko et al., 1996; Coles et al., 2004; Johns, 1977; Martinez-Navarrete et al., 2008; Reh and Fischer, 2001; Straznicky and Gaze, 1971; Tropepe et al., 2000; Xu et al., 2007). The identification and characterization of neural progenitors stem cells in the eye may open new avenues for the treatment of several ocular diseases characterized by neuronal death, such as retinitis pigmentosa, age-related macular degeneration, diabetic retinopathy and glaucoma (Ahmad, 2001; Bernardos et al., 2007; Ohta et al., 2008; Ooto et al., 2004; Tropepe et al., 2000).

Some studies suggest that NPY might have an important role on progenitor cells proliferation and/or differentiation in the nervous tissue (Baptista et al., 2012; Doyle et al., 2012; Thiriet et al., 2011). In cultured rat retinal cells, we have shown that NPY stimulates the proliferation of neuronal progenitor cells (BrdU+/nestin+ cells), which means that NPY promotes the proliferation of committed neural immature cells, with this effect being mediated by the activation of the nitric oxide synthase - soluble guanylyl cyclase (NOS–sGC) and ERK 1/2 signaling pathways (Alvaro et al., 2008a). Additionally, NPY, through Y_1 and Y_5 receptor activation, has the potential of maintaining self-renewal and pluripotency of human embryonic stem cells (hESC) (Son et al., 2011). NPY signaling can be useful in the development of defined and xeno-free culture conditions for the large-scale propagation of undifferentiated hESCs (Son et al., 2011). Thus, the NPY system is a putative target to develop new strategies to increase retinal progenitor cells proliferation.

Neuroprotection is an important strategy to prevent cell death occurring in neurological disorders. The neuroprotective effects of NPY against excitotoxicity are well known both in different brain regions, and also in the retina (Alvaro et al., 2008b; Alvaro et al., 2009; Silva et al., 2003; Silva et al., 2005; Smialowska et al., 2009; Xapelli et al., 2006; Xapelli et al., 2007). In rat and mouse organotypic hippocampal cultures, NPY is able to reduce cell death induced by glutamate receptor agonists, through activation of NPY Y_1, Y_2 and/or Y_5 receptors (Silva et al., 2003; Smialowska et al., 2009; Xapelli et al., 2007). NPY also exerts a neuroprotective effect against toxicity (necrosis and apoptosis) induced by 3,4-methylenedioxymethamphetamine (MDMA) in rat retinal neural cell culture (neurons, astrocytes, Müller cells (GFAP-positive cells) and microglial cells) (Alvaro et al., 2008b). However the mechanism underlying this neuroprotective effect of NPY against MDMA toxicity has not yet been clarified (Alvaro et al., 2008b). As mentioned above, we have shown that NPY inhibits the increase in $[Ca^{2+}]_i$ in rat retinal neurons through the activation of NPY Y_1, Y_4, and Y_5 receptor subtypes. Since sustained elevated cytosolic $[Ca^{2+}]_i$ levels have been linked to cell death, this inhibitory effect of NPY may also contribute to its neuroprotective effect in these cells (Alvaro et al., 2009). More recently, we also showed that NPY has a protective role against glutamate-induced toxicity in rat retinal cells (*in vitro* and in an animal model) (Santos-Carvalho et al., 2013). In rat retinal neural cell cultures, the activation of NPY Y_2, Y_4 and Y_5 receptors inhibited necrotic cell death, while apoptotic cell death was only prevented by the activation of NPY Y_5 receptor. Moreover, NPY neuroprotective effect was mediated by the activation of PKA and p38K. In the animal model, NPY inhibited the increase in the number of apoptotic cell death induced by glutamate (Santos-Carvalho et al., 2013).
In retinal slices, it has also been shown that NPY, through the release of glutamate and ATP, inhibits osmotic swelling of Müller cells. This inhibitory effect was mediated by NPY Y_1, but not NPY Y_2 or Y_5 receptors, expressed in retinal neurons. This glial volume regulation may

contribute to the neuroprotective effects of NPY in the retina (Uckermann et al., 2006). Subsequently, the same group found that this neuroprotective effect of NPY was also detected in freshly isolated rat Müller cells, which suggests that NPY receptors of rat Müller cells were directly activated (Linnertz et al., 2011), and that rat Müller cells express NPY Y_1 receptor. Thus, NPY receptor agonists might be viewed as putative therapeutic drugs against neural cell degeneration occurring in several retinal degenerative diseases, such as glaucoma and diabetic retinopathy.

5. NPY involvement in retinal pathologies

A genetic study in a Finnish population has shown that a Leu^7Pro polymorphism in the NPY gene (substitution of a leucine to proline in human prepro-NPY) is associated with an increased predisposition to develop diabetic retinopathy (DR) in Type 2 diabetic patients (Koulu et al., 2004; Niskanen et al., 2000), and could be used to predict earlier onset of type 2 diabetes and retinopathy (Jaakkola et al., 2006). In contrast, it has been shown that this polymorphism is not a risk factor for exudative age related macular degeneration (Kaarniranta et al., 2007).
Retinal NPY and NPY Y_2 receptor expression are increased in oxygen-reared animals compared with room-air reared ones, in the hyperoxic vasoconstrictive phase (P12) and the period of retinal neovascularization (P17) of the development of oxygen-induced retinopathy of this mouse model. Therefore, NPY and NPY Y_2 receptor could be associated with angiogenesis and vasoconstriction in this mouse model of oxygen-induced retinopathy (Yoon et al., 2002).
In another study, Koulu and collaborators, using $Y_2^{-/-}$ mice and rats treated with NPY Y_2 receptor mRNA targeted antisense oligonucleotide, demonstrated that the NPY Y_2 receptor plays an important role in hyperoxia-induced retinal neovascularization (Koulu et al., 2004). Thus, they corroborate the contribution of NPY Y_2 receptor in neovascularization processes in

the progression of diabetic retinopathy and the contribution of NPY gene in type 2 diabetes diabetic retinopathy (Koulu et al., 2004).

However, more recently, another group found decreased levels of NPY and NPY Y_2 receptor in a similar model of oxygen-induced retinopathy to that used by Yoon and collaborators. (Schmid et al., 2012). The authors justify this discrepancy by the fact that Yoon et al. measured mRNA levels by quantitative RT-PCR while Schmidt et al measured protein levels by radioimmunoassay. They suggested, therefore, that NPY is a mediator of physiological but not pathological angiogenesis, thus explaining the absence of this peptide in abnormal vessel formation in retinopathy (Schmid et al., 2012).

In humans, retinas of patients with proliferative vitreoretinopathy present NPY Y_1 receptor immunoreactivity in reactive and proliferating Müller cells. This immunoreactivity was not detected in normal human retina. Therefore, the presence of this receptor may be related to the proliferation of Müller cells, the regrowth of proliferative vitreoretinopathy membranes, and the consequent secondary retinal detachments (Canto Soler et al., 2002).

In conclusion, the available data suggests a relevant role of NPY on the development of some retinal disease, but further studies are needed to clarify the mechanisms involved.

6. Conclusions

NPY and NPY receptors are expressed in the retina of several species, in neurons, astrocytes, and Müller and microglial cells. Activation of NPY receptors appears to mediate several, potentially important effects in the retina, including cell proliferation, neurotransmitter modulation and neuroprotection summarized in Table 3. Future studies are likely to uncover several further functions of NPY in retinal physiology and pathophysiology.

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References

Agasse, F., Bernardino, L., Kristiansen, H., Christiansen, S. H., Ferreira, R., Silva, B., Grade, S., Woldbye, D. P. and Malva, J. O. (2008) Neuropeptide Y Promotes Neurogenesis in Murine Subventricular Zone. *Stem cells (Dayton, Ohio)* 26, 1636-1645

Ahmad, I. (2001) Stem cells: new opportunities to treat eye diseases. *Invest Ophthalmol Vis Sci* 42, 2743-2748.

Allen, Y. S., Adrian, T. E., Allen, J. M., Tatemoto, K., Crow, T. J., Bloom, S. R. and Polak, J. M. (1983) Neuropeptide Y distribution in the rat brain. *Science* 221, 877-879.

Alvaro, A. R., Martins, J., Araujo, I. M., Rosmaninho-Salgado, J., Ambrosio, A. F. and Cavadas, C. (2008a) Neuropeptide Y stimulates retinal neural cell proliferation - involvement of nitric oxide. *Journal of neurochemistry* 105, 2501-2510.

Alvaro, A. R., Martins, J., Costa, A. C., Fernandes, E., Carvalho, F., Ambrosio, A. F. and Cavadas, C. (2008b) Neuropeptide Y protects retinal neural cells against cell death induced by ecstasy. *Neuroscience* 152, 97-105.

Alvaro, A. R., Rosmaninho-Salgado, J., Ambrosio, A. F. and Cavadas, C. (2009) Neuropeptide Y inhibits [Ca2+]i changes in rat retinal neurons through NPY Y1, Y4, and Y5 receptors. *Journal of neurochemistry* 109, 1508-1515.

Alvaro, A. R., Rosmaninho-Salgado, J., Santiago, A. R., Martins, J., Aveleira, C., Santos, P. F., Pereira, T., Gouveia, D., Carvalho, A. L., Grouzmann, E., Ambrosio, A. F. and Cavadas, C. (2007) NPY in rat retina is present in neurons, in endothelial cells and also in microglial and Muller cells. *Neurochem Int* 50, 757-763.

Ammar, D. A., Hughes, B. A. and Thompson, D. A. (1998) Neuropeptide Y and the retinal pigment epithelium: receptor subtypes, signaling, and bioelectrical responses. *Invest Ophthalmol Vis Sci* 39, 1870-1878.

Ault, D. T. and Werling, L. L. (1999) Phencyclidine and dizocilpine modulate dopamine release from rat nucleus accumbens via sigma receptors. *Eur J Pharmacol* 386, 145-153.

Bagnoli, P., Dal Monte, M. and Casini, G. (2003) Expression of neuropeptides and their receptors in the developing retina of mammals. *Histol Histopathol* 18, 1219-1242.

Baptista, S., Bento, A. R., Goncalves, J., Bernardino, L., Summavielle, T., Lobo, A., Fontes-Ribeiro, C., Malva, J. O., Agasse, F. and Silva, A. P. (2012) Neuropeptide Y promotes neurogenesis and protection against methamphetamine-induced toxicity in mouse dentate gyrus-derived neurosphere cultures. *Neuropharmacology* 62, 2413-2423.

Bernardos, R. L., Barthel, L. K., Meyers, J. R. and Raymond, P. A. (2007) Late-stage neuronal progenitors in the retina are radial Muller glia that function as retinal stem cells. *J Neurosci* 27, 7028-7040.

Bitran, M., Tapia, W., Eugenin, E., Orio, P. and Boric, M. P. (1999) Neuropeptide Y induced inhibition of noradrenaline release in rat hypothalamus: role of receptor subtype and nitric oxide. *Brain Res* 851, 87-93.

Bleakman, D., Harrison, N. L., Colmers, W. F. and Miller, R. J. (1992) Investigations into neuropeptide Y-mediated presynaptic inhibition in cultured hippocampal neurones of the rat. *Br J Pharmacol* 107, 334-340.

Bruun, A. and Ehinger, B. (1993) NPY-induced neurotransmitter release from the rabbit and chicken retina. *Acta Ophthalmol (Copenh)* 71, 590-596.

Bruun, A., Ehinger, B. and Ekman, R. (1991) Characterization of neuropeptide Y-like immunoreactivity in vertebrate retina. *Exp Eye Res* 53, 539-543.

Bruun, A., Ehinger, B., Sundler, F., Tornqvist, K. and Uddman, R. (1984) Neuropeptide Y immunoreactive neurons in the guinea-pig uvea and retina. *Invest Ophthalmol Vis Sci* 25, 1113-1123.

Bruun, A., Ehinger, B., Sytsma, V. and Tornqvist, K. (1985) Retinal neuropeptides in the skates, Raja clavata, R. radiata, R. oscellata (Elasmobranchii). *Cell Tissue Res* 241, 17-24.

Bruun, A., Tornqvist, K. and Ehinger, B. (1986) Neuropeptide Y (NPY) immunoreactive neurons in the retina of different species. *Histochemistry* 86, 135-140.

Canto Soler, M. V., Gallo, J. E., Dodds, R. A., Hokfelt, T., Villar, M. J. and Suburo, A. M. (2002) Y1 receptor of neuropeptide Y as a glial marker in proliferative vitreoretinopathy and diseased human retina. *Glia* 39, 320-324.

Cepko, C. L., Austin, C. P., Yang, X., Alexiades, M. and Ezzeddine, D. (1996) Cell fate determination in the vertebrate retina. *Proceedings of the National Academy of Sciences of the United States of America* 93, 589-595.

Cerda-Reverter, J. M. and Larhammar, D. (2000) Neuropeptide Y family of peptides: structure, anatomical expression, function, and molecular evolution. *Biochem Cell Biol* 78, 371-392.

Chen, J., Zhang, Y. and Shen, P. (2008) A protein kinase C activity localized to neuropeptide Y-like neurons mediates ethanol intoxication in Drosophila melanogaster. *Neuroscience* 156, 42-47.

Coles, B. L., Angenieux, B., Inoue, T., Del Rio-Tsonis, K., Spence, J. R., McInnes, R. R., Arsenijevic, Y. and van der Kooy, D. (2004) Facile isolation and the characterization of human retinal stem cells. *Proceedings of the National Academy of Sciences of the United States of America* 101, 15772-15777.

D'Angelo, I. and Brecha, N. C. (2004) Y2 receptor expression and inhibition of voltage-dependent Ca2+ influx into rod bipolar cell terminals. *Neuroscience* 125, 1039-1049.

D'Angelo, I., Oh, S. J., Chun, M. H. and Brecha, N. C. (2002) Localization of neuropeptide Y1 receptor immunoreactivity in the rat retina and the synaptic connectivity of Y1 immunoreactive cells. *J Comp Neurol* 454, 373-382.

De Berardinis, E. and Auricchio, G. (1951) [Hydrolysis of adenosinetriphosphoric acid (ATP) in the retina and its biological significance]. *Ann Ottalmol Clin Ocul* 77, 430-453.

Decressac, M., Prestoz, L., Veran, J., Cantereau, A., Jaber, M. and Gaillard, A. (2009) Neuropeptide Y stimulates proliferation, migration and differentiation of neural precursors from the subventricular zone in adult mice. *Neurobiol Dis* 34, 441-449.

Decressac, M., Wright, B., David, B., Tyers, P., Jaber, M., Barker, R. A. and Gaillard, A. (2011) Exogenous neuropeptide Y promotes in vivo hippocampal neurogenesis. *Hippocampus* 21, 233-238.

Doyle, K. L., Hort, Y. J., Herzog, H. and Shine, J. (2012) Neuropeptide Y and peptide YY have distinct roles in adult mouse olfactory neurogenesis. *J Neurosci Res* 90, 1126-1135.

Duner, H., Von Euler, U. S. and Pernow, B. (1954) Catechol amines and substance P in the mammalian eye. *Acta Physiol Scand* 31, 113-114.

Emsley, J. G. and Hagg, T. (2003) Endogenous and exogenous ciliary neurotrophic factor enhances forebrain neurogenesis in adult mice. *Exp Neurol* 183, 298-310.

Ferreira, R., Xapelli, S., Santos, T., Silva, A. P., Cristovao, A., Cortes, L. and Malva, J. O. (2010) Neuropeptide Y modulation of interleukin-1 beta (IL-1{beta})-induced nitric oxide production in microglia. *J Biol Chem* 285, 41921-41934.

Ferriero, D. M. and Sagar, S. M. (1989) Development of neuropeptide Y-immunoreactive neurons in the rat retina. *Brain Res Dev Brain Res* 48, 19-26.

Gammon, C. M., Lyons, S. A. and Morell, P. (1990) Modulation by neuropeptides of bradykinin-stimulated second messenger release in dorsal root ganglion neurons. *Brain Res* 518, 159-165.

Grandt, D., Schimiczek, M., Rascher, W., Feth, F., Shively, J., Lee, T. D., Davis, M. T., Reeve, J. R., Jr. and Michel, M. C. (1996) Neuropeptide Y 3-36 is an endogenous ligand selective for Y2 receptors. *Regul Pept* 67, 33-37.

Greber, S., Schwarzer, C. and Sperk, G. (1994) Neuropeptide Y inhibits potassium-stimulated glutamate release through Y2 receptors in rat hippocampal slices in vitro. *Br J Pharmacol* 113, 737-740.

Gu, J., Polak, J. M., Adrian, T. E., Allen, J. M., Tatemoto, K. and Bloom, S. R. (1983) Neuropeptide tyrosine (NPY)--a major cardiac neuropeptide. *Lancet* 1, 1008-1010.

Haeggendal, J. and Malmfors, T. (1963) Evidence of Dopamine-Containing Neurons in the Retina of Rabbits. *Acta Physiol Scand* 59, 295-296.

Hansel, D. E., Eipper, B. A. and Ronnett, G. V. (2001a) Neuropeptide Y functions as a neuroproliferative factor. *Nature* 410, 940-944.

Hansel, D. E., Eipper, B. A. and Ronnett, G. V. (2001b) Regulation of olfactory neurogenesis by amidated neuropeptides. *J Neurosci Res* 66, 1-7.

Hastings, J. A., Morris, M. J., Lambert, G., Lambert, E. and Esler, M. (2004) NPY and NPY Y1 receptor effects on noradrenaline overflow from the rat brain in vitro. *Regul Pept* 120, 107-112.

Hendry, S. H., Jones, E. G., DeFelipe, J., Schmechel, D., Brandon, C. and Emson, P. C. (1984) Neuropeptide-containing neurons of the cerebral cortex are also GABAergic. *Proceedings of the National Academy of Sciences of the United States of America* 81, 6526-6530.

Hiscock, J. and Straznicky, C. (1989) Neuropeptide Y-like immunoreactive amacrine cells in the retina of Bufo marinus. *Brain Res* 494, 55-64.

Hiscock, J. and Straznicky, C. (1990) Neuropeptide Y- and substance P-like immunoreactive amacrine cells in the retina of the developing Xenopus laevis. *Brain Res Dev Brain Res* 54, 105-113.

Hodges, G. J., Jackson, D. N., Mattar, L., Johnson, J. M. and Shoemaker, J. K. (2009) Neuropeptide Y and neurovascular control in skeletal muscle and skin. *Am J Physiol Regul Integr Comp Physiol* 297, R546-555.

Hokfelt, T., Aman, K., Arvidsson, U., Bedecs, K., Ceccatelli, S., Hulting, A. L., Langel, U., Meister, B., Pieribone, V. and Bartfai, T. (1992) Galanin message-associated peptide (GMAP)- and galanin-like immunoreactivities: overlapping and differential distributions in the rat. *Neurosci Lett* 142, 139-142.

Howell, O. W., Doyle, K., Goodman, J. H., Scharfman, H. E., Herzog, H., Pringle, A., Beck-Sickinger, A. G. and Gray, W. P. (2005) Neuropeptide Y stimulates neuronal precursor proliferation in the post-natal and adult dentate gyrus. *Journal of neurochemistry* 93, 560-570.

Howell, O. W., Scharfman, H. E., Herzog, H., Sundstrom, L. E., Beck-Sickinger, A. and Gray, W. P. (2003) Neuropeptide Y is neuroproliferative for post-natal hippocampal precursor cells. *Journal of neurochemistry* 86, 646-659.

Howell, O. W., Silva, S., Scharfman, H. E., Sosunov, A. A., Zaben, M., Shatya, A., McKhann, G., 2nd, Herzog, H., Laskowski, A. and Gray, W. P. (2007) Neuropeptide Y is important for basal and seizure-induced precursor cell proliferation in the hippocampus. *Neurobiol Dis* 26, 174-188.

Hutsler, J. J. and Chalupa, L. M. (1994) Neuropeptide Y immunoreactivity identifies a regularly arrayed group of amacrine cells within the cat retina. *J Comp Neurol* 346, 481-489.

Hutsler, J. J. and Chalupa, L. M. (1995) Development of neuropeptide Y immunoreactive amacrine and ganglion cells in the pre- and postnatal cat retina. *J Comp Neurol* 361, 152-164.

Isayama, T. and Eldred, W. D. (1988) Neuropeptide Y-immunoreactive amacrine cells in the retina of the turtle Pseudemys scripta elegans. *J Comp Neurol* 271, 56-66.

Isayama, T., Polak, J. and Eldred, W. D. (1988) Synaptic analysis of amacrine cells with neuropeptide Y-like immunoreactivity in turtle retina. *J Comp Neurol* 275, 452-459.

Jaakkola, U., Pesonen, U., Vainio-Jylha, E., Koulu, M., Pollonen, M. and Kallio, J. (2006) The Leu7Pro polymorphism of neuropeptide Y is associated with younger age of onset of type 2 diabetes mellitus and increased risk for nephropathy in subjects with diabetic retinopathy. *Exp Clin Endocrinol Diabetes* 114, 147-152.

Jen, P. Y., Li, W. W. and Yew, D. T. (1994) Immunohistochemical localization of neuropeptide Y and somatostatin in human fetal retina. *Neuroscience* 60, 727-735.

Jin, K., Zhu, Y., Sun, Y., Mao, X. O., Xie, L. and Greenberg, D. A. (2002) Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America* 99, 11946-11950.

Johns, P. R. (1977) Growth of the adult goldfish eye. III. Source of the new retinal cells. *J Comp Neurol* 176, 343-357.

Jotwani, G., Itoh, K. and Wadhwa, S. (1994) Immunohistochemical localization of tyrosine hydroxylase, substance P, neuropeptide-Y and leucine-enkephalin in developing human retinal amacrine cells. *Brain Res Dev Brain Res* 77, 285-289.

Kaarniranta, K., Holopainen, J. M., Karvonen, M. K., Koulu, M., Kallio, J., Pesonen, U., Terasvirta, M., Uusitalo, H. and Immonen, I. (2007) Leucine 7-proline 7 polymorphism in the signal peptide of neuropeptide Y is not a risk factor for exudative age-related macular degeneration. *Acta Ophthalmol Scand* 85, 188-191.

Kang, W. S., Lim, M. Y., Lee, E. J., Kim, I. B., Oh, S. J., Brecha, N. C., Park, C. B. and Chun, M. H. (2001) Light- and electron-microscopic analysis of neuropeptide Y-immunoreactive amacrine cells in the guinea pig retina. *Cell Tissue Res* 306, 363-371.

Keffel, S., Schmidt, M., Bischoff, A. and Michel, M. C. (1999) Neuropeptide-Y stimulation of extracellular signal-regulated kinases in human erythroleukemia cells. *The Journal of pharmacology and experimental therapeutics* 291, 1172-1178.

Kiyama, H., Katayama, Y., Hillyard, C. J., Girgis, S., MacIntyre, I., Emson, P. C. and Tohyama, M. (1985) Occurrence of calcitonin gene-related peptide in the chicken amacrine cells. *Brain Res* 327, 367-369.

Kiyama, H., Shiosaka, S., Kuwayama, Y., Shibasaki, T., Ling, N. and Tohyama, M. (1984) Corticotropin-releasing factor in the amacrine cells of the chicken retina. *Brain Res* 298, 197-200.

Kojima, K., Iida, M., Majima, Y., Okada, S., Yoshida, N. and Kiribuchi, K. (1961) [Histochemical studies on monoamine oxydase (serotonin) in the retina]. *Nihon Ganka Kiyo* 12, 861-866.

Koulu, M., Movafagh, S., Tuohimaa, J., Jaakkola, U., Kallio, J., Pesonen, U., Geng, Y., Karvonen, M. K., Vainio-Jylha, E., Pollonen, M., Kaipio-Salmi, K., Seppala, H., Lee, E. W., Higgins, R. D. and Zukowska, Z. (2004) Neuropeptide Y and Y2-receptor are

involved in development of diabetic retinopathy and retinal neovascularization. *Ann Med* 36, 232-240.

Larhammar, D., Blomqvist, A. G., Yee, F., Jazin, E., Yoo, H. and Wahlested, C. (1992) Cloning and functional expression of a human neuropeptide Y/peptide YY receptor of the Y1 type. *J. Biol. Chem.* 267, 10935-10938.

Lindeman, V. F. (1947) The cholinesterase and acetylcholine content of the chick retina, with especial reference to functional activity as indicated by the pupillary constrictor reflex. *Am J Physiol* 148, 40-44.

Linnertz, R., Wurm, A., Pannicke, T., Krugel, K., Hollborn, M., Hartig, W., Iandiev, I., Wiedemann, P., Reichenbach, A. and Bringmann, A. (2011) Activation of voltage-gated Na(+) and Ca(2)(+) channels is required for glutamate release from retinal glial cells implicated in cell volume regulation. *Neuroscience* 188, 23-34.

Loren, I., Tornqvist, K. and Alumets, J. (1980) VIP (vasoactive intestinal polypeptide)-immunoreactive neurons in the retina of the rat. *Cell Tissue Res* 210, 167-170.

Magni, P. (2003) Hormonal control of the neuropeptide Y system. *Curr Protein Pept Sci* 4, 45-57.

Martin, S. E. and Patterson, R. E. (1989) Coronary constriction due to neuropeptide Y: alleviation with cyclooxygenase blockers. *Am J Physiol* 257, H927-934.

Martinez-Navarrete, G. C., Angulo, A., Martin-Nieto, J. and Cuenca, N. (2008) Gradual morphogenesis of retinal neurons in the peripheral retinal margin of adult monkeys and humans. *J Comp Neurol* 511, 557-580.

Martire, M., Pistritto, G., Mores, N., Agnati, L. F. and Fuxe, K. (1995) Presynaptic A2-adrenoceptors and neuropeptide Y Y2 receptors inhibit [3H]noradrenaline release from rat hypothalamic synaptosomes via different mechanisms. *Neurosci Lett* 188, 9-12.

Mathieu, M., Tagliafierro, G., Bruzzone, F. and Vallarino, M. (2002) Neuropeptide tyrosine-like immunoreactive system in the brain, olfactory organ and retina of the zebrafish, Danio rerio, during development. *Brain Res Dev Brain Res* 139, 255-265.

Mazzocchi, G., Malendowicz, L. K., Macchi, C., Gottardo, G. and Nussdorfer, G. G. (1996) Further investigations on the effects of neuropeptide Y on the secretion and growth of rat adrenal zona glomerulosa. *Neuropeptides* 30, 19-27.

McDonald, A. J. (1996) Localization of AMPA glutamate receptor subunits in subpopulations of non-pyramidal neurons in the rat basolateral amygdala. *Neurosci Lett* 208, 175-178.

Medeiros, M. D. and Turner, A. J. (1994) Processing and metabolism of peptide-YY: pivotal roles of dipeptidylpeptidase-IV, aminopeptidase-P, and endopeptidase-24.11. *Endocrinology* 134, 2088-2094.

Medeiros Mdos, S. and Turner, A. J. (1996) Metabolism and functions of neuropeptide Y. *Neurochem Res* 21, 1125-1132.

Michel, M. C. (1991) Receptors for neuropeptide Y: multiple subtypes and multiple second messengers. *Trends in pharmacological sciences* 12, 389-394.

Michel, M. C., Beck-Sickinger, A., Cox, H., Doods, H. N., Herzog, H., Larhammar, D., Quirion, R., Schwartz, T. and Westfall, T. (1998) XVI. International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. *Pharmacol Rev* 50, 143-150.

Michel, M. C. and Rascher, W. (1995) Neuropeptide Y: a possible role in hypertension? *Journal of hypertension* 13, 385-395.

Milenkovic, I., Weick, M., Wiedemann, P., Reichenbach, A. and Bringmann, A. (2004) Neuropeptide Y-evoked proliferation of retinal glial (Muller) cells. *Graefes Arch Clin Exp Ophthalmol* 242, 944-950.

Millar, B. C., Weis, T., Piper, H. M., Weber, M., Borchard, U., McDermott, B. J. and Balasubramaniam, A. (1991) Positive and negative contractile effects of neuropeptide Y on ventricular cardiomyocytes. *Am J Physiol* 261, H1727-1733.

Muske, L. E., Dockray, G. J., Chohan, K. S. and Stell, W. K. (1987) Segregation of FMRF amide-immunoreactive efferent fibers from NPY-immunoreactive amacrine cells in goldfish retina. *Cell Tissue Res* 247, 299-307.

Negishi, K., Kiyama, H., Kato, S., Teranishi, T., Hatakenaka, S., Katayama, Y., Miki, N. and Tohyama, M. (1986) An immunohistochemical study on the river lamprey retina. *Brain Res* 362, 389-393.

Negishi, K. and Wagner, H. J. (1995) Differentiation of photoreceptors, glia, and neurons in the retina of the cichlid fish Aequidens pulcher; an immunocytochemical study. *Brain Res Dev Brain Res* 89, 87-102.

Niskanen, L., Voutilainen-Kaunisto, R., Terasvirta, M., Karvonen, M. K., Valve, R., Pesonen, U., Laakso, M., Uusitupa, M. I. and Koulu, M. (2000) Leucine 7 to proline 7 polymorphism in the neuropeptide y gene is associated with retinopathy in type 2 diabetes. *Exp Clin Endocrinol Diabetes* 108, 235-236.

Oh, S. J., D'Angelo, I., Lee, E. J., Chun, M. H. and Brecha, N. C. (2002) Distribution and synaptic connectivity of neuropeptide Y-immunoreactive amacrine cells in the rat retina. *J Comp Neurol* 446, 219-234.

Ohta, K., Ito, A. and Tanaka, H. (2008) Neuronal stem/progenitor cells in the vertebrate eye. *Dev Growth Differ* 50, 253-259.

Olasmaa, M. and Terenius, L. (1986) Neuropeptide Y receptor interaction with beta-adrenoceptor coupling to adenylate cyclase. *Prog Brain Res* 68, 337-341.

Onali, P. and Olianas, M. C. (1994) PACAP is a potent and highly effective stimulator of adenylyl cyclase activity in the retinas of different mammalian species. *Brain Res* 641, 132-134.

Ooto, S., Akagi, T., Kageyama, R., Akita, J., Mandai, M., Honda, Y. and Takahashi, M. (2004) Potential for neural regeneration after neurotoxic injury in the adult mammalian retina. *Proceedings of the National Academy of Sciences of the United States of America* 101, 13654-13659.

Osborne, N. N., Beaton, D. W., Boyd, P. J. and Walker, R. J. (1986) Substance P-like immunoreactivity in the retina and optic lobe of the squid. *Neurosci Lett* 70, 65-68.

Osborne, N. N., Patel, S., Terenghi, G., Allen, J. M., Polak, J. M. and Bloom, S. R. (1985) Neuropeptide Y (NPY)-like immunoreactive amacrine cells in retinas of frog and goldfish. *Cell Tissue Res* 241, 651-656.

Overdick, B., Kirchmair, R., Marksteiner, J., Fischer-Colbrie, R., Troger, J., Winkler, H. and Saria, A. (1996) Presence and distribution of a new neuropeptide, secretoneurin, in human retina. *Peptides* 17, 1-4.

Pirone, A., Lenzi, C., Marroni, P., Betti, L., Mascia, G., Giannaccini, G., Lucacchini, A. and Fabiani, O. (2008) Neuropeptide Y in the brain and retina of the adult teleost gilthead seabream (Sparus aurata L.). *Anat Histol Embryol* 37, 231-240.

Pons, J., Kitlinska, J., Jacques, D., Perreault, C., Nader, M., Everhart, L., Zhang, Y. and Zukowska, Z. (2008) Interactions of multiple signaling pathways in neuropeptide Y-mediated bimodal vascular smooth muscle cell growth. *Can J Physiol Pharmacol* 86, 438-448.

Potter, E. (1987) Presynaptic inhibition of cardiac vagal postganglionic nerves by neuropeptide Y. *Neurosci Lett* 83, 101-106.

Prada Oliveira, J. A., Verastegui Escolano, C., Gomez Luy, C. and Collantes Ruiz, J. (2003) Ontogenic attendance of neuropeptides in the embryo chicken retina. *Histol Histopathol* 18, 1013-1026.

Prod'homme, T., Weber, M. S., Steinman, L. and Zamvil, S. S. (2006) A neuropeptide in immune-mediated inflammation, Y? *Trends Immunol* 27, 164-167.

Pruitt, K. D., Tatusova, T., Brown, G. R. and Maglott, D. R. (2012) NCBI Reference Sequences (RefSeq): current status, new features and genome annotation policy. *Nucleic Acids Res* 40, D130-135.

Reh, T. A. and Fischer, A. J. (2001) Stem cells in the vertebrate retina. *Brain Behav Evol* 58, 296-305.

Rettori, V., Milenkovic, L., Aguila, M. C. and McCann, S. M. (1990a) Physiologically significant effect of neuropeptide Y to suppress growth hormone release by stimulating somatostatin discharge. *Endocrinology* 126, 2296-2301.

Rettori, V., Milenkovic, L., Riedel, M. and McCann, S. M. (1990b) Physiological role of neuropeptide Y (NPY) in control of anterior pituitary hormone release in the rat. *Endocrinologia experimentalis* 24, 37-45.

Rodi, D., Mazzuferi, M., Bregola, G., Dumont, Y., Fournier, A., Quirion, R. and Simonato, M. (2003) Changes in NPY-mediated modulation of hippocampal [3H]D-aspartate outflow in the kindling model of epilepsy. *Synapse* 49, 116-124.

Rodrigo, C., Zaben, M., Lawrence, T., Laskowski, A., Howell, O. W. and Gray, W. P. (2010) NPY augments the proliferative effect of FGF2 and increases the expression of FGFR1 on nestin positive postnatal hippocampal precursor cells, via the Y1 receptor. *Journal of neurochemistry* 113, 615-627.

Rorstad, O. P., Brownstein, M. J. and Martin, J. B. (1979) Immunoreactive and biologically active somatostatin-like material in rat retina. *Proceedings of the National Academy of Sciences of the United States of America* 76, 3019-3023.

Rosmaninho-Salgado, J., Alvaro, A. R., Grouzmann, E., Duarte, E. P. and Cavadas, C. (2007) Neuropeptide Y regulates catecholamine release evoked by interleukin-1beta in mouse chromaffin cells. *Peptides* 28, 310-314.

Rosmaninho-Salgado, J., Araujo, I. M., Alvaro, A. R., Mendes, A. F., Ferreira, L., Grouzmann, E., Mota, A., Duarte, E. P. and Cavadas, C. (2009) Regulation of catecholamine release and tyrosine hydroxylase in human adrenal chromaffin cells by interleukin-1beta: role of neuropeptide Y and nitric oxide. *Journal of neurochemistry* 109, 911-922.

Santos-Carvalho, A., Aveleira, C. A., Elvas, F., Ambrosio, A. F. and Cavadas, C. (2012) Neuropeptide Y receptors Y1 and Y2 are present in neurons and glial cells in rat retinal cells in culture. *Invest Ophthalmol Vis Sci*.

Santos-Carvalho, A., Elvas, F., Alvaro, A. R., Ambrosio, A. F. and Cavadas, C. (2013) Neuropeptide Y receptors activation protects rat retinal neural cells against necrotic and apoptotic cell death induced by glutamate. *Cell Death Dis* 4, e636.

Schmid, E., Leierer, J., Kieselbach, G., Teuchner, B., Kralinger, M., Fischer-Colbrie, R., Krause, J. E., Nguyen, Q. A., Haas, G., Stemberger, K. and Troger, J. (2006) Neurokinin A and neurokinin B in the human retina. *Peptides* 27, 3370-3376.

Schmid, E., Nogalo, M., Bechrakis, N. E., Fischer-Colbrie, R., Tasan, R., Sperk, G., Theurl, M., Beer, A. G., Kirchmair, R., Herzog, H. and Troger, J. (2012) Secretoneurin, substance P and neuropeptide Y in the oxygen-induced retinopathy in C57Bl/6N mice. *Peptides* 37, 252-257.

Schwertfeger, E., Klein, T., Vonend, O., Oberhauser, V., Stegbauer, J. and Rump, L. C. (2004) Neuropeptide Y inhibits acetylcholine release in human heart atrium by activation of Y2-receptors. *Naunyn-Schmiedeberg's archives of pharmacology* 369, 455-461.

Senanayake, P., Drazba, J., Shadrach, K., Milsted, A., Rungger-Brandle, E., Nishiyama, K., Miura, S., Karnik, S., Sears, J. E. and Hollyfield, J. G. (2007) Angiotensin II and its receptor subtypes in the human retina. *Invest Ophthalmol Vis Sci* 48, 3301-3311.

Silva, A. P., Carvalho, A. P., Carvalho, C. M. and Malva, J. O. (2001) Modulation of intracellular calcium changes and glutamate release by neuropeptide Y1 and Y2 receptors in the rat hippocampus: differential effects in CA1, CA3 and dentate gyrus. *Journal of neurochemistry* 79, 286-296.

Silva, A. P., Pinheiro, P. S., Carvalho, A. P., Carvalho, C. M., Jakobsen, B., Zimmer, J. and Malva, J. O. (2003) Activation of neuropeptide Y receptors is neuroprotective against excitotoxicity in organotypic hippocampal slice cultures. *FASEB J.* 17, 1118-1120.

Silva, A. P., Xapelli, S., Grouzmann, E. and Cavadas, C. (2005) The putative neuroprotective role of neuropeptide Y in the central nervous system. *Curr Drug Targets CNS Neurol Disord* 4, 331-347.

Sinclair, J. R., Jacobs, A. L. and Nirenberg, S. (2004) Selective ablation of a class of amacrine cells alters spatial processing in the retina. *J Neurosci* 24, 1459-1467.

Sinclair, J. R. and Nirenberg, S. (2001) Characterization of neuropeptide Y-expressing cells in the mouse retina using immunohistochemical and transgenic techniques. *J Comp Neurol* 432, 296-306.

Smialowska, M., Domin, H., Zieba, B., Kozniewska, E., Michalik, R., Piotrowski, P. and Kajta, M. (2009) Neuroprotective effects of neuropeptide Y-Y2 and Y5 receptor agonists in vitro and in vivo. *Neuropeptides* 43, 235-249.

Son, M. Y., Kim, M. J., Yu, K., Koo, D. B. and Cho, Y. S. (2011) Involvement of neuropeptide Y and its Y1 and Y5 receptors in maintaining self-renewal and proliferation of human embryonic stem cells. *J Cell Mol Med* 15, 152-165.

Stanic, D., Paratcha, G., Ledda, F., Herzog, H., Kopin, A. S. and Hokfelt, T. (2008) Peptidergic influences on proliferation, migration, and placement of neural progenitors in the adult mouse forebrain. *Proceedings of the National Academy of Sciences of the United States of America* 105, 3610-3615.

Stone, R. A., Kuwayama, Y. and Laties, A. M. (1987) Regulatory peptides in the eye. *Experientia* 43, 791-800.

Straznicky, C. and Hiscock, J. (1989) Neuropeptide Y-like immunoreactivity in neurons of the human retina. *Vision Res* 29, 1041-1048.

Straznicky, C. and Hiscock, J. (1994) Neuropeptide Y-immunoreactive neurons in the retina of two Australian lizards. *Arch Histol Cytol* 57, 151-160.

Straznicky, K. and Gaze, R. M. (1971) The growth of the retina in Xenopus laevis: an autoradiographic study. *J Embryol Exp Morphol* 26, 67-79.

Subhedar, N., Cerda, J. and Wallace, R. A. (1996) Neuropeptide Y in the forebrain and retina of the killifish, Fundulus heteroclitus. *Cell Tissue Res* 283, 313-323.

Tatemoto, K., Carlquist, M. and Mutt, V. (1982) Neuropeptide Y--a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* 296, 659-660.

Thiriet, N., Agasse, F., Nicoleau, C., Guegan, C., Vallette, F., Cadet, J. L., Jaber, M., Malva, J. O. and Coronas, V. (2011) NPY promotes chemokinesis and neurogenesis in the rat subventricular zone. *Journal of neurochemistry* 116, 1018-1027.

Tropepe, V., Coles, B. L., Chiasson, B. J., Horsford, D. J., Elia, A. J., McInnes, R. R. and van der Kooy, D. (2000) Retinal stem cells in the adult mammalian eye. *Science* 287, 2032-2036.

Tsuda, K., Tsuda, S., Goldstein, M. and Masuyama, Y. (1995) Sodium ions attenuate the inhibitory effects of neuropeptide Y on norepinephrine release in rat hypothalamus. *Am J Hypertens* 8, 1135-1140.

Uckermann, O., Wolf, A., Kutzera, F., Kalisch, F., Beck-Sickinger, A. G., Wiedemann, P., Reichenbach, A. and Bringmann, A. (2006) Glutamate release by neurons evokes a purinergic inhibitory mechanism of osmotic glial cell swelling in the rat retina: activation by neuropeptide Y. *J Neurosci Res* 83, 538-550.

Wetzel, R. K. and Eldred, W. D. (1997) Specialized neuropeptide Y- and glucagon-like immunoreactive amacrine cells in the peripheral retina of the turtle. *Vis Neurosci* 14, 867-877.

Xapelli, S., Agasse, F., Ferreira, R., Silva, A. P. and Malva, J. O. (2006) Neuropeptide Y as an endogenous antiepileptic, neuroprotective and pro-neurogenic peptide. *Recent patents on CNS drug discovery* 1, 315-324.

Xapelli, S., Bernardino, L., Ferreira, R., Grade, S., Silva, A. P., Salgado, J. R., Cavadas, C., Grouzmann, E., Poulsen, F. R., Jakobsen, B., Oliveira, C. R., Zimmer, J. and Malva, J. O. (2008) Interaction between neuropeptide Y (NPY) and brain-derived neurotrophic factor in NPY-mediated neuroprotection against excitotoxicity: a role for microglia. *Eur J Neurosci* 27, 2089-2102.

Xapelli, S., Silva, A. P., Ferreira, R. and Malva, J. O. (2007) Neuropeptide Y can rescue neurons from cell death following the application of an excitotoxic insult with kainate in rat organotypic hippocampal slice cultures. *Peptides* 28, 288-294.

Xiong, Z. and Cheung, D. W. (1995) ATP-Dependent inhibition of Ca2+-activated K+ channels in vascular smooth muscle cells by neuropeptide Y. *Pflugers Arch* 431, 110-116.

Xu, H., Sta Iglesia, D. D., Kielczewski, J. L., Valenta, D. F., Pease, M. E., Zack, D. J. and Quigley, H. A. (2007) Characteristics of progenitor cells derived from adult ciliary body in mouse, rat, and human eyes. *Invest Ophthalmol Vis Sci* 48, 1674-1682.

Yamada, T., Brecha, N., Rosenquist, G. and Basinger, S. (1981) Cholecystokinin-like immunoreactivity in frog retina: localization, characterization, and biosynthesis. *Peptides* 2 Suppl 2, 93-97.

Yang, X. L. (2004) Characterization of receptors for glutamate and GABA in retinal neurons. *Prog Neurobiol* 73, 127-150.

Yoon, H. Z., Yan, Y., Geng, Y. and Higgins, R. D. (2002) Neuropeptide Y expression in a mouse model of oxygen-induced retinopathy. *Clin Experiment Ophthalmol* 30, 424-429.

Zhou, Z., Zhu, G., Hariri, A. R., Enoch, M. A., Scott, D., Sinha, R., Virkkunen, M., Mash, D. C., Lipsky, R. H., Hu, X. Z., Hodgkinson, C. A., Xu, K., Buzas, B., Yuan, Q., Shen, P. H., Ferrell, R. E., Manuck, S. B., Brown, S. M., Hauger, R. L., Stohler, C. S., Zubieta, J. K. and Goldman, D. (2008) Genetic variation in human NPY expression affects stress response and emotion. *Nature*.

Zhu, B. S. and Gibbins, I. (1995) Synaptic circuitry of neuropeptide-containing amacrine cells in the retina of the cane toad, Bufo marinus. *Vis Neurosci* 12, 919-927.

Zhu, B. S. and Gibbins, I. (1996) Muller cells in the retina of the cane toad, Bufo marinus, express neuropeptide Y-like immunoreactivity. *Vis Neurosci* 13, 501-508.

Fig. 1 - The structural organization of the retina. Diagram illustrating the distribution of retinal neurons (photoreceptors, bipolar cells, ganglion cells, horizontal and amacrine cells), macroglia and microglial cells in organized layers in the retina. Retinal cells are well organized in several layers: retinal pigment epithelium (RPE); photoreceptor outer segment (POS); outer nuclear layer (ONL); outer plexiform layer (OPL); inner nuclear layer (INL); inner plexiform layer (IPL) and ganglion cell layer (GCL). The astrocytes are in the GCL, while the Müller cells extend through the entire thickness of the retina, extending processes from the outer until the inner limiting membrane. Microglial cells are mainly in OPL, IPL and GCL.

Fig. 2 – Localization of NPY-IR in different mammalian retinas (red depicted). A - In the human retina, NPY-IR is present in amacrine cell bodies in INL and GCL, amacrine processes in IPL and ganglion cells in GCL. NPY-immunoreactive fibers are found between INL and OPL and also crossing the INL. NPY-IR is also found in RPE; B - In rat retina, NPY-IR is present in amacrine cells in INL and GCL; C – In mouse retina, NPY-IR is found in amacrine cell bodies in INL and GCL, and processes in IPL; D – In baboon and pig retina, NPY-IR is localized in amacrine cell bodies in INL and processes in IPL; E – In cat retina, NPY-IR is found in amacrine cells in INL, in processes in IPL and in gamma-type retinal ganglion cells. F – In bovine retina, NPY-IR is present in RPE cells; G - In guinea-pig retina, NPY–immunoreactive fibers form a single layer in IPL, and NPY-IR cell bodies are in INL. NPY-IR is also present in some bipolar cells; H – In dolphin and dog retina, NPY-IR is located in medium to large ganglion cells with processes extended to IPL, in some areas of the retina. Only a few cells in the INL are NPY-immunoreactive.

Highlights (Review)

- NPY and NPY receptors are present in the retina of several species;
- NPY modulates neurotransmitters release from retinal cells;
- NPY protects retinal neural cells against toxic insults;
- NPY induces proliferation of retinal progenitor cells.

Figure 1

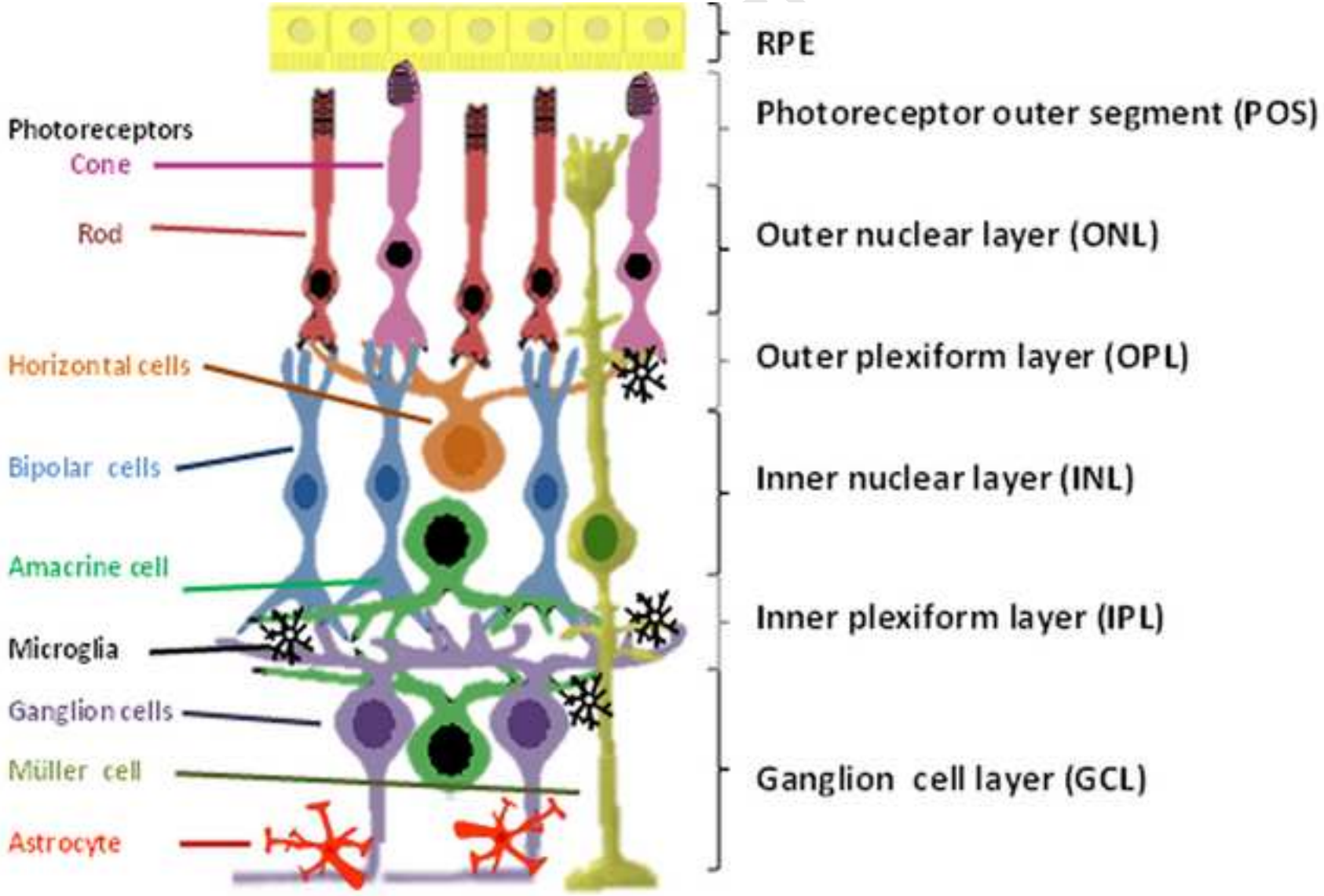

Figure 1, Santos-Carvalho, *Progress in Neurobiology*

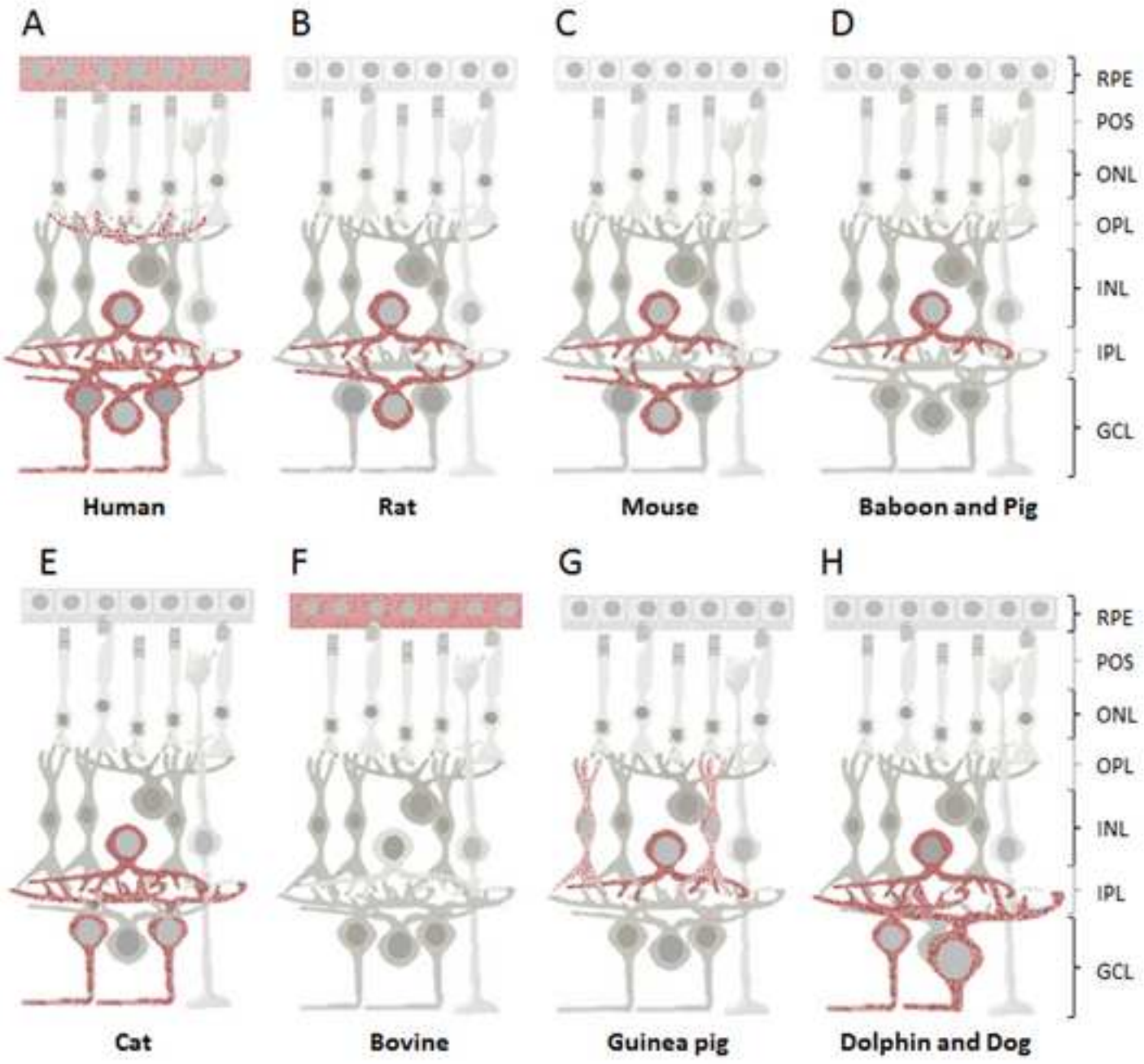

Figure 2, Santos-Carvalho, *Progress in Neurobiology*

Table 1 - NPY-IR localization in the retina of several non-mammalian species

		Species	NPY-IR localization in the retina	References
Non Mammals	Fishes	Blue acara (*Aequidens pulcher*)	amacrine cells and IPL	(Negishi and Wagner, 1995)
		Carp	cell bodies of amacrine cells in INL and processes in the IPL	(Bruun et al., 1986)
		Gilthead seabream (*Sparus aurata L.)*	amacrine cells	(Pirone et al., 2008)
		Goldfish	cell bodies of amacrine in INL and cell processes in two layers in IPL	(Bruun et al., 1986; Osborne et al., 1985) (Muske et al., 1987)
		Killifish (*Fundulus heteroclitus*)	amacrine cell fibers in IPL	(Subhedar et al., 1996)
		Lamprey (*Lampreta fluviatilis*)	pyriform subclass of amacrine cells	(Negishi et al., 1986; Rawitch et al., 1992)
		Skates (*Raja clavata, Raja radiate and Raja oscellata*)	amacrine cells in the innermost part of INL and fibers in IPL	(Bruun et al., 1985)
		Squid	no NPY-IR	(Osborne et al., 1986)
		Trout	cell bodies of amacrine cells in INL and processes in IPL	(Bruun et al., 1986)
		Zebrafish	amacrine cells	(Mathieu et al., 2002)
	Anurans	Frogs (*Bufo marinus and Xenopus laevis*)	cell bodies of amacrine cells in INL and cell processes in IPL; bipolar-like cell bodies in the middle of INL and sparsely in GCL; processes ramifying in three sublayers in IPL; Müller cells within the INL and processes in IPL; co-localization of GABA in all NPY-IR amacrine cells of anuran retina.	(Bruun et al., 1991; Bruun et al., 1986; Hiscock and Straznicky, 1989, 1990; Osborne et al., 1985; Zhu and Gibbins, 1995, 1996)
	Reptiles	Lizards (*Pogona vitticeps and Varanus gouldii*)	amacrine cells: type A and type B in INL and displaced at GCL	(Straznicky and Hiscock, 1994)
		Turtle	three types of amacrine cells: type A, at INL, IPL and occasional processes at GCL; type B, at INL and IPL; type C, at the periphery of retina. bipolar cells	(Isayama and Eldred, 1988 Isayama et al., 1988; Wetzel and Eldred, 1997)
	Birds	Chicken	cell bodies of amacrine cells in the middle and innermost INL and processes in the IPL	(Bruun et al., 1986)
		Pigeon	cell bodies of amacrine cells in INL and processes in the IPL	(Bruun *et al.*, 1986; Verstappen *et al.*, 1986)

Table 2 – Localization of NPY receptors in mammalian retina

NPY Receptors	Mammal Species	Localization of NPY receptors in mammalian retina	References
NPY Y_1	Mouse	mRNA expression in room air reared mouse retina.	(Yoon et al., 2002)
	Rat	Immunoreactivity in horizontal cell bodies in INL and processes in OPL, in cholinergic amacrine cell processes in IPL and in all calbindin horizontal cells in rat retina;	(D'Angelo et al., 2002)
		mRNA in rat retinas and cultured rat retinal cells;	(Alvaro et al., 2008; Alvaro et al., 2009; Alvaro et al., 2007)
		Immunoreactivity in photoreceptors, bipolar, horizontal, amacrine and ganglion cells as well as in macroglial and microglial cells of rat retinal cells in culture.	(Santos-Carvalho et al., 2012)
	Guinea Pig	NPY Y_1 receptor in cultured Müller cells.	(Milenkovic et al., 2004)
	Bovine	mRNA in cultured RPE cells.	(Ammar et al., 1998)
	Human	mRNA in human RPE;	(Ammar et al., 1998)
		Immunoreactivity in Müller cells in retina with proliferative vitreoretinopathy.	(Canto Soler et al., 2002)
NPY Y_2	Mouse	mRNA expression in post-natal oxygen-reared mice.	(Yoon et al., 2002)
	Rat	mRNA in intact retinas and cultured rat retinal cells;	(Alvaro et al., 2008; Alvaro et al., 2009; Alvaro et al., 2007; D'Angelo and Brecha, 2004)
		Immunoreactivity in photoreceptors, bipolar, horizontal, amacrine and ganglion cells as well as macroglial and microglial cells of rat retinal cells in culture.	(Santos-Carvalho et al., 2012)
	Bovine	mRNA in bovine RPE and cultured RPE cells;	(Ammar et al., 1998)
	Human	mRNA in human RPE.	(Ammar et al., 1998)
NPY Y_4	Rat	mRNA in rat retina and cultured rat retinal cells.	(Alvaro et al., 2008; Alvaro et al., 2009; Alvaro et al., 2007; D'Angelo and Brecha, 2004)
NPY Y_5	Rat	mRNA in rat retina and cultured rat retinal cells;	(Alvaro et al., 2008; Alvaro et al., 2009; Alvaro et al., 2007; D'Angelo and Brecha, 2004)
	Human	mRNA in human RPE.	(Ammar et al., 1998)

Table 3 – Roles of NPY receptors in mammalian retina

NPY Receptors	Mammal Species	Function of NPY receptors in mammalian retina	References
NPY Y_1	Rat	Receptor activation inhibits the increase in $[Ca^{2+}]_i$ in rat retinal neurons;	(Alvaro et al., 2009)
		Proliferation of rat retinal cells in culture;	(Alvaro et al., 2008)
		Receptor activation in neurons increases glutamate release that activates glutamate mGlu receptors of Müller cells and inhibits their osmotic swelling.	(Uckermann et al., 2006)
	Guinea Pig	NPY has a biphasic effect on Muller cells proliferation through NPY Y_1 receptor activation.	(Milenkovic et al., 2004)
NPY Y_2	Mouse	NPY Y_2 mRNA expression increase in post-natal oxygen-reared mice, suggesting that may have a dual role, vasoconstriction and angiogenesis, in the evolution of oxygen-induced retinopathy.	(Yoon et al., 2002)
	Rat	Inhibition of voltage-dependent Ca^{2+} influx into rod bipolar cell terminals;	(D'Angelo and Brecha, 2004)
		NPY Y_2 receptor antisense oligonucleotide prevents hyperoxia-induced retinal neovascularization;	(Koulu et al., 2004)
		Proliferation of rat retinal cells in culture;	(Alvaro et al., 2008)
		Receptor activation inhibits the necrotic cell death induced by glutamate in rat retinal cells in culture.	(Santos-Carvalho et al., 2013)
	Bovine	NPY signaling in cultured bovine RPE occurs mainly through NPY Y_2 receptor.	(Ammar et al., 1998)
NPY Y_4	Rat	Receptor activation inhibits the increase in $[Ca^{2+}]_i$ in rat retinal neurons.	(Alvaro et al., 2009)
		Receptor activation inhibits the necrotic cell death induced by glutamate in rat retinal cells in culture.	(Santos-Carvalho et al., 2013)
NPY Y_5	Rat	Receptor activation inhibits the increase in $[Ca^{2+}]_i$ in rat retinal neurons;	(Alvaro et al., 2009)
		Proliferation of rat retinal cells in culture.	(Alvaro et al., 2008)
		Receptor activation inhibits the necrotic and apoptotic cell death induced by glutamate in rat retinal cells in culture.	(Santos-Carvalho et al., 2013)

Abbreviation List

BrdU	5-Bromo-2'-deoxyuridine
$[Ca^{2+}]_i$	Intracellular calcium
CNS	Central nervous system
DPP-IV	Dipeptidyl peptidase IV
E18	Embrionic day 18
ERK	Extracellular signal-regulated kinases
GABA	γ-Aminobutyric acid
GAD 65	Glutamic acid decarboxylase 65
GAT-1	GABA transporter 1
GCL	Ganglion cell layer
hESC	Human embryonic stem cells
iGluRs	Ionotropic glutamate receptors
INL	Inner nuclear layer
IPL	Inner plexiform layer
Leu	Leucine
MAPK	Mitogen activated protein kinase
MDMA	3,4-Methylenedioxymethamphetamine
mGLuRs	Metabotropic glutamate receptors
mRNA	Messenger ribonucleic acid
NO	Nitric oxide
NOS–sGC	Nitric oxide synthase - soluble guanylyl cyclase
NPY	Neuropeptide Y
NPY–IR	Neuropeptide Y immunoreactivity
NPY Y_1	NPY receptor type 1
ONL	Outer nuclear layer
OPL	Outer plexiform layer
P7	Postnatal day 7
PKA	Protein Kinase A
POS	Photoreceptor outer segment
Pro	Proline
RPE	Retinal pigmented epithelium