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Prog Retin Eye Res. Author manuscript; available in PMC 2019 May 01.

Published in final edited form as:

Prog Retin Eye Res. 2018 May ; 64: 96–130. doi:10.1016/j.preteyeres.2017.12.001.

Neural control of choroidal blood flow

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Abstract

The choroid is richly innervated by parasympathetic, sympathetic and trigeminal sensory nerve fibers that regulate choroidal blood flow in birds and mammals, and presumably other vertebrate classes as well. The parasympathetic innervation has been shown to vasodilate and increase choroidal blood flow, the sympathetic input has been shown to vasoconstrict and decrease choroidal blood flow, and the sensory input has been shown to both convey pain and thermal information centrally and act locally to vasodilate and increase choroidal blood flow. As the choroid lies behind the retina and cannot respond readily to retinal metabolic signals, its innervation is important for adjustments in flow required by either retinal activity, by fluctuations in the systemic blood pressure driving choroidal perfusion, and possibly by retinal temperature. The former two appear to be mediated by the sympathetic and parasympathetic nervous systems, via central circuits responsive to retinal activity and systemic blood pressure, but adjustments for ocular perfusion pressure also appear to be influenced by local autoregulatory myogenic mechanisms. Adaptive choroidal responses to temperature may be mediated by trigeminal sensory fibers. Impairments in the neural control of choroidal blood flow occur with aging, and various ocular or systemic diseases such as glaucoma, age-related macular degeneration (AMD), hypertension, and diabetes, and may contribute to retinal pathology and dysfunction in these conditions, or in the case of AMD be a precondition. The present manuscript reviews findings in birds and mammals that contribute to the above-summarized understanding of the roles of the autonomic and sensory innervation of the choroid in controlling choroidal blood flow, and in the importance of such regulation for maintaining retinal health.

Keywords

Ciliary ganglion; Pterygopalatine ganglion; Superior cervical ganglion; Parasympathetic; Sympathetic; Choroidal blood flow; Ocular blood flow; Uvea

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1. Overview of ocular blood supplies and their neural control in mammals and birds

1.1. Why mammals and birds?

In this review on the innervation of the choroid, the central circuitry regulating the choroidal innervation, and the importance of such regulation for retinal health, we summarize findings in both mammals and birds for several reasons. First, our initial studies of neural control of choroidal blood flow ensued from our circuitry studies in pigeons on the inputs and outputs of the preganglionic nucleus of Edinger-Westphal (EW). Unexpectedly and as detailed later in this review, these circuitry studies revealed a bisynaptic retinal input to the medial part of EW that had output via the ciliary ganglion to blood vessels of the choroid (Gamlin et al., 1982). At that time, this was the first clear evidence for a central circuit involved in control of choroidal blood flow in any species. As evidence was also emerging at that time from studies by others of substantial autonomic innervation of choroid in mammals, it seemed likely that central circuits also existed in mammals for regulating choroidal blood flow (ChBF) via its autonomic input, but remained to be discovered. Because of the unknown nature of these central circuits in mammals, we took advantage of our discovery in birds to explore the role and importance of neurogenic ChBF control by means of studies of the EW-ciliary ganglion-choroid circuit in birds. We believed such studies would provide general insight into the signals that drive autonomic control of ChBF and the importance of such control for retinal health. Our findings in the latter regard provide the second reason for including our studies of birds in this review. We eventually expanded our efforts to include additional autonomic circuits in birds, and central circuitry controlling ChBF in mammals. Our studies and relevant studies of others are summarized below. Note that although forebrain cytoarchitecture in birds differs from that in mammals (Reiner et al., 2004, 2005), fundamental similarities exist between birds and mammals in retinal structure, choroidal structure, and choroidal innervation, as also detailed below, which support the relevance of our choroidal studies in birds.

1.2. The retinal vascular supply and retinal thickness in mammals and birds

The retina has two vascular supplies in most placental mammalian species, the choroidal vasculature and the vessels of the inner retina (Fig. 1) (Chase, 1982; Bill, 1984). The blood supply to the inner retina is via the central retinal artery (which arises from the ophthalmic artery), whose branches radiate from the optic nerve head onto the inner retinal surface and then give rise to branches that penetrate into the retina through the depth of the inner nuclear layer, ramifying in the inner and outer plexiform layers, and supply blood to the inner half of the retina (Figs. 1 and 2) (Alm, 1992). Because retinal blood vessels are located directly within the retina itself, they are able to respond to the local concentrations of carbon dioxide and oxygen, and to regulate blood flow accordingly (called metabolic coupling), as typical of most vascular beds (Bill, 1984; Bill and Sperber, 1990), or by neurovascular coupling (Metea and Newman, 2006; Biesecker et al., 2016), as in the brain (Takano et al., 2006; Filosa et al., 2006; Iadecola and Nedergaard, 2007). The retinal vessels of mammals are themselves not innervated, but the central retinal artery is innervated by parasympathetic, sympathetic and sensory nerve fibers (Laties, 1967; Kumagai et al., 1988; Bergua et al.,

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2003). The central retinal artery is also the blood supply of the postlaminar and laminar parts of the optic nerve, and the postlaminar part of the optic nerve head additionally receives contributions from the short ciliary arteries that supply the choroid (Fig. 3) (Reiner et al., 2012). By contrast, the prelaminar part of the optic nerve head is supplied by the central retinal artery and by the choroid, the latter of which is also innervated by parasympathetic, sympathetic and sensory nerve fibers. Thus, the blood supply to the optic nerve and optic nerve head are under neural influence, and blood flow in the retinal vessels themselves is thereby affected by the control exerted at the level of the central retinal artery, short ciliary arteries, and choroid (Strohmaier et al., 2016), as well as by the above noted local retinal metabolic regulatory mechanisms and neurovascular coupling.

A retinal vascular supply appears to be necessary for a thick retina that allows detailed form vision, given the reported limit of intraretinal oxygen diffusion from the choroid under normoxia of ~150 μm (Dollery et al., 1969; Landers, 1978; Linsenmeier and Zhang, 2017). This is thought to explain why retinal thickness is only about 150 μm in mammals with so-called avascular retinas, such as monotremes, most marsupials, and some placental mammals, and in the avascular fovea of foveate placental mammals (Chase, 1982). Although birds entirely lack retinal blood vessels, they nonetheless have relatively thick retinas (e.g. 300–400 μm in pigeon or chicken depending on the region) and good acuity (Fig. 1) (Donovan, 1978; McKibbin et al., 2014). Instead of retinal vessels, birds possess a richly vascularized pleated fan-shaped structure called the pecten that protrudes from the optic nerve head into the vitreous (Fig. 1). Its capillaries are highly permeable, and supplied by branches of the ophthalmotemporal artery that enter the eye along the edges of the vertically elongate optic nerve head (Fig. 4), which are thus collectively comparable to the central retinal artery of mammals (Wingstrand and Munk, 1965; Hosler and Olson, 1984; Meyer, 1977). The highly permeable blood vessels of the pecten are thought to enable it to secrete oxygen and other nutrients into the vitreous for the inner retina, and thereby play a similar role as the retinal vasculature in mammals (Brach, 1977; Pettigrew et al., 1990; Meyer, 1977). This specialization for providing nutrients to the inner retinas is thought to facilitate the high acuity vision of birds by eliminating the need for an inner retinal vascular network that could interfere with passage of light through the retina for image formation (Brach, 1977). Although it might be thought that the pecten itself would interfere with retinal image formation for the central retina, this appears not to be the case since its shadow seems to mainly fall on the optic nerve head (Brach, 1977; Meyer, 1977).

1.3. The choroidal vascular supply in mammals and birds

The blood supply to the choroid in mammals arises from the ophthalmic branch of the internal carotid artery, via the long and short ciliary arteries (Fig. 2), while in birds it arises from multiple choroidal branches of the ophthalmotemporal artery from the external ophthalmic branch of the internal carotid artery (Fig. 4) (Reiner et al., 2012). The choroid is one of the most highly vascularized structures in the body of mammals and birds, and also contains fibroblasts, melanocytes, resident immunocompetent cells, pericytes, and supporting connective tissue (De Stefano and Mugnaini, 1997; Nickla and Wallman, 2010). In birds and primates, the choroid has also been reported to contain extravascular smooth muscle cells and lymphatic lacunae (Poukens et al., 1998; Nickla and Wallman, 2010).

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The choroid is organized into an outer layer of large blood vessels (called Haller's Layer in mammals), a middle layer of branches of the larger vessels (called Sattler's layer in mammals), and the choriocapillaris arising from vessels of the middle layer and juxtaposed to Bruch's membrane (Fig. 1). Vessels of the choriocapillaris possess fenestrations facing Bruch's membrane that facilitate fluid and protein exchange with the retina. Although choroidal thickness varies among species, choroidal thickness tends to be proportionally greater in birds than mammals, with choroidal thickness in pigeons and chickens for example being comparable to that in humans (about 200 μm) despite the much greater eye size in humans.

The choroid accounts for about 85% of the blood supply to the retina, and its major role involves supplying oxygen and nutrients to the retinal pigment epithelium (RPE) and photoreceptors of the outer retina (Bill, 1984, 1985). The choroid is, however, also the major or exclusive vascular supply for both outer and inner retina in regions poor in or lacking retinal vessels (such as the fovea in primates), and in animal groups in which retinal vessels are sparse (rabbits). Although the pecten in birds plays a role similar to that of the retinal vasculature in mammals, it is uncertain if it is of comparable effectiveness in its ability to provide nutrients deep into the retina. Assuming a similar oxygen depth from the choriocapillaris as in mammals, it seems possible that the choroid in birds supplies at least part of the inner retina in thinner peripheral parts of the retina. The overall importance of the choroid is emphasized by the rapid photoreceptor loss that occurs with severely diminished choroidal blood flow (Gay et al., 1964; Collier, 1967; Gaudric et al., 1982; McCulloch, 1988). Photoreceptors are particularly dependent on the choroid because of their high metabolism and continuous outer segment renewal (Bill, 1984; Young, 1978).

1.4. Innervation of the choroid in mammals and birds

Although it was once thought that choroidal blood flow (ChBF) so exceeds retinal requirements as to obviate need for its regulation (Bill, 1984, 1985), it now seems clear that: 1) high ChBF is essential as the driving force for diffusion of oxygen and nutrients through Bruch's membrane, the retinal pigment epithelium (RPE), and the depth of the outer neural retina, and to the inner retina in the case of retinal regions devoid of or poor in retinal vessels (Bill, 1984, 1985; Cringle et al., 1999; Yu et al., 2005); 2) ChBF must be stably matched to the thermal and metabolic needs of the retina (Bill, 1984, 1985; Tillis et al., 1988); and 3) disturbances in ChBF impair outer retinal function (Steinberg, 1987; Yancey and Linsenmeier, 1988, 1989; Lovasik et al., 2005a; Lovasik and Kergoat, 2012). Thus, the ability of ChBF to respond adaptively to variations in retinal need imposed by changes in illumination level and retinal activity, and to maintain stable flow despite fluctuations in bodily state and time of day, are likely to be important for maintaining the health and proper functioning of the RPE and outer retina.

In contrast to the retinal vessels, the choroid and its capillary bed, the choriocapillaris, are separated from the outer retina by Bruch's membrane (BM) and the RPE (Nickla and Wallman, 2010). This barrier and the high blood flow rate in the choroid make ChBF largely unable to be regulated by vasogenic metabolites derived from the outer retina (Bill and Sperber, 1990). Instead, the choroid is under neural control, although myogenic

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mechanisms are thought to contribute as well (Kiel and Shepherd, 1992; Kiel, 1994; Kiel and van Heuven, 1995). Three major types of nerve fibers innervate the choroid in mammals and birds (Table 1, Fig. 5), and presumably members of other vertebrate classes as well: 1) parasympathetic fibers arising from the pterygopalatine ganglion (PPG) that co-contain vasoactive intestinal polypeptide (VIP), neuronal nitric oxide synthase (nNOS) and acetylcholine (Stone et al., 1987; Ruskell, 1971; Yamamoto et al., 1993; Cuthbertson et al., 1997); 2) noradrenergic sympathetic fibers from the superior cervical ganglion that also contain neuropeptide Y (NPY) (Guglielmone and Cantino, 1982; Stone et al., 1987); and 3) sensory fibers from the trigeminal ganglion co-containing substance P (SP) and calcitonin gene-related peptide (CGRP) (Stone et al., 1987; Shih et al., 1999). These parasympathetic, sympathetic, and sensory fibers and their terminals tend to be localized to the walls of the arteries and veins of the choroid, but not the choriocapillaris. Pericytes along choroidal vessels, which exert contractile effects, have also been reported to receive terminals (Schrödl et al., 2014). In addition, cholinergic parasympathetic fibers to the choroid also arise from the ciliary ganglion in birds, and perhaps in some mammalian groups as well (Stjernschantz et al., 1976; Meriney and Pilar, 1987; Stone et al., 1987; Koss et al., 1991; Reiner et al., 1991; Cuthbertson et al., 1996; May, 1997). The nNOS+ and VIP + PPG fibers have a vasodilatory influence on choroidal vessels and increase ChBF (Bill, 1984, 1991; Bill and Sperber, 1990), while adrenergic fibers have a vasoconstrictory action that decreases ChBF (Bill, 1984, 1991; Bill and Sperber, 1990; Koss and Gherezghiher, 1993). The input to the choroid from the ciliary ganglion also has a vasodilatory influence, mediated by muscarinic elicitation of endothelial NO release (Fitzgerald et al., 1990b; Zagvazdin et al., 1996a, 2000; Cuthbertson et al., 1999). The sensory fibers in the choroid have a vasodilatory influence as well. The anatomy and function of these various inputs to choroid in birds and mammals are detailed in the following sections.

1.5. Brain and retina in mammals and birds

As reviewed extensively elsewhere (Reiner et al., 2004, 2005), the pallial part of the telencephalon in birds is organized cytoarchitectonically into nuclear groups that perform the same general sensory, motor and cognitive functions as the neocortex in mammals. Although differences also exist between birds and mammals in the cytoarchitecture of the diencephalon and midbrain, one-to-one homologues can be identified between mammals and birds for the vast majority of midbrain and hindbrain cell groups, including those associated with the cranial nerve nuclei, and the parasympathetic and sympathetic nervous systems (Reiner et al., 2004, 2005). Retinal lamination and the major cell types are also similar between mammals and birds, with the retina in birds being cone-rich (Meyer, 1977), similar to that of diurnal mammals including most primates but unlike that of nocturnal mammals including most rodents (Jacobs, 2009). Due to the various similarities outlined above, we thus believe our studies in birds on choroidal blood flow and its health-related significance have relevance to mammals.

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2. Facial nucleus parasympathetic input to choroid

2.1. Peripheral anatomy of facial circuitry for control of ChBF in mammals

The distributed orbital neurons composing the PPG profusely innervate choroidal blood vessels in all mammalian groups studied, including rats, guinea pigs, cats, squirrels, monkeys and humans, with postganglionic axons from the PPG entering the choroid directly, on ciliary vessels, and by joining the short ciliary nerves arising from the ciliary ganglion (Ruskell, 1970, 1971; Uddman et al., 1980; Shimizu, 1982; Terenghi et al., 1982; Uusitalo et al., 1984; Stone, 1986; Lin et al., 1988; Ten Tusscher et al., 1990; Beckers et al., 1993; Reiner et al., 2012). Additional PPG fibers pass through the ciliary ganglion on their way to the choroid (Beckers et al., 1993). The PPG neurons innervating choroid in rats are found within the proximal part of the PPG and scattered along the greater petrosal nerve itself, in which the preganglionic fibers to the PPG travel (Cuthbertson et al., 2003). Small accessory ganglia near the ciliary ganglion may be part of the PPG system as well, since in rats they project to the eye and are VIP+ (Kuwayama et al., 1987; Stone et al., 1987; Ten Tusscher et al., 1990). PPG neurons are VIP+ and their extirpation has been shown in cats to eliminate VIP + fibers from choroid (Uddman et al., 1980; Butler et al., 1984). The PPG is also the source of a profuse nNOS + innervation of choroid, and all nNOS + PPG neurons have been found to also contain VIP in rats (Fig. 6) (Yamamoto et al., 1993; Cuthbertson et al., 2003). Additionally, PPG neurons and their input to choroid appear to be cholinergic as well (Terenghi et al., 1982; Uusitalo et al., 1984; Hara et al., 1985; Stone, 1986; Cuthbertson et al., 2003). As evidenced by VIP immunolabeling, PPG input to choroid in mammals ends on arteries, veins and melanocytes (Fig. 7) (Beckers et al., 1993). The distribution within PPG of neurons innervating choroid overlaps those innervating the iris (Ten Tusscher et al., 1990), Meibomian glands (LeDoux et al., 2001), and cerebral vasculature (Suzuki et al., 1988). Thus, it is uncertain if the PPG neurons controlling the choroid only subserve the choroid. Based on the number of retrogradely labeled neurons we observed in the PPG and greater petrosal nerve after intrachoroidal fluorogold injection, and based on the extent of the fluorogold injections, it appears that at least 200–300 PPG neurons innervate choroid in rats (Cuthbertson et al., 2003). Additionally, PPG neurons innervate orbital vessels feeding into choroid and thereby exert a further influence on ChBF (Stone et al., 1987; Toda et al., 1998).

2.2. Central anatomy of facial circuitry for control of ChBF in mammals

The PPG in mammals receives its preganglionic parasympathetic input from the superior salivatory nucleus (SSN) subdivision of the facial nucleus motor complex (Table 1) (Ten Tusscher et al., 1990). We have used transneuronal retrograde pathway tracing in rats with the Bartha K strain of pseudorabies virus (PRV) to identify the SSN neurons innervating those PPG neurons projecting to choroid, and to identify central neurons projecting to that part of SSN (Cuthbertson et al., 2003; Li et al., 2015a). Double-labeling immunofluorescence for PRV + neurons in SSN at relatively short survival times after minute injections of PRV restricted to choroid (to ensure only neurons in SSN projecting bisynaptically to choroid were labeled) showed that they were cholinergic, confirming their preganglionic identity (Fig. 8). Moreover, the choroidal SSN neurons appeared to be slightly more rostral and medial in location than those observed after injection of PRV into other

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peripheral PPG targets, such as the Meibomian glands (LeDoux et al., 2001) and the lacrimal gland (Tóth et al., 1999), suggesting choroidal control may be localized to a select subpopulation of SSN neurons. The choroidal SSN neurons commonly were also nNOS+, and the majority of nNOS + neurons within the SSN were PRV+, indicating nNOS to be a relatively selective marker of choroidal neurons in rat SSN (Cuthbertson et al., 2003).

We also characterized the central neurons projecting to the SSN neurons innervating choroidal PPG neurons, using a combination of pathway tracing and immunolabeling (Li et al., 2015a). As part of these studies, we examined the higher order labeling beyond SSN after minute injections of PRV into choroid in rats in which the superior cervical ganglia had been excised (to prevent labeling of sympathetic circuitry). Several neuronal populations beyond the choroidal part of ipsilateral SSN showed selective transneuronal labeling. For example, the dorsal part of the paraventricular nucleus (PVN) of the hypothalamus notably contained PRV + neurons bilaterally (more heavily ipsilaterally) (Fig. 9). The input of PVN to choroidal SSN is of interest, because PVN is responsive to systemic blood pressure (BP) and plays a role in maintaining stable systemic BP (Porter and Brody, 1986; Wyss et al., 1990; Krukoff et al., 1997; Badoer and Merolli, 1998), and the PVN region exerts a vasodilatory influence on cerebral blood flow (CBF) (Golanov et al., 2001). We also observed PRV + neurons in the caudolateral part of the nucleus of the solitary tract (NTS) known to receive aortic baroreceptor input via the vagus nerve and respond to BP fluctuation (Fig. 9) (Rogers et al., 1993; Ciriello, 1983). Like the PVN, the NTS exerts a vasodilatory influence on cerebral blood flow (Nakai and Ogino, 1984) and has been shown to project directly to the SSN by anterograde labeling methods (Agassandian et al., 2002). In addition, we observed PRV + neurons in the periaqueductal gray, the raphe magnus (RaM), the B3 serotonergic cell group of the pons, the A5 noradrenergic group of the pons, and the rostral ventrolateral medulla (RVLM). The PRV + neurons were located in the parts of these cell groups that are known to be responsive to systemic BP signals and previously reported to be involved in systemic BP regulation by the sympathetic nervous system (Finley and Katz, 1992; Dampney, 1994; Jansen et al., 1995; Guyenet, 2006). Using PRV labeling and/or conventional pathway tracing in combination with immunolabeling and confocal laser scanning microscope viewing, we found that PVN neurons projecting to SSN were oxytocinergic and glutamatergic, RaM neurons projecting to SSN were serotonergic, and NTS neurons projecting to SSN were glutamatergic (Fig. 10). Thus, all of these inputs appear to have an excitatory influence on SSN neurons.

Our results suggest that blood pressure and blood volume signals can drive parasympathetic vasodilation of the choroidal vasculature. Of particular note, all of these same regions that project to SSN can also drive sympathetic constriction of the systemic vasculature in response to low BP signals. The phenomenon of blood flow stability over a range of systemic BPs has been well documented for the cerebral vasculature, and cerebral compensation for low BP is thought to maintain cerebral function and prevent ischemic cerebral injury (Paulson et al., 1990). As noted above, the PPG innervates the choroid, orbital vessels, the lacrimal gland and Meibomian glands (Ten Tusscher et al., 1990; LeDoux et al., 2001), as well as nonorbital tissues such as the cerebral vasculature and the nasal mucosa (Walters et al., 1986). Given that PVN stimulation or NTS stimulation increases cerebral blood flow (Ciriello, 1983; Nakai and Ogino, 1984; Krukoff et al., 1997; Rogers

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et al., 1993; Golanov et al., 2001), the BP responsiveness of PVN and NTS suggests that their activation may drive cerebral blood flow increases in response to low systemic BP, while also driving peripheral vasoconstriction (Calarescu et al., 1984; Porter and Brody, 1986; Goldstein and Kopin, 1990). The combination of increased vasodilation in the cerebral vasculature combined with peripheral vasoconstriction would work together to maintain a stable cerebral blood flow despite BP declines.

It may be that the inputs of PVN, NTS, RaM, B3, A5 and RVLM to choroidal SSN play a similar role in maintaining choroidal blood flow during normal or accidental (e.g. due to hemorrhage) bouts of low systemic BP. In fact, the possibility exists that the same populations of PPG neurons and SSN neurons that regulate choroidal blood flow also regulate cerebral blood flow. The fact that the brain and eye are both neural tissues with high metabolic requirements calling for a stable blood supply is concordant with this possibility. Consistent with this, ChBF stability during BP variation has been reported in humans, rabbits, rats and pigeons (Kiel and Shepherd, 1992; Kiel and van Heuven, 1995; Riva et al., 1997a, b; Reiner et al., 2010a, 2011). Such reflexive PPG-mediated choroidal vasodilation could be important for maintaining metabolic support of the retina during the routine, daily bouts of low BP, such as might occur during inactivity, rest, or sleep, as well as for preventing retinal ischemia during extreme BP-lowering events due to injury or blood loss (Bill, 1985; Bill and Sperber, 1990). It seems, therefore, that neurogenic mechanisms are likely to contribute significantly to the stability of both choroidal and cerebral blood flow during downward fluctuations in systemic BP, as discussed further in the following section on the function of the SSN-PPG circuit.

Because of the evidence that retinal illumination can increase ChBF via central circuits in birds, as discussed in more detail in the section on ciliary ganglion innervation of the choroid (Fitzgerald et al., 1990b, 1996; Shih et al., 1993b), because of the evidence that light or transition from dark to light increases ChBF in mice, rabbits, monkeys, and humans (Parver et al., 1982, 1983; Tillis et al., 1988; Bill and Sperber, 1990; Longo et al., 2000; Fuchsjäger-Mayrl et al., 2001; Huemer et al., 2007; Berkowitz et al., 2016), and because of the evidence that flicker-modulated regulation of blood flow occurs in the short ciliary arteries supplying choroid and in the central retinal artery (Michelson et al., 2002; Zeitz et al., 2008), and perhaps in choroid itself in at least some mammalian species and under some conditions (Garhöfer et al., 2002; Lovasik et al., 2005b), visual inputs to choroidal SSN neurons are of interest. Retinal activity-dependent regulation of ChBF, mediated by retinal input to central autonomic circuitry, may serve as a replacement for the direct metabolic or neurovascular coupling evident between retina and the inner retinal circulation, which is not possible for the choroidal circulation due to its position behind the retina. While photoreceptor metabolism is high in the dark and declines in the light (Voaden et al., 1983; Linsenmeier, 1986; Tillis et al., 1988; Braun et al., 1995; Medrano and Fox, 1995; Wang et al., 1997; Cringle et al., 1999), flickering light (which may better simulate a changing visual scene than constant illumination) results in a considerable increase in inner retinal metabolism with little change in photoreceptor metabolism (Bill and Sperber, 1990; Braun et al., 1995). Retinal activity-mediated increases in ChBF, in those species or circumstances when they may occur, may be especially important for driving nutrients and oxygen to inner retina under these circumstances, again for species poor in retinal vessels and for retinal

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regions poor in retinal vessels. The neural mechanisms that might mediate such a light response in mammals are unclear. One possibility is that, like in birds and as discussed below, a circuit involving suprachiasmatic nucleus (SCN) input to the ciliary ganglion via the preganglionic neurons of the nucleus of Edinger-Westphal (EW) is responsible for light-mediated ChBF increases. We have not, however, observed PRV + neurons in SCN or EW following minute injections of PRV confined to superior choroid in rats (Li et al., 2015a). Thus, unlike in birds (Reiner et al., 1983, 1991; Cuthbertson et al., 1996), the EW-ciliary ganglion circuit in rats does not appear to exert a direct influence on ChBF. This raises the possibility that the light-mediated regulation of ChBF reported in various mammalian species (Parver et al., 1982, 1983; Bill and Sperber, 1990; Stiris et al., 1991; Longo et al., 2000) is not mediated by either SCN or EW.

It may be that PPG input to choroid can respond to visual signals, and thereby mediate the visual control of ChBF. This might occur by a visual input to SSN from light-responsive PVN neurons. The retina is known to project to ipsilateral PVN in rodents (Hermes et al., 1996; Youngstrom et al., 1987), and it is possible the PVN retinorecipient neurons include those that project to choroidal neurons of SSN. The finding that the ChBF changes that occur in human macula during dark – light transitions can be blocked by NOS inhibition is consistent with the possible involvement of the PPG input to the choroid in light-mediated control of ChBF (Huemer et al., 2007). On the other hand, it is also possible that the PRV + neurons in the ventrocaudal part of the spinal trigeminal nucleus we have observed after PRV injections confined to ipsilateral superior choroid mediate a light response. This region receives corneal input (Aicher et al., 2014) and responds to bright light (Okamoto et al., 2009, 2010). It may thus be that a corneal response to bright light, which appears to be mediated via an innervation from melanopsin-containing trigeminal fibers (Matynia et al., 2016), can drive ChBF increases via a projection from cornea-responsive trigeminal nucleus neurons to the choroidal SSN. Finally, while light-mediated increases in ChBF have been demonstrated in many studies in mammals, light-mediated ChBF increases have not been observed in some studies in rabbits and newborn piglets (Stjernschantz et al., 1977; Stiris et al., 1991), nor were ChBF increases in response to diffuse luminance flicker observed in cats (Riva et al., 1994), and only small, non-statistically significant increases were seen in a study in humans (Garhöfer et al., 2002).

2.3. Functional studies of facial parasympathetic control of ChBF in mammals

The influence of the facial parasympathetic input to choroid on choroidal blood flow has been studied pharmacologically in mammals with drugs that affect or mimic the actions of the peripheral vasodilators used by the PPG (VIP, NO and acetylcholine), and by stimulation of the preganglionic neurons at the level of the facial nerve or the SSN. The latter studies have also examined the role of VIP, NO and acetylcholine in mediating the effects of facial nerve or SSN stimulation on ChBF. In early studies, Ruskell (1970) showed that severing PPG input to the choroid yielded diminished intraocular pressure (IOP). While the basis of this effect was uncertain at that time, Ruskell (1971) suggested that the reduced choroidal volume stemming from reduced ChBF, as caused by loss of basal PPG vasodilatory influences on the choroid, was the cause of the lessened IOP, since the volume of blood in the choroid contributes to IOP. This interpretation is consistent with the slight

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rise in IOP in rabbits, cats and monkeys caused by facial nerve stimulation (Gloster, 1960; Nilsson et al., 1985). It is possible, however, that the PPG input also acts more directly on mechanisms controlling IOP that involve aqueous production or outflow facility. In any case, numerous researchers subsequently carried out detailed studies on the physiology and pharmacology of PPG input to the choroid.

Intravenous delivery of VIP in rabbits has been shown to increase ChBF and IOP (Nilsson and Bill, 1984). Other studies in various mammalian species have shown that NO also plays a role in maintaining basal ChBF (Deussen et al., 1993; Mann et al., 1995; Koss, 1998; Luksch et al., 2000), and that NO mediates the cholinergic neurogenic vasodilation of choroidal vessels (Koss, 1998; Gherezghiher et al., 1991; Deussen et al., 1993; Nilsson, 1996; Steinle et al., 2000). For example, Deussen et al. (1993) showed that inhibition of NOS in dogs with the nonselective inhibitor LNAME (N^G-nitro-L-arginine methyl ester) increases mean systemic arterial blood pressure (ABP) by 20% (thereby increasing choroidal perfusion pressure) and yet decreases ChBF by 40–50%. The systemic hypertensive effect appears to occur due to the peripheral vasoconstrictory effects of eNOS inhibition, while the diminished ChBF seems to reflect the vasoconstrictory effects of nNOS and/or eNOS inhibition in choroid. Similarly, NOS inhibition substantially decreases basal ChBF in humans (Luksch et al., 2000, 2003), rabbits (Seligsohn and Bill, 1993), piglets (Jacot et al., 1998), and rats (O'Brien et al., 1997; Kelly et al., 1998; Koss, 1998).

Two studies in cats using laser Doppler flowmetry (LDF) to measure ChBF showed that intravenous acetylcholine increases ChBF, despite its systemic hypotensive effect that decreases choroidal perfusion pressure (Riva et al., 1994; Mann et al., 1995). Mann et al. (1995) showed that the ChBF increase with intravenous acetylcholine in cats was attenuated 50% by NOS inhibition with LNA (N^G-nitro-L-arginine) despite a 40% increase in blood pressure with LNA. Nilsson (2000) showed in cats that both nonselective NOS inhibition as well as atropine blockade of muscarinic cholinergic control of the choroid reduced ChBF. These results, and the evidence that NOS inhibition with LNAME yields endothelium-dependent vasoconstriction of pig ophthalmic arteries (Wang et al., 1993; Bakken et al., 1995), suggests that at least part of the effect of NO on basal choroidal tone might be mediated by endothelial release of NO in response to acetylcholine release from PPG terminals (Yao et al., 1991). Thus, both acetylcholine and NO release from PPG terminals appear to influence ChBF, the former by stimulating endothelial NO release. These influences can occur both at the level of the choroid as well as at the level of the vessels feeding the choroid. The ophthalmic artery and its short and long ciliary branches supplying the choroid are innervated by VIP+, nNOS+ and cholinergic nerves of PPG origin, and they show NO-mediated relaxation in response to transmural activation of these nerves or to cholinergic stimulation (Nyborg and Nielsen, 1990; Yao et al., 1991; Yu et al., 1992; Haefliger et al., 1993, 2001; Bakken et al., 1995; Toda et al., 1995, 1997, 1998). Acetylcholine-evoked release of endothelial NO from intraorbital vessels, however, may not occur for all such vessels in all species, since NO-mediated relaxation of the intraocular part of the bovine long posterior ciliary arteries (using isolated artery preparations) was found to be driven by neurally derived NO, but not endothelial NO (Wiencke et al., 1994).

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Activation of preganglionic input to the PPG by facial nerve stimulation or by stimulation of the preganglionic neurons within SSN has also been shown to increase ChBF in mammals, and be dependent upon NO release (presumably from PPG terminals on orbital and/or choroidal blood vessels). For example, facial nerve stimulation in rabbits increases ChBF, and also yields a slight IOP rise (Stjernschantz and Bill, 1980). A later study reported that facial nerve activation in cats, rabbits or monkeys yields a ChBF increase that is preventable with ganglionic blockade with hexamethonium (Nilsson et al., 1985). Nilsson (1996) showed that 2 Hz facial nerve stimulation in rabbits increases ChBF, with the increase reduced 50% by nonselective NOS inhibition using LNAME or LNA, both of which inhibit both nNOS and eNOS. A role of some vasodilator in addition to NO was implied by the further finding by Nilsson (1996) that a ChBF increase with a slightly higher facial nerve stimulation frequency (5 Hz) was only minimally suppressed by NOS inhibition. In a subsequent study in cats, Nilsson (2000) similarly reported differential effects of low versus high frequency facial nerve stimulation on ChBF. In this case, ChBF increases with a lower facial nerve stimulation frequency (5 Hz) could be completely blocked by systemic NOS inhibition with LNA, but ChBF increases with a higher stimulation frequency (10 Hz) were reduced but not blocked by LNA alone. The results in rabbits and cats can be interpreted to suggest that the different vasoactive substances released by PPG terminals play roles at different nerve firing frequencies, with vasodilation caused by NO predominant at low frequencies (either endothelially or neurally derived), and with vasodilation caused by VIP predominant at higher frequencies. Studies on control of blood flow and salivary secretion in the submandibular and parotid glands, respectively, have yielded a similar result, showing that low frequency firing of parasympathetic postganglionic fibers may preferentially yield vasodilation and salivation via a muscarinic cholinergic action that stimulates endothelial NO release, while high frequency firing of parasympathetic postganglionic fibers may preferentially yield vasodilation and salivation by VIP release (Lundberg et al., 1982; Fazekas et al., 1987; Mansson and Ekstrom, 1991).

Direct activation of the SSN neurons projecting to those PPG neurons innervating choroid also increases choroidal vessel vasodilation in rats, as measured by transcleral laser Doppler flowmetry (LDF) from anterior sites along the upper surface of the eye (Steinle et al., 2000; Fitzgerald et al., 2002b) and from more posterior sites as well (Fitzgerald et al., 2002b), yielding large blood flow increases above baseline. Since the retinal vasculature is meager at the LDF sites used (Zhang, 1994), the SSN-elicited blood flow increases in rats are mainly attributable to ChBF increases (Koss, 1998; Steinle et al., 2000; Fitzgerald et al., 2002b). Note that Steinle et al. (2000) did not detect ChBF increases in posterior choroid with SSN stimulation, but their approach of slitting the cornea to access the posterior retina may have compromised IOP and thereby compromised choroidal vasodilation in response to preganglionic activation. Intravenous administration of the general NOS inhibitor LNAME or the nNOS-selective inhibitor TRIM (1-(2-Trifluoromethylphenyl)imidazole) both significantly decreased basal ChBF in rats and attenuated the ChBF increases yielded by SSN stimulation (Steinle et al., 2000). The results employing the selective nNOS inhibitor TRIM indicate that the increase in ChBF with SSN stimulation involves NO release from intrachoroidal, as well as possibly from intraorbital, PPG fibers. Given the evidence for acetylcholine-mediated vasodilation of intraorbital vessels, a role for cholinergic endothelial

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NO-mediated vasodilation in the ChBF increases with SSN stimulation cannot be ruled out. In their study in rats, however, Steinle et al. (2000) reported that atropine did not block SSN-elicited choroidal vasodilation in rat. It is uncertain, however, if this was the case because of the stimulation frequencies examined or because of the atropine dose used. In chronically sympathectomized rats, cholinergic PPG mechanisms are clearly revealed, as M3 muscarinic receptor antagonists and atropine block ChBF increases with SSN stimulation (Steinle and Smith, 2000). This result is consistent with the finding that PPG neurons also appear to be cholinergic (Suzuki et al., 1990; Cuthbertson et al., 1997) and muscarinic agonists yield choroidal vasodilation (Gherezghiher et al., 1991; Mann et al., 1995).

Since the projection from NTS to choroidal SSN provides a means by which BP signals can modulate ChBF, we investigated if activation of baroreceptive NTS evokes ChBF increases in rat eye, using LDF to measure ChBF transclerally (Li et al., 2016a). We found that electrical activation of ipsilateral baroresponsive NTS, as defined by the c-fos response to hypotension, and its efferent fiber pathway to choroidal SSN increased mean ChBF by about 40–80% above baseline, depending on current level (Fig. 11). The ChBF responses obtained with stimulation of baroresponsive NTS were driven by increases in both choroidal blood volume (ChBVol) presumably caused by choroidal vasodilation, and choroidal blood velocity (ChBVel) presumably caused by orbital vessel dilation. Stimulation of baroresponsive NTS, by contrast, yielded no significant mean increases in systemic arterial blood pressure that might itself drive a ChBF increase. The increases in ChBF with NTS stimulation were significantly reduced by intravenous administration of the neuronal NO synthase (nNOS) inhibitor Nω-propyl-l-arginine (NPA), thus implicating intrachoroidal and orbital nitrergic PPG terminals in the NTS-elicited ChBF increases. Our results show that NTS neurons projecting to choroidal SSN do mediate increase in ChBF, and they thus suggest a role of baroresponsive NTS in the blood pressure-dependent regulation of ChBF. We performed similar studies to show that activation of PVN or RaM also drives increases in ChBF (Fitzgerald et al., 2012a, 2012b). Studies by others have also shown that baroreceptive NTS also drives cerebral vasodilation (Nakai, 1985; Agassandian et al., 2003).

To determine how low BP signals to NTS from the aortic depressor nerve (ADN), which fires at a low rate during systemic hypotension (Zhang and Mifflin, 2000), could yield increased firing in the NTS output to SSN, we investigated the hypothesis that SSN-projecting NTS neurons receive input from inhibitory ADN-receptive NTS neurons (Scheuer et al., 1996), which as a result inverts the sign of the ADN activity for SSN-projecting NTS neurons (Fitzgerald et al., 2010; Li et al., 2016b). To evaluate this hypothesis (Fig. 12), we determined if SSN-projecting NTS neurons are under prominent inhibitory control. We found that SSN-projecting NTS perikarya and their proximal dendrites identified by retrograde labeling from SSN are densely coated with inhibitory GAD65 + terminals, but only lightly coated with excitatory VGLUT2+ terminals. As NTS is rich in GABAergic neurons (Blessing et al., 1984), this inhibitory input is likely to arise from within NTS. Consistent with basal GABAergic suppression of NTS neurons that drive ChBF increases, we found that injection of the GABA-A receptor antagonist GABAzine into NTS yielded a significant 75% increase in ChBF, as measured using LDF. These results support our hypothesis that low BP signals from the ADN cause vasodilation in choroid by means of ADN input to GABAergic inhibitory NTS neurons that themselves control the activity

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of NTS neurons that have excitatory output to SSN neurons controlling ChBF. Our findings may help explain instances in which sustained high-dose long-term treatment with benzodiazepines, which are GABA-A receptor agonists, has been associated with macular degeneration (see Li et al., 2016b). The benzodiazepines may act by inhibiting the GABA-receptive NTS neurons that project to choroidal SSN, resulting in reduced excitatory drive to SSN and reduced ChBF. Moreover, as cerebral blood flow appears to be controlled by the same NTS-SSN circuit as ChBF (Agassandian et al., 2003), our findings also help explain why high benzodiazepine doses reduce cerebral blood flow and alter its baroregulation, since the same GABA-receptive NTS neurons that regulate ChBF via SSN may also regulate cerebral blood flow by means of this same projection (see Li et al., 2016b).

2.4. ChBF regulation in response to blood pressure fluctuation and the PPG input to choroid in mammals

The issue of ChBF compensation for ocular perfusion pressure changes so as to maintain ChBF near basal levels had been somewhat controversial. The phenomenon whereby blood flow in a vascular bed is maintained at a constant level despite variations in perfusion pressure is typically termed autoregulation, based on the assumption that it is mediated by mechanisms intrinsic to the blood vessels (namely a myogenic response to perfusion pressure). Although some authors also include regulation by local factors such as oxygen or carbon dioxide levels as autoregulation, for purposes of this paper we will refer to such regulation as metabolic regulation and restrict the term autoregulation to local regulation to perfusion pressure changes. Early studies on ChBF reported that it decreased linearly with reductions in choroidal perfusion pressure caused by acute hemorrhage (that lowered blood pressure and thereby perfusion pressure) or by increased IOP (which reduces choroidal perfusion pressure) (Alm and Bill, 1970; Bill, 1984, 1985; Alm, 1992). These observations had, in fact, led to a dogma that while cerebral blood flow and retinal blood flow do autoregulate to maintain stable flow when perfusion pressure is reduced, as demonstrated by numerous studies (Alm and Bill, 1972; Alm, 1992; Paulson et al., 1990), ChBF does not. It became increasingly evident from subsequent studies and is now widely accepted, however, that ChBF does compensate for perfusion pressure changes. For example, some degree of autoregulation with IOP elevation (which causes choroidal perfusion pressure reduction) has been noted in cats (Friedman, 1970; Weiter et al., 1973), rabbits (Chou et al., 2002), and humans (Riva et al., 1997a,b). When ocular perfusion pressure is experimentally reduced by lowering BP rather than by raising IOP, studies in rabbits have shown that stable ChBF over a blood pressure (BP) range of 40–50 mm Hg below basal BP is observed (Kiel and Shepherd, 1992). Kiel and van Heuven (1995) hypothesized that the compensation for BP reduction stemmed from myogenic mechanisms causing vasodilation in response to low vessel transmural pressure. The initial failure to detect ChBF compensation for perfusion pressure changes may have, in part, stemmed from methodological differences. Studies that did not report compensation tended to use the microsphere method, for which each animal typically provides only one data point, thus making it difficult to study dynamic processes such as autoregulation. By contrast, studies that did detect autoregulation have used laser Doppler Flowmetry, which is much better suited for studying dynamic changes in blood flow. More detailed descriptions of various ChBF methods are provided in Schmetterer and Kiel (2012). Kiel (2012) has also suggested that anesthetic may have played a role

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in the failure to detect ChBF compensation for perfusion pressure changes. Moreover, the studies by Kiel show that manipulating choroidal perfusion pressure by changing BP yields better ChBF compensation than does manipulating choroidal perfusion pressure by changing IOP. The prior studies that did not detect autoregulation tended to use IOP to manipulate choroidal perfusion pressure.

The observation that manipulation of BP does not produce an equivalent outcome to manipulation of IOP in terms of the efficacy of autoregulation suggests that more than local autregulatory mechanisms are involved in the compensation for BP change, since if myogenic mechanisms alone were involved in both the compensation would be comparable. Given our anatomical data, it seems likely that neurogenic mechanisms contribute to ChBF regulation to BP fluctuation. Consistent with this, Kiel (1999) noted that both NOS inhibition and ganglionic blockade diminished the ChBF compensation to systemic hypotension in rabbit, implying some involvement of neurogenic vasodilatory mechanisms in the compensation. By contrast, ChBF compensation in piglet to perfusion pressure changes caused by IOP manipulation do not involve NO release, and thus seemingly at least in part involve a different mechanism than is involved in choroidal compensation for blood pressure fluctuations (Jacot et al., 1998). Because of their likely differing mechanisms and their different driving stimuli, we refer to the blood flow compensation for a change in perfusion pressure as caused by a change in systemic BP as baroregulation, and we refer to blood flow compensation for a change in choroidal perfusion pressure as caused by change in IOP as autoregulation. As discussed in more detail below, this terminological distinction is at least in part motivated by the recognition that the strictly local control implied by the term auto-regulation can be misleading when applied to ChBF compensation for BP fluctuation, given the evident involvement of centrally mediated autonomic reflexes in ChBF compensation for BP changes.

In hindsight, it should have always seemed unlikely that ChBF would not show compensation for reduced ocular perfusion pressure, given the potentially adverse consequences of either supranormal ChBF or subnormal ChBF on short term and long term retinal health and function. Without baroregulation, high BP, for example, would yield an ocular perfusion pressure resulting in excessively high ChBF, causing fluid accumulation in retina and abnormal exchange of wastes and nutrients between retina and choroid (Bill and Sperber, 1990; Kiel, 1994). Similarly, without baroregulation, low BP would yield an ocular perfusion pressure yielding low ChBF, causing retinal hypoxia and impaired retinal function (Steinberg, 1987; Yancey and Linsenmeier, 1988, 1989; Lovasik et al., 2005a; Lovasik and Kergoat, 2012).

Given the input of hypothalamic and solitary nucleus blood pressure- sensitive sites to the choroidal neurons of the SSN (Hosoya et al., 1984; Spencer et al., 1990; Ito and Seki, 1998; Cuthbertson et al., 2003; Li et al., 2015a), at least part of the choroidal compensation to BP declines is likely to be mediated by the SSN-PPG circuit. Consistent with this possibility, prior studies have suggested that at least part of the vasodilatory compensation of cerebral blood vessels for declines in BP may be mediated by the PPG (Gotoh and Tanaka, 1988; Morita et al., 1995). Interestingly, systemic hypotension does not activate sympathetic input to the choroid, but does cause peripheral vasoconstriction to mitigate the systemic

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hypotension (Bill and Nilsson, 1985). Thus, the eye (like the brain) is a privileged tissue during systemic hypotension. Nonetheless, Linder (1981) found that although facial nerve stimulation increased ChBF in rabbits with systemic hypotension, facial nerve section did not reduce ChBF in rabbits with either systemic hypotension or in normotensive rabbits. These results seem inconsistent with the vasodilatory influence of the SSN-PPG circuit on the choroid and a role for it in ChBF compensation for low systemic blood pressure, at least in rabbits. Given the anatomical evidence for inputs to the SSN from BP-sensitive brain sites, however, we believed further studies were needed to assess the contribution of the SSN-PPG circuit in mammals to ChBF baroregulation.

To this end, we investigated the role of neuronally derived NO in ChBF baroregulation in rats (Reiner et al., 2010a). ChBF was continuously monitored using LDF in anesthetized rats, and arterial BP (ABP) measured via the femoral artery. At multiple intervals over a 2–4 h period during which ABP varied freely, ChBF and ABP were sampled and the results compiled across rats. We found that ChBF remained near baseline over an ABP range from 40 mm Hg above basal ABP (i.e. 90–100 mm Hg) to 40 mm Hg below basal ABP, but largely followed ABP linearly below 60 mm Hg (Fig. 13). Choroidal vascular resistance increased linearly as ABP increased above 100 mm Hg (indicating active vasoconstriction), and decreased linearly as ABP declined from basal to 60 mm Hg (indicating active vasodilation), but resistance declined no further below 60 mm Hg (reflecting a vasodilation limit). Inhibition of nitric oxide (NO) formation using either a selective inhibitor of nNOS (NPA) or a nonselective inhibitor of both nNOS and eNOS (LNAME) did not affect compensation above 100 mm Hg ABP, but did cause ChBF to linearly follow declines in BP below 90 mm Hg (Fig. 13). In NOS-inhibited rats, vascular resistance increased linearly with BP above 100 mm Hg (indicating intact vasoconstriction to mediate baroregulation during high ABP), but remained at baseline below 90 mm Hg (indicating a failure of vasodilation). These findings reveal that ChBF in rats, as in rabbits, humans and pigeons, compensates for rises and/or declines in arterial blood pressure so as to remain relatively stable within a physiological range of ABPs. The ChBF compensation for low ABP in rats is dependent on choroidal vasodilation caused by neuronally derived NO formation but not the compensation for elevated BP, implicating parasympathetic nervous system vasodilation in the ChBF compensation to low ABP.

Note that ChBF does not appear to regulate (decrease) to high oxygen levels in adult mammals, but does regulate (increase) in response to high CO2 (hypercapnia). For example, ChBF is unaltered in response to breathing 100% oxygen in humans (Riva et al., 1994; Kergoat and Faucher, 1999; Geiser et al., 2000), but is increased by breathing carbogen (95% O2–5% CO2) (Geiser et al., 2000). Similarly, hypercapnia increases ChBF in cats, sheep, baboons, and newborn piglets (Alm and Bill, 1972; Wilson et al., 1977; Milley et al., 1984; Stiris et al., 1991). High CO2 is known to increase cerebral blood flow as well (Wilson et al., 1977). The mechanism of the increased ChBF with hypercapnia is uncertain. Schmetterer et al. (1997) reported that NO is involved in hypercapnia-mediated increases in blood flow in the human ophthalmic artery, raising the possibility that the same is true for the choroid. Cyclooxygenase products do not appear to mediate the hypercapnic ChBF increase in newborn piglets (Stiris et al., 1992). Whether vasodilatory PPG input plays a role in hypercapnic ChBF increases is unknown. It is possible SSN neurons controlling ChBF

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receive input from NTS neurons that themselves receive input from chemoreceptive carotid and/or aortic body cells.

2.5. Retinal function and the PPG input to choroid in mammals

We investigated if impairment of adaptive control of ChBF by the SSN-PPG circuit was damaging to the retina (Reiner et al., 2010b). The right SSN was stereotaxically lesioned in male rats, and histological analysis of the retina carried out 8–10 weeks later. Prior to sacrifice, full field scotopic flash ERGs were measured for both eyes at 2, 4 and/or 7–10 weeks after the SSN lesion. ChBF evaluation was performed in some of these rats prior to sacrifice, which confirmed that they were defective in ChBF baroregulation during low systemic BP. Our ERG data showed that destruction of all or most of right choroidal SSN (SSN-Lx) significantly diminished the right eye ERG a-wave and b-wave amplitudes by 8–10 weeks after ipsilateral SSN destruction, compared to sham lesions or lesions that missed right choroidal SSN (SSN-miss) (Fig. 14). Left eye b-wave abnormalities were also seen in the SSN-Lx rats. The right eye a-wave and b-wave deficits grew progressively worse from 2 to 8 weeks. In the histological analysis, GFAP (glial fibrillary acidic protein) immunolabeling was analyzed blinded using a scoring system for the extent of GFAP labeling in the Müller cells and the abundance of labeled Müller cells that we described previously (Kimble et al., 2006). Seven to ten weeks after destruction of choroidal SSN (n = 12), GFAP was significantly upregulated in retinal Müller cells throughout the ipsilateral retina in SSN-Lx rats (Fig. 14) to double that in controls (n = 11), as well as slightly upregulated in contralateral retina as well. By contrast, in control cases, which include cases with no lesions and cases without choroidal SSN damage, GFAP immunolabeling of Müller cells was mainly limited to their vitreal endfeet. Our findings indicate that impairment of the adaptive control of ChBF by the facial parasympathetic input to the choroid adversely affects retinal health. Thus, the underperfusion of the choroid during low BP that occurs with dysfunction of the SSN-PPG circuit appears to harm the retina. Preliminary studies also indicate an increase in choroidal macrophages in eyes ipsilateral to SSN destruction, as detected by IBA1 immunolabeling, suggesting early signs of an outer retinal inflammatory process as well.

In studies published in abstract form, we also investigated the long-term impact of impaired NO-mediated parasympathetic control of choroidal blood vessels on retinal health and visual function (Li et al., 2017). To this end, we studied young and aged mice lacking one or two nNOS alleles (Huang et al., 1993). Using transcleral LDF, we found that basal ChBF was ~60% of WT in both nNOS +/− and nNOS−/− mice at 3 months of age, but baroregulation appeared to be largely intact (perhaps due to compensation by VIP or cholinergic mechanisms). For these studies, blood pressure was sampled using a tail cuff, and concurrent ChBF tabulated. Visual acuity (as assessed using Optomotry), and thickness of retinal layers and photoreceptor abundance (as assessed in 1-μm thick toluidine blue-stained sections of plastic embedded retinas) did not differ between wild-type (WT) and mutant mice at 3 months of age. By contrast, visual acuity was only 60% of age-matched WT in nNOS +/− mice at 200–500 days of age. Surprisingly, visual acuity in nNOS−/− mice at 200–500 days of age was only reduced to ~85% of WT, which was nonetheless statistically significant. Similarly, a significant decline in photoreceptor

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abundance and retinal ganglion cell layer thickness was seen between 200 and 500 days of age in nNOS +/− mice, but not in nNOS−/− mice. The basis of the greater visual deficit and accelerated age-related ganglion cell and photoreceptor pathology in nNOS +/− mice is uncertain but may relate to the more greatly diminished nitrosylative oxidative damage attending the nullizygous nNOS state (Martínez-Lazcano et al., 2007). Thus, parasympathetic choroidal vasodilation appears important for long-term outer retinal health, and NO-mediated vasodilation is an important contributor to this. Further studies are needed to determine if baroregulation fails prematurely in older nNOS-deficient mice, and determine why hemizygous nNOS-KO should be more deleterious than homozygous nNOS-KO.

2.6. Peripheral anatomy of facial circuitry for control of ChBF in birds

The pigeon PPG consists of an interconnected series of three-four microganglia of about 50–200 neurons each and numerous lesser microganglia (Fig. 4) (Cuthbertson et al., 1997). The main microganglia of the PPG network in pigeons lie along the superior aspect of the Harderian gland. Neurons of all of these microganglia are extremely rich in VIP and nNOS, and moderate in choline acetyltransferase (ChAT, the enzyme responsible for acetylcholine synthesis), with the majority co-containing VIP and nNOS. In pigeons and chickens, the PPG has been shown to innervate choroidal vessels, as well as orbital vessels supplying the choroid (Cuthbertson et al., 1997; Fischer and Stell, 1999). Axons containing VIP and nNOS extend from the PPG network to perivascular fiber plexi on orbital blood vessels (Cuthbertson et al., 1997). These orbital vessels, many of which enter the choroid posteriorly and nasally, are a conduit by which PPG postganglionic fibers reach the choroid. Within the choroid, VIP+ and nNOS + fibers are widely scattered but sparse, and most abundant in nasal choroid. These results suggest that PPG neurons in birds use VIP and NO, and also possibly acetylcholine, to exert vasodilatory control over blood flow to and within the avian choroid, as also is the case in mammals. Additionally, a few VIP+ and nNOS + neurons have been also observed in the choroid of pigeons and chickens. In some avian groups, however, such as ducks, many more intrinsic choroidal neurons co-containing VIP and nNOS have been reported (Bergua et al., 1996; Schrödl et al., 2001a,b; 2004), as described in more detail in the later section on intrinsic choroidal neurons.

2.7. Central anatomy of facial circuitry for control of ChBF in birds

Several studies have suggested that the preganglionic neurons innervating the PPG in birds reside in the superior salivatory nucleus, in a similar brainstem location as in mammals (Ganchrow et al., 1987; Medina and Reiner, 1994; Schrödl et al., 2006). Among these, Schrödl et al. (2006) carried out a detailed anatomical study on the localization of the avian SSN. ChAT + neurons in brainstem were retrogradely labeled via the radix autonomica of the facial nerve, which conveys preganglionic axons from the SSN to the PPG. The SSN neurons were located dorsolateral to somatic facial motoneurons, as they are in mammals. As in mammals, the SSN region receives input from the nucleus of the solitary tract (Arends et al., 1988), the parabrachial region (Wild et al., 1990), and the SSN (Korf, 1984). Also as in mammals, the parabrachial region receives input from the nucleus of the solitary tract, which receives baroreceptive input (Katz and Karten, 1979; Berk et al., 1993).

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Thus, the avian SSN-PPG circuit, like that in mammals, may be responsive to baroreceptor information and regulate ChBF as a function of systemic BP.

2.8. Functional studies of facial parasympathetic control of ChBF in bird

We have used transcleral LDF to measure ChBF in pigeons while systematically electrically stimulating brainstem in the vicinity of the facial motor nucleus (Reiner et al., 2012), focusing on the region of small cholinergic neurons comprising the SSN in chickens that have been shown to project to the PPG (Ganchrow et al., 1987; Schrödl et al., 2006). We found this region of the SSN of birds was effective for eliciting ChBF increases (100% or more) without significant concomitant BP increases. The NOS inhibitor 7-nitroindazole (7NI) greatly attenuated (about 50%) the ChBF increases that could be elicited from this region, consistent with an involvement of PPG NOS + neurons in the increases. Note that although 7NI has widely been referred to as a selective nNOS inhibitor, our own studies show that 7NI does induce a clear pressor effect in rats, that this pressor effect is peripherally mediated, and that 7NI does inhibit endothelium-dependent cholinergic vasodilation, all suggesting that the 7NI does inhibit eNOS (Zagvazdin et al., 1996b), and is thus not nNOS selective (Alderton et al., 2001; Ayajiki et al., 2001).

2.9. Choroidal baroregulation and the PPG input to choroid in birds

As part of our interest in the significance of the facial parasympathetic control of ChBF, we investigated if ChBF in pigeons showed baroregulation (i.e. compensation for perfusion pressure changes caused by BP changes so as to maintain ChBF near basal levels). In one line of study (Reiner et al., 2003), we determined whether ChBF in pigeons compensates for an acute decrease in arterial BP and remains stable. ChBF was measured using transcleral LDF in anesthetized pigeons, and a stable decrease in arterial BP was produced by blood withdrawal from the brachial artery. During the withdrawal itself, ABP decreased rapidly, but the ChBF decline was typically not as great as the arterial BP decline and showed recovery once ABP had stabilized at its lower level. In the case of blood withdrawal that did not cause ABP to go below 40 mm Hg, the ChBF decline at one minute after blood withdrawal was only about 50% of the ABP decline. When the ABP declined to a level below 40 mm Hg, the magnitude of the ChBF was proportional to ABP decline. These results confirmed for pigeons that ChBF does significantly compensate for ABP decline over a certain range. We further found that NOS inhibition with LNAME eliminated ChBF baroregulation to an ABP drop induced by blood withdrawal, suggesting a role of NO-mediated mechanisms in the process (Reiner et al., 2003). In a second set of studies (Reiner et al., 2003, 2011), we determined if ChBF baroregulation occurred during spontaneous ABP fluctuation. We found that significant ChBF baroregulation does occur in pigeons when the ABP deviates +30 or −40 from basal ABP (Fig. 15). Our overall results for the SNN-PPG circuit of birds thus indicate that while intrachoroidal PPG fibers may be fewer in at least some avian species than commonly the case in mammals, the central circuitry and role of the PPG input to orbital and choroidal blood vessels in ChBF baroregulation appears similar to that in mammals. Such ChBF baroregulation would prevent underperfusion-related ischemia during low ABP or overperfusion-related edema and impaired fluid-tissue exchange during high ABP in the outer retina (Johnson, 1980; Bill and Sperber, 1990; Kiel, 1994), and thus seemingly be an important ocular homeostatic

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mechanism. For the cerebral vasculature, in which baroregulation is also evident, both an intrinsic vascular smooth muscle myogenic mechanism (which acts to maintain vessel wall stretch within a preferred range) and a PPG-mediated neurogenic mechanism have been proposed to contribute to baroregulation during low systemic BP (Ishitsuka et al., 1986; Gotoh and Tanaka, 1988; Paulson et al., 1990). In addition to a choroidal neurogenic mechanism we have described above, myogenic mechanisms have also been proposed to play a role in choroidal baroregulation (Kiel and Shepherd, 1992; Kiel, 1994).

3. Oculomotor nucleus parasympathetic input to choroid

3.1. Anatomical evidence for and against ciliary ganglion input to choroid in mammals

Birds also possess a parasympathetic input to the choroid via the ciliary ganglion, which receives its preganglionic input from the nucleus of Edinger-Westphal (EW) of the oculomotor nuclear complex (Fig. 16) (Reiner et al., 1983). While avian choroid receives both ciliary ganglion and PPG input, the ciliary ganglion input is the more prominent parasympathetic input. In mammals, the PPG typically gives rise to a massive choroidal innervation, but it has remained uncertain if the ciliary ganglion also innervates the choroid. Various lines of evidence suggest it does, but none of the evidence is definitive.

The ciliary ganglion innervates the muscles of accommodation and the sphincter muscle of the iris in mammals and birds. The ciliary ganglion postganglionic nerves to these structures travel via the short ciliary nerves, which penetrate the posterior globe, and course to the front of the eye, to reach these intraocular muscle groups. In birds, two distinct neuron types are present in the ciliary ganglion, a smaller one innervating choroid called choroidal neurons and a larger one innervating the muscles of accommodation and the iris sphincter muscle called ciliary neurons (Fig. 16) (Reiner et al., 1983, 1991; Meriney and Pilar, 1987). Moreover, preganglionic EWin birds consists of two clear cytoarchitectonic subdivisions, the medial of which projects to the choroidal neurons of the ciliary ganglion and the lateral of which projects to the ciliary neurons of the ciliary ganglion (Reiner et al., 1983; Reiner et al., 1991). In mammals, no such distinctly different neuron types have been reported in the ciliary ganglion, and no distinct subdivision of EW that does not subserve pupil constriction and accommodation and thus might potentially subserve choroidal blood flow regulation has been identified (Gamlin, 2000; May et al., 2008). May (1997), nonetheless, noted numerous references in the literature that the short ciliary nerves that arise from the ciliary ganglion have branches to choroidal blood vessels. The ciliary ganglion neurons in birds and mammals are cholinergic, and Imai (1977) identified cholinergic nerves by acetylcholinesterase staining in albino rats and rabbits that enter the choroid via the short ciliary nerves and ramify on choroidal blood vessels. Imai noted a similar result in pigeons, and in pigmented rabbits and rats, in which the nerves were more difficult to discern due to the choroidal pigmentation. Imai (1977) also noted cholinergic nerves in monkey choroid. Using biochemical methods, Mindel and Mittag (1976) confirmed the presence of ChAT in the choroid of rabbits, cows and humans, consistent with the presence of cholinergic nerves in choroid. In all of these instances of possible ciliary ganglion input to choroidal blood vessels via the short ciliary nerves in mammals, however, an interpretation difficulty is posed by the fact that some PPG postganglionic branches join and travel with the short

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ciliary nerves, and others travel through the ciliary ganglion to emerge in the short ciliary nerves (Ruskell, 1970; Kuwayama et al., 1987; Lin et al., 1988; Ten Tusscher et al., 1990). Thus, the cholinergic fibers in the short ciliary nerves described in some or all of these prior studies in mammals might be of PPG origin.

Additional apparent support for ciliary ganglion control of choroid in mammals involves the evidence for an input from SCN to EW in mammals (Pickard et al., 2002). This input resembles that from SCN to EW in birds (see below) and could thus be part of an EW circuit mediating control of choroidal blood flow. If the facial parasympathetic input to choroid mainly mediates systemic BP-dependent control of ChBF, an additional circuit would in principle be needed for retinal activity-dependent control of ChBF. The SCN input to a putative ChBF-control part of EW would provide such a mechanism, and as discussed below there is evidence for both EW and ciliary ganglion involvement in choroidal vasodilation in mammals. Note, however, in our own PRV studies of central circuitry controlling ChBF, we did not see any PRV + labeled neurons in SCN following minute injections of PRV restricted to the choroid of the superior quadrant of the eye (Li et al., 2015a). Thus, although SCN may project to EW in mammals, we found no evidence in rats that those EW neurons receiving a putative SCN input also project to ciliary ganglion neurons projecting to choroid. If such EW neurons existed and they received SCN input, then PRV would have transneuronally labeled them from the choroid via the ciliary ganglion, and yielded transneuronal retrograde labeling of their afferent SCN neurons as well. Note that the EW and ciliary ganglion vary widely among mammals, being poorly developed and cell sparse in rodents and rabbits, but much better developed in primates. It may be that an EW-ciliary ganglion-choroid circuit is absent in nocturnal small-eyed mammals without fovea such as rodents, but present in diurnal foveate species such as many primates (including humans). Studies to determine if EW in any primates contains separate regions responsible for pupil constriction, accommodation, and ChBF control are needed. Although pupil and accommodation control neurons have been demonstrated in EW in macaques, whether they reside in separate parts of EW is uncertain, and whether there is a separate part of EW that mediates neither is uncertain (McDougal and Gamlin, 2015).

3.2. Functional evidence for an EW – ciliary ganglion circuit to choroid in mammals

As noted above in the section on the PPG, there is evidence that ChBF can be increased by muscarinic cholinergic activation. Additionally, Alm et al. (1973) showed that preventing acetylcholine breakdown by corneal administration of the cholinesterase inhibitor neostigmine or ocular muscarinic activation by corneal administration of the agonist pilocarpine causes a slight increase in ChBF, despite the distance from the choroid. These ChBF increases mediated by cholinergic drugs might occur by their action on choroidal vessels post-synaptic to cholinergic ciliary ganglion input. Consistent with this possibility, Stjernschantz et al. (1976) showed that intracranial stimulation of the oculomotor nerve in rabbits increased blood flow in the choroid. These authors were surprised by this effect, and speculated that perhaps their stimulation had inadvertently activated the nearby trigeminal nerve. Bill et al. (1976) showed, however, that ganglionic blockade with hexamethonium or peripheral muscarinic blockade with biperiden in rabbits slightly reduced the vasodilatory effect on the choroid of intracranial stimulation of the oculomotor nerve,

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implicating a ciliary ganglion role and peripheral cholinergic mechanisms in the ChBF increase. Hexamethonium also reduced basal ChBF. In a later study, however, Stjernschantz and Bill (1979) reported that intracranial stimulation of the oculomotor nerve failed to yield a ChBF increase in rabbits, cats or monkeys. It was uncertain why the ChBF increase in rabbits was no longer evident. Similarly, Bill (1962) directly stimulated the ciliary ganglion input to the choroid in rabbits and cats, and collected ocular blood efflux to measure flow. He reported no increase in ChBF with ciliary ganglion activation, but method sensitivity might be an issue. Gherezghiher et al. (1990) reported that oculomotor nerve stimulation in cats increased IOP (they did not measure ChBF). This effect could be blocked by hexamethonium and they suggested the IOP increase might have been due to an increase in choroidal volume due to vasodilation. Consistent with these results, ciliary ganglion removal causes an IOP drop in cats and monkeys (Colasanti and Powell, 1985; Erickson-Lamy and Kaufman, 1988). Stjernschantz (1976), however, reported that stimulation of the intracranial part of the oculomotor nerve in rabbits had minimal impact on IOP, and in fact slightly decreased it. Nakanome et al. (1995) found that upon short ciliary nerve stimulation in cats at a point just before the eye and distal to the ciliary ganglion, capsaicin-sensitive increases in ChBF were obtained. The capsaicin-sensitivity indicates that the ChBF increase was largely mediated by trigeminal sensory fibers that had joined the short ciliary nerves just prior to scleral penetration. By contrast, stimulation of the short ciliary nerves just after their emergence from the ciliary ganglion yielded a prominent capsaicin-insensitive vasodilation in the choroid that was parasympathetic in origin (sensory fibers being absent at this point). While this is again consistent with a ciliary ganglion innervation of the choroid, the possibility remains that it was PPG fibers traveling with the short ciliary nerves that were responsible for the evoked choroidal vasodilation. Thus, none of the lines of functional evidence for a ciliary ganglion influence on ChBF in mammals is unambiguous, and it remains uncertain if there is EW-ciliary ganglion control of ChBF in mammals. As noted above, it would be useful to determine if EW in any primates contains separate regions responsible for pupil constriction, accommodation, and ChBF control.

3.3. Anatomy of ciliary ganglion circuit to choroid in birds

In birds, we have identified the major central components of a circuit regulating ChBF via the ciliary ganglion (Gamlin et al., 1982; Reiner et al., 1983, 1991; Cuthbertson et al., 1996, 1999). The central components of this circuit are (arbitrarily beginning with the right eye): the right retina - the left suprachiasmatic nucleus (SCN) - the right (and to a lesser extent the left) medial subdivision of the nucleus of Edinger-Westphal (EWM) (Fig. 16). Neurons of EWM, in turn, innervate those neurons of the ipsilateral ciliary ganglion that give rise to an extensive cholinergic innervation of ipsilateral choroidal blood vessels. By contrast, neurons of lateral EW (EWL) innervate the ciliary neurons of the ciliary ganglion, some of which innervate the iris sphincter muscle and the rest of which innervate the muscles of accommodation. Those ciliary ganglion neurons controlling the iris receive their input from caudolateral EWL, which in turn receives its input from a retinorecipient pretectal cell group, called area pretectalis in birds, of the contralateral side of the brain (Reiner et al., 1983; Gamlin et al., 1984). Area pretectalis receives mainly contralateral retinal input. The more rostromedial part of EWL controls accommodation and receives input from the reticular midbrain, which appears to receive input from the arcopallium, a motor area of

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the telencephalon (Reiner et al., 1983; Gamlin and Reiner, 1991). Stimulation of the pigeon arcopallium yields accommodation (Reiner et al., 1983). Note that our efforts to delineate the neurochemistry, connectivity and function of the different parts of pigeon EW were aided to no small extent by the large and well-defined nature of this cell group, and the cytoarchitectonic distinctness of its parts. By contrast, in many mammals preganglionic EW is small and illdefined, although it is somewhat larger and more distinct in primates (May et al., 2008; Kozicz et al., 2011).

Based on recent analysis of the hypothalamic circadian control system in birds, Cantwell and Cassone (2006a,b) have suggested that the mammalian SCN is a composite of two hypothalamic regions in birds. A medial region in birds termed the medial SCN (mSCN) appears to be responsible for generating circadian rhythms but it receives only meager retinal input, while the more lateral region projecting to EWM was termed the visual SCN (vSCN) by them and receives substantial retinal input (Cantwell and Cassone, 2006a,b). These two SCN regions are interconnected, and together they have the neurochemistry, connections and function of the mammalian SCN. For these reasons, we will henceforth term the part of avian SCN controlling ChBF the vSCN. Given that melanopsin-expressing retinal ganglion cells in birds drive the pupil light reflex, as they do in mammals, presumably by a projection to the area pretectalis (Verra et al., 2011; Valdez et al., 2015; Diaz et al., 2016), it seems likely that the visual drive to vSCN in birds (like that to mammalian SCN) also derives from intrinsically photosensitive melanopsin-expressing retinal ganglion cells. The anatomy of the vSCN-EWM-Ciliary Ganglion circuit clearly suggests that it might be involved in the light-regulated control of ChBF, and in studies described below we have shown this is the case.

Neurons of EW are cholinergic and possess AMPA type glutamate receptors, implying the inputs from SCN, AP and reticular formation are in large part glutamatergic (Meriney and Pilar, 1987; Reiner et al., 1991; Toledo et al., 2002). Choroidal neurons release acetylcholine (Meriney and Pilar, 1987), and cholinergic fibers from the ciliary ganglion are widespread in avian choroid (Cuthbertson et al., 1996). In pigeons, they are most abundant in the superior and temporal ocular quadrants (Cuthbertson et al., 1996). We found that nNOS is present in only a small fraction of ciliary neurons, and is absent from choroidal neurons (Cuthbertson et al., 1999), and thus not a source of nNOS + fibers in avian choroid. Consistent with the cholinergic projection of the ciliary ganglion to the eye (Imai, 1977; Meriney and Pilar, 1987; Reiner et al., 1991), Fischer et al. (1998) found that M2, M3 and M4 receptors are present in chick choroid and ciliary body by Western blots and immunolabeling.

3.4. Function of vSCN-EWM-ciliary ganglion circuit in birds

Our functional studies confirm the anatomical implication that the vSCN-EWM circuit participates in the light-regulated control of ChBF (Reiner et al., 1983). For example, we showed that electrical stimulation of EWM increases ChBF in the ipsilateral eye, as measured by LDF (Fitzgerald et al., 1990b). In Fitzgerald et al. (1996), we also showed that activation by electrical stimulation of what we now call vSCN yields increases in ChBF in the opposite eye, while retinal illumination yields ChBF increases in the illuminated eye, as would be predicted by the doubly crossed layout of the vSCN-EWM circuit. Furthermore,

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the vSCN-elicited and light-elicited increases could be blocked reversibly by lidocaine injection into the EWM ipsilateral to the recorded eye. Control studies confirmed that the light-elicited increases were not artifactually generated by transocular illumination of the LDF probe. In a study on chicks, we showed that severing the ciliary nerves permanently dilates the pupil and causes increased ChBF (Shih et al., 1993a). We interpreted the ChBF increase to stem from the increased illumination falling on the retina due to the chronically dilated pupil. An opaque occluder that diminished light entry in chick eyes with severed ciliary nerves eliminated the ChBF increase (Shih et al., 1994). These results indicate that the vSCN-EWM-Ciliary Ganglion circuit regulates ChBF based on the pattern and/or intensity of retinal illumination (Fitzgerald et al., 1996), and perhaps flicker may as well (Shih et al., 1997). Such reflexive responses to increased retinal illumination or increased complexity of the patterns imaged on the retina may be adaptive, since such retinal activation alters the metabolic and/or thermal demands on the retina (Linsenmeier, 1986; Steinberg, 1987; Bill and Sperber, 1990).

We also found that the non-selective NOS inhibitors 7NI and LNAME each attenuate the EWM-evoked ChBF increase by about 80%. In light of our findings that choroidal neurons do not contain NOS or make NO (Cuthbertson et al., 1999), these results indicate that it is endothelially derived NO that must mediate EWM-elicited choroidal vasodilation. Since choroidal neurons of the avian ciliary ganglion do release acetylcholine (Meriney and Pilar, 1987), and since acetylcholine is known to stimulate endothelial NO release (Moncada et al., 1991), we studied the role of muscarinic cholinergic mechanisms in ciliary ganglion-mediated ChBF increases in pigeon (Zagvazdin et al., 2000). Using LDF and atropine as well as selective blockers of the M3-type muscarinic receptor (4-diphenyl-acetoxy-N-methylpiperedine, 4DAMP) and the M2-type muscarinic receptor (himbacine), we found that atropine and M3-type muscarinic receptor blockade greatly (by about 90%) inhibited EWM-evoked increases in ChBF, while M2-type receptor inhibition increased ChBF by about 100%. Based on our findings that the ciliary ganglion input to choroid does not synthesize NO but inhibitors of NO production do block EWM-evoked choroidal vasodilation, it seems likely that the M3 receptors acted on by 4DAMP are present on choroidal endothelial cells, and the ciliary ganglion thereby mediates choroidal vasodilation via M3 cholinergic stimulation of endothelial NO release. In contrast, M2 muscarinic receptors may play a presynaptic role in downregulating EWM-evoked parasympathetic cholinergic vasodilation in avian choroid. Inhibiting M2 muscarinic receptors thus would be a means to potentiate ChBF. Our finding that EW lesions significantly diminish ChBF in pigeons indicates that basal activity in the preganglionic input to the ciliary ganglion is needed to maintain basal choroidal tone (Fitzgerald et al., 1996), and our finding that severing the choroidal nerves from the choroidal neurons of the ciliary ganglion to the choroid in chickens vastly diminishes ChBF supports the view that ciliary ganglion input prominently controls basal choroidal tone, as well as mediates evoked increases (Shih et al., 1993b).

3.5. Retinal function and ChBF control by the vSCN-EWM-ciliary ganglion circuit in birds

While large reductions in ChBF lead to severe photoreceptor loss (Gay et al., 1964; McCulloch, 1988), even slight reductions in ChBF hinder the ability of oxygen and nutrients

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to reach the outer retina, and such ChBF reductions thereby have rapid and deleterious functional consequences for the outer retina (Steinberg, 1987; Yancey and Linsenmeier, 1988, 1989; Lovasik et al., 2005a; Lovasik and Kergoat, 2012). ChBF is also vital for supporting the inner retina in those retinal regions or species poor in retinal vessels (Bill, 1984; Kiel and Shepherd, 1992). It seems likely then that adaptive neural regulation of ChBF is important for the long-term health of the retina. We have specifically addressed this issue in studies on the avian vSCN-EWM-Ciliary Ganglion system in which we destroyed EWM. Such lesions reduce basal ChBF to 50–75% of normal in the ipsilateral eye, and block adaptive ChBF regulation by the SCN-EWMCiliary Ganglion circuit (e.g. light-mediated ChBF increases) (Fitzgerald et al., 1996). In pigeon eyes affected by EWM lesions, we have found evidence of retinal functional disturbance and pathology, including: 1) increased glial fibrillary acidic protein (GFAP) in retinal Müller cells (Fitzgerald et al., 1990a; Kimble et al., 2006); 2) losses in behaviorally assessed visual acuity (Hodos et al., 1998); and photoreceptor loss (Reiner et al., 2016). In the former studies, we examined the effects of EWM lesions on retinal GFAP immunolabeling in birds housed under normal circadian lighting conditions (Kimble et al., 2006). We found that the GFAP increases in Müller cells following EWM destruction are progressive up to 24 weeks, and occur preferentially in superior/temporal retina, which is heavily innervated by the ciliary ganglion in pigeons (Fig. 17). After 24 months, GFAP expression begins to diminish, but the GFAP upregulation is still evident one year after the lesion. In behavioral studies, we examined the effects of EWM lesions on visual acuity in pigeons (Hodos et al., 1998). Bilateral lesions of EWM were made electrolytically, and visual acuity for high contrast, square-wave gratings was determined behaviorally about one year later and compared to that in a group of pigeons that had received sham lesions of EW about one year prior to acuity testing. Because lesions targeting EWM invariably result in damage to the adjoining EWL, two additional control groups were studied. In one control group, bilateral lesions in area pretectalis (AP), which innervates the pupillary control part of EWL and thereby controls pupillary constriction (Reiner et al., 1983), were made and the effects on visual acuity determined about one year later. In the second additional control group, the effects of acute accommodative and pupillary dysfunction on acuity were studied in cyclopleged pigeons. The mean acuities of birds with AP lesions (9.1 ± 1.4 cycles/degree) and sham lesions (7.1 ± 1.5 cycles/degree) were not significantly different from normal. In contrast, pigeons with lesions that completely destroyed EW bilaterally showed visual acuity (2.7 ± 0.1 cycles/degree) that was well below the acuity of the sham and AP-lesion control groups. The acuity of the cyclopleged pigeons (4.8 ± 0.3 cycles/degree) and one pigeon with a nearly complete bilateral EWL but a unilateral EWM lesion (6.4 cycles/degree) indicated that only about half of the loss with a bilateral EW lesion could be attributed to accommodative dysfunction. Thus, bilateral destruction of EWM led to a loss in visual acuity, suggesting that disruption of adaptive neural regulation of ChBF causes retinal injury that impairs vision.

More recently, we characterized the effect of loss of EWM-mediated ChBF regulation on photoreceptor health in pigeons housed in either moderate intensity diurnal or constant light (Reiner et al., 2016). Photoreceptor abundance following complete EWM destruction was compared to that following a lesion in the pupil control circuit (as a control for spread

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of EWM lesions to the nearby pupil-controlling lateral EW) or following no EW damage. Birds were housed post lesion in a 12 h 400 lux light/12 h dark light cycle for up to 16.5 months, or in constant 400 lux light for up to 3 weeks. Disruption of pupil control had no adverse effect on photoreceptor outer segment abundance in either diurnal light or constant light, but EWM destruction led to 50–60% loss of blue/violet cone outer segments in both light conditions, and a 42% loss of principal cone outer segments in constant light. The findings indicate that adaptive parasympathetic regulation of ChBF in birds plays a role in maintaining photoreceptor health, and mitigates the harmful effect of light on photoreceptors, especially in the case of short wavelength-sensitive cone photoreceptors.

Our studies in birds, therefore, show that interrupting parasympathetic neural control of ChBF by the vSCN-EWM-Ciliary Ganglion circuit is harmful for the retina. It seems likely therefore that retinopathy would also ensue from central or peripheral damage to parasympathetic facial, sensory or sympathetic circuits controlling ChBF (Potts, 1966). The precise nature of the retinal injury and the circumstances under which the impaired neural control of ChBF might be especially harmful, however, are uncertain and would depend on the precise role that the damaged circuit plays in supporting ocular health and on the ongoing presence of exteroceptive or interoceptive signals that might engage those circuits. It is possible that the retinal impairments observed with EWM lesions that disable parasympathetic control of ChBF stem from ChBF insufficiency that renders the retina chronically hypoxic and ischemic (Yancey and Linsenmeier, 1988, 1989; Fitzgerald et al., 1990a). Impaired parasympathetic control of ChBF may also result in harmful accumulation of waste products in the outer retina or an inadequate nutrient supply for outer retina renewal (e.g. amino acids, sugars and fats) (Herron et al., 1969; LaVail, 1981). Consistent with this, an increase in lipid granules was seen by us in the RPE of EWM eyes by light microscopy (LM) and electron microscopy (EM) beginning already by 4 weeks postlesion. Regardless of the basis of the retinal damage that occurs with disturbed ciliary ganglion-mediated control of ChBF in birds, it is likely that these same potentially damaging processes are normally held in check by intact adaptive ciliary ganglion-mediated control of ChBF.

4. Sympathetic superior cervical ganglion input to choroid

Sympathetic noradrenergic nerve fibers from the superior cervical ganglion innervate the choroid in mammals (Stone et al., 1987), and birds (Guglielmone and Cantino, 1982). In birds and mammals, the innervation is to blood vessels, and in birds to the smooth muscle of the choroidal stroma as well. Mammalian groups in which sympathetic innervation of the choroid has been demonstrated include rats, guinea pigs, rabbits, cats, and monkeys (Fig. 18) (Malmfors, 1965; Laties and Jacobowitz, 1966; Ehinger, 1966; Fitzgerald et al., 1992; Li and Grimes, 1993; Klooster et al., 1996). The sympathetic nerve fibers utilize noradrenaline as a neurotransmitter, and they thus contain the enzymes involved in its synthesis, such as tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH), and the transporter involved in the packaging of noradrenaline into vesicles, namely the vesicular monoamine neurotransmitter – 2 (VMAT2). The sympathetic nerve fibers from the superior cervical ganglion travel to the choroid via orbital blood vessels or by joining the ophthalmic nerve in both mammals and birds (Smith and Fan, 1996). Consistent with the sympathetic innervation of choroid and vessels supplying the choroid, cervical sympathetic stimulation in

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rats, rabbits, cats and monkeys increases uveal resistance and decreases ChBF (Bill, 1962; Alm and Bill, 1973; Alm, 1977, 1992; Riva et al., 1994; Abe et al., 1995; Steinle et al., 2000). The choroidal vasoconstriction caused by direct administration of noradrenaline or by activation of sympathetic nerves to the choroid is mediated by alpha-adrenergic receptors on blood vessels (Bill, 1984; Gherezghiher et al., 1991; Koss and Gherezghiher, 1993; Koss, 1994; Abe et al., 1995; Kawarai and Koss, 1998; Steinle et al., 2000). Consistent with a role of alpha-adrenergic receptors on choroidal vessels in ChBF control, Kiel and Lovell (1996) reported that alpha-adrenoreceptor blockade increased ChBF in rabbits. Blockers of beta-adrenergic receptors, by contrast, have been shown in pig to be only marginally effective in dilating the short posterior ciliary arteries (Braakman et al., 1999), and thus unlikely to have a significant vascular role in sympathetic choroidal control. The sympathetic co-transmitter NPY also has a role in ChBF regulation, since intravenous NPY in rabbits decreases ChBF 50% (Nilsson, 1991). NPY may be responsible for that part of choroidal sympathetic vasoconstriction that is not blocked by aphaadrenergic receptor antagonists (Alm, 1992), and particularly contribute to choroidal vasoconstriction with high sympathetic nerve firing rates, with noradrenaline playing its role during low firing rates (Bill, 1991).

Several studies have examined the impact on ChBF of removing the sympathetic input to choroid. Using LDF, Chou and coworkers found no evident increase in ChBF at basal perfusion pressures in rabbits one week after bilateral removal of the superior cervical ganglion (SCG) (Chou et al., 2000), or one week after unilateral removal of the SCG (Chou et al., 2002). Zhan et al. (2002) reported that basal ChBF in rabbits was also unchanged 24 h after sympathetic denervation by SCG removal. Similarly, Bill and coworkers have noted that sympathetic input does not substantially affect basal choroidal tone at normal systemic blood pressure in rabbits (Bill, 1984, 1985; Bill and Nilsson, 1985). Chou et al. (2000, 2002) did, however, note that as ocular perfusion pressure was lowered to zero by increasing IOP, ChBF remained higher in the rabbits with unilateral or bilateral sympathetic denervation as ChBF fell below about 50 mm Hg. Thus, sympathetic input exerts a constrictory effect on the tone of choroidal vessels that is manifest at low perfusion pressures, at least in rabbits. By contrast, Steinle et al. (2002) reported increased choroidal vessel luminal diameters and increased blood flow six weeks after ipsilateral cranial sympathetic transection in rats. In our own work in rats with bilateral SCG removal (SCGx), we observed by LDF that baseline ChBF was slightly but persistently elevated in SCGx compared to sham rats (by an average of 14.5%) at 2–3 months after surgery (n = 14 sham and 14 SCGx eyes), and this difference was significant over time by ANOVA ($p = 0.000015$). Thus, the sympathetic input appears to exert a small but steady vasoconstrictory effect on choroid under basal conditions, at least in rats. This vasoconstrictory influence apparently can also be revealed in rabbits if choroidal perfusion pressure is lowered by increasing IOP.

Bill (1984, 1985) has suggested that the sympathetic innervation of choroid becomes particularly activated with elevated systemic blood pressure, which acts to vasoconstrict the choroid to prevent the over-perfusion that would otherwise occur with the increased perfusion pressure caused by the elevated systemic BP. Increases in systemic blood pressure can occur naturally during stress or heightened activity levels. Several studies in humans have shown, in fact, that the choroid vasoconstricts after exercise-induced increases in systemic blood pressure and thereby compensates for the increased perfusion pressure

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(Riva et al., 1997a; Lovasik et al., 2003). Fuchsjäger-Mayrl et al. (2003) reported that endothelial cell release of the vasoconstrictor endothelin-1 plays a role in this effect. It may also be that central baroreceptor-responsive circuitry acting via the sympathetic input to choroid contributes to choroidal baroregulation during high systemic BP. The details of such circuitry, however, need elucidation. In particular, since low BP signals cause systemic sympathetic vasoconstriction, but it is high BP that drives choroidal vasoconstriction, there must be a difference between inputs to the central neurons giving rise to the sympathetic postganglionic outflow to the systemic versus the choroidal vasculature. In the absence of sympathetic choroidal vasoconstriction during high systemic BP, the resulting sustained elevations in ChBF would be harmful for retinal health and function, as they would lead to overperfusion and breakdown of the blood-retinal barrier. Ocular overperfusion and/or vascular leakiness has in fact, been demonstrated in sympathectomized rabbits with systemic blood pressure elevation caused by aortic clamping (Bill and Linder, 1976; Bill and Nilsson, 1985) and in sympathectomized monkeys subjected to systolic hypertension (Ernest, 1977).

Consistent with the above considerations, removal of the sympathetic input to choroid has an adverse impact on retinal health. For example, Steinle et al. (2005) noted that there was a significant reduction in photoreceptor cell numbers and the width of the retinal outer nuclear layer in sympathectomized rat eyes. Increased Müller cell immunostaining for GFAP spanning the ganglion cell layer and inner nuclear layer was also noted after sympathectomy by Steinle et al. (2005). We examined the effects of sympathetic denervation of choroid on ChBF baroregulation, as well as on retinal function (Fitzgerald et al., 2012a; Li et al., 2015b). Rats received bilateral superior cervical ganglionectomy (SCGx) (n = 7), which depleted the choroid of sympathetic innervation (vMat2+ fibers) but not parasympathetic innervation (VIP+ and NOS + fibers) in both eyes. Control rats received sham surgery (n = 7). The flash-evoked scotopic electroretinogram (ERG) was measured for both eyes 2–3 months after SCGx, and visual acuity was assessed for both eyes by OptoMotry. ChBF was assessed using LDF. To determine the effectiveness of ChBF baroregulation, arterial blood pressure (BP) was raised above basal by intravenous LNAME (10 mg/kg). Arterial blood pressure (BP) was measured via the femoral artery, as previously described (Reiner et al., 2010a). Subsequently, eyes from these and additional SCGx and sham rats were harvested for histological evaluation. Baseline ChBF prior to LNAME administration was persistently and significantly elevated in SCGx eyes compared to sham rat eyes by 14.5%, as noted above (Fig. 19). Over the first five minutes of the rapid ABP rise caused by LNAME, ChBF rose briefly and minimally in sham eyes, before returning to baseline, and then remained below baseline as the high ABP was maintained (Fig. 19). By contrast, in SCGx mice, ChBF increased considerably more, and did not return to baseline until about 5 min after LNAME administration. Even then, ChBF remained elevated above that in sham eyes. These results show that SCGx impaired choroidal baroregulation during ABP rise, and led to a slight sustained elevation in ChBF during high ABP as well. Impairments in visual function were associated with the deficits in baroregulation after SCGx. For example, the scotopic ERG b-wave amplitude was significantly reduced by 10–15% at the brightest 3 light intensities (Fig. 19), and the scotopic a-wave was significantly reduced by 20–30% at the brightest two light intensities, compared to sham rat eyes. Regression analysis showed that the failure of baroregulation (as assessed by increased correlation of ABP with ChBF) was significantly

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linked to a slowing of the latency and a reduction in the peak of the a-wave and b-wave. Visual acuity was also reduced for SCGx eyes, by ~50%, and this deficit too was statistically associated with baroregulatory failure. Photoreceptors, however, seemed more affected by the increase in baseline ChBF in SCGx eyes, as basal ChBF was significantly inversely correlated with photoreceptor abundance across sham and SCGx eyes (i.e. the higher basal ChBF caused by SCGx was harmful for photoreceptors). In the retina, Müller cell GFAP immunolabeling was significantly upregulated 2.4 fold (Fig. 19), and IBA1+ microglia were significantly increased 2.6-fold in SCGx eyes and doubled in the outer retina. These studies indicate that sympathetic denervation of choroid raises basal ChBF and impairs the ChBF baroregulatory response to increased ABP, both leading to choroidal overperfusion. The elevated basal ChBF seems to drive the slight ~15% photoreceptor loss we observed, while the baroregulatory failure seems more linked to the acuity and ERG deficits. The means by which choroidal overperfusion injures retina is uncertain, but it is likely it causes lipid peroxidation in outer retina (Bill and Sperber, 1990). Consistent with this possibility, we have observed an increase in autofluorescent RPE lipofuscin in SCGx rat eyes. Our observation of increases in outer retinal microglia as detected by immunolabeling for the macrophage/microglia marker IBA1 following SCGx is consistent with the possibility that the outer retinal debris provokes the early stages of an inflammatory response, as is the observation of a substantial increase in MHCII + activated macrophages in choroid as detected by double immunofluorescence for the macrophage/microglia marker IBA1 and for the MHCII antibody OX6 in SCGx rat eyes. These results are of interest in light of the finding that individuals with a genetic predisposition to age-related macular degeneration (AMD), which is mediated by outer retinal inflammation, exhibit defective sympathetic ChBF baroregulation beginning early in life (Told et al., 2013).

5. Trigeminal sensory input to choroid

Sensory nerve fibers from the trigeminal ganglion co-containing SP and CGRP innervate the choroid in mammals and birds (Stone et al., 1987; Corvetti et al., 1988; Shih et al., 1999). These mainly represent branches of the ophthalmic nerve, but some sensory axons that reach the orbit arise from the maxillary nerve. Like sensory fibers from dorsal root ganglia, peripheral trigeminal fibers respond to heat, cold, pain or touch. The ophthalmic nerve fibers typically join the meshwork of nerves behind the eye, with one prominent branch entering the ciliary ganglion. Nerve fibers reach the choroid by traveling with the short ciliary nerves and on blood vessels of the orbit. Among mammals, SP+ and CGRP + fibers to the choroid have been observed in diverse primate and non-primate species (Stone and Kuwayama, 1985; Kuwayama and Stone, 1987; Stone and McGlinn, 1988; Fitzgerald et al., 1992). Sensory fibers can influence their innervation territory in either of two ways – by sending a message centrally to initiate a reflex response, or by direct local release of SP/CGRP in response to activating stimuli. The central target of ophthalmic trigeminal sensory fibers is the trigeminal sensory nucleus of the brainstem, by which trigeminal fibers can elicit ocular reflexes, such as blinking and tearing, via projections of the trigeminal sensory nucleus to motor cell groups of the brainstem (Belmonte et al., 1997; Gallar et al., 2003). Orbital sensory fibers can also release SP and CGRP in response to stimuli and cause local effects, which can include an action on the vasculature (Belmonte et al., 1997).

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Consistent with a local action of intrachoroidal SP+/CGRP + fibers, the choroid has been shown to possess SP receptors (Denis et al., 1991) and CGRP receptors (Heino et al., 1995). The neuropeptides SP and CGRP are vasodilators and their release from intrachoroidal trigeminal sensory fibers would be expected to increase ChBF (Bill, 1984, 1991; Stone et al., 1987; Bill and Sperber, 1990; Bill, 1991; Shih et al., 1999). Consistent with this, stimulation of the ophthalmic nerve in rabbit increases ChBF (Stjernschantz et al., 1979), and is associated with uveal release of SP (Bill and Nilsson, 1985). Orbital vessels too are sites at which sensory fibers can affect blood flow to the choroid. For example, Bakken et al. (1995) showed that the pig ophthalmic artery dilates in response to CGRP. Additionally, trigeminal fibers have SP+ and CGRP + terminals in the PPG, which could be a basis of peripheral sensory-autonomic reflexes that affect ChBF (Suzuki et al., 1989).

A number of investigators, including ourselves, have raised the possibility that orbital trigeminal fibers, including those within the choroid, participate in temperature-driven ChBF reflexes, although some have argued against the likelihood of this (Kiel, 2012). Our work in chicks with myopia-inducing goggles and ophthalmic nerve cuts, for example, suggests that the sensory input to the choroid may be involved in temperature-dependent regulation of ChBF (Shih et al., 1999). Consistent with the idea that ChBF can respond to temperature, ChBF in rabbits has been reported to increase with retinal cooling below or heating above 34 °C, and thereby maintain constant retinal temperature with ambient temperatures ranging from 30 to 40 °C (Tamai et al., 1999). Similarly, Stiehl et al. (1986) showed that retinal temperature remains relatively constant during cooling of the conjunctiva in cats by up to 6 °C. Since vasoconstrictors prevented this compensation, the authors concluded that the retinal thermoregulation was mediated by an increase in ChBF. Other studies have shown that defects in ChBF adversely affect retinal temperature. For example, reduced ocular blood flow caused by elevated IOP has been shown to result in reduced scleral and retinal temperature (Auker et al., 1982; Bill et al., 1983). Moreover, heating an eye in which blood flow has been reduced by elevation of IOP (which reduces ocular perfusion pressure and ChBF) results in an exaggerated rise in ocular temperature compared to eyes at normal IOP, suggesting that ChBF can play a role in thermoregulatory ocular cooling as well (Bill et al., 1983), as indicated also by Tamai et al. (1999). ChBF-dependent thermoregulation may be important for retinal homeostasis and function (Parver et al., 1980). For example, reducing vitreal temperature in humans to 27–28 °C results in an abnormal ERG (Horiguchi and Miyake, 1991). Similarly, reducing body temperature stepwise in mice from 38 °C to 18 °C results in a steady reduction in the ERG a-wave and b-wave (Horiguchi and Miyake, 1991; Mizota and Adachi-Usami, 2002).

The specific role of the trigeminal input to the choroid in the apparent temperature responsiveness of ChBF, however, has not yet been demonstrated experimentally. In this regard, it would be of interest to know if the trigeminal sensory axons to the choroid, like those to other cranial targets including cornea, also possess the transient receptor potential channels by which heat sense (TRPV1-4) and the cold sense (TRPA1 and TRPM8) are mediated (Vass et al., 2004; Voets et al., 2004; Murata and Masuko, 2006; Yeo et al., 2010; Belmonte et al., 2015; Dussor and Cao, 2016). It also uncertain how the trigeminal input to the choroid might mediate the temperature responsiveness of ChBF. One possibility is that the sensory fibers within the choroid themselves release the vasodilators SP and CGRP

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in response to activation by heating or cooling. It also may be the case that the central connections of the choroidal sensory fibers mediate an alteration in ChBF via autonomic circuitry. In our studies in rat in which we mapped the central regions projecting to choroidal SSN using minute injections of PRV into choroid (Li et al., 2015a), we observed that a ventrocaudal part of the spinal trigeminal nucleus contained PRV-labeled neurons. This region of the spinal trigeminal nucleus is known to receive corneal sensory input (Aicher et al., 2014) and respond to bright light (Okamoto et al., 2009, 2010), and thus potentially could mediate parasympathetic choroidal vasodilation via the SSN-PPG circuit in response to light or corneal irritation. The response to light seems to occur due to the presence of melanopsin in the trigeminal fibers innervating cornea (Matynia et al., 2016). The central projections of the trigeminal fibers innervating the choroid itself are, however, not known. If they project to the same ventrocaudal part of the spinal trigeminal nucleus as the corneal sensory fibers, then the choroidal sensory fibers could mediate choroidal vasodilation in response to thermal signals or inflammatory signals. In this light, choroidal thickening during viral fever is of interest, since it could be driven by either the thermal signals or inflammatory signals caused by the viral illness (Kaya et al., 2016). Note also that Gallar et al. (2003) reported the presence of cold sensing scleral and episcleral fibers at the back of the eye in cats and noted that these fibers respond to cooling associated with experimentally diminished choroidal blood flow. As the fibers analyzed lie too deep to be affected by external temperature, the authors suggested that the sensory fibers may sense drops in posterior ocular surface temperature stemming from drops in ChBF and drive a compensatory centrally mediated thermoregulatory vasodilatory reflex that serves to maintain constant ChBF and ocular temperature. This too could be mediated via facial parasympathetic circuitry that receives input from the trigeminal sensory nucleus, and serve retinal thermoregulation via ChBF modulation.

6. Intrinsic choroidal neurons

Studies over the past 25 years have proven that the choroid in some mammalian and many avian species contains neuronal cells that make many of the various neuroactive substances typical of parasympathetic PPG neurons. Although intrinsic choroidal neurons were initially reported by silver staining methods in the 19th century (see Schrödl et al., 2003), Terenghi et al. (1982) first noted them for their neuroactive substance content (VIP) in guinea pig choroid after colchicine treatment (which prevents transport of neuroactive substances out of the perikarya). Shortly thereafter, Miller et al. (1983) and Stone et al. (1986) reported VIP + neurons in human choroid. This phenomenon was subsequently more extensively studied by several groups. Using NADPHd histochemistry, which detects neurons that use NOS to synthesize NO, Bergua et al. (1993) reported the presence of many isolated or grouped NADPHd + neurons in human choroid, with their perikarya being about 30 μm in diameter. Flügel et al. (1994) made similar observations of human choroid and reported that VIP was typically also present in the NOS + intrinsic choroidal neurons. The ganglion cells tended to be most numerous in the central retina in the macular region around the human fovea (Flügel et al., 1994; Triviño et al., 2002; May et al., 2004). Flügel-Koch et al. (1994) reported that NOS+ and VIP + intrinsic choroidal ganglion cells are also present in cynomolgus monkeys, which have a fovea centralis. No such neurons were, however,

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observed in afoveate mammalian species such as rats, rabbits, tree shrews, cats, pigs or owl monkeys (Flügel et al., 1994; Flügel-Koch et al., 1994). Since these neurons were absent from primate and non-primate species lacking a fovea centralis, these authors suggested that the plexus was associated with choroidal blood flow control in the region of the fovea. Consistent with this, the intrinsic choroidal ganglion cells in humans give rise to processes that can be observed to join the perivascular network of nNOS+ and VIP + fibers in the choroid, and observed to end on arteries. The intrinsic choroidal ganglion cells in humans have themselves been noted to receive terminals, some of which contain NOS or VIP (Flügel et al., 1994). Schrödl et al. (2003) used immunolabeling for nNOS or VIP, as well as single-cell filling, to study the connectivity of the intrinsic choroidal neurons in human eye in more detail. They reported that intrinsic choroidal neurons send processes to other ganglion cells, as well as to vascular and nonvascular smooth muscle of the choroid. CGRP + sensory boutons, TH +/VMAT2+ contacts from presumptive sympathetic postganglionic terminals, and terminals containing vesicular acetylcholine transporter that could be parasympathetic pre- or postganglionic endings have also been observed on intrinsic choroidal neurons (Lütjen-Drecoll, 2006). The evidence of sensory input to intrinsic choroidal ganglion cells suggests they could have a role in reflexive intrachoroidal modulation of ChBF in response to sensory stimuli such as heat or cold detected by the sensory fibers, while the apparent parasympathetic and sympathetic input suggests they could be under central autonomic control too.

Intrinsic choroidal neurons are also present in birds and of a similar size (30 μm) and neurochemistry (nNOS+ and VIP+) as in mammals (Bergua et al., 1996; Cuthbertson et al., 2003; Schrödl et al., 2004; Stübinger et al., 2010). These neurons give rise to processes that contact choroidal blood vessels, and they receive CGRP + contacts from presumptive sensory terminals, TH+/VMAT2+ contacts from presumptive sympathetic postganglionic terminals, and ChAT + terminals that could be parasympathetic preganglionic or postganglionic endings (Schrödl et al., 2001a,b; Stübinger et al., 2010). The evidence for sensory input to intrinsic choroidal ganglion cells in birds suggests that they too may have a role in reflexive intrachoroidal modulation of ChBF in response to sensory stimuli such as heat or cold, while the parasympathetic input in birds suggests they could also be under central control, possibly by SSN. The sympathetic nerve terminals on intrinsic choroidal neurons indicates their sympathetic modulation as well, perhaps reducing their vasodilatory drive during activation of sympathetic input to choroid, such as during high systemic BP (Schrödl et al., 2001b). The intrinsic choroidal neurons vary among avian groups in their abundance, with the highest abundance observed being in goose, the next highest in turkeys and chickens, and the lowest in ducks (Schrödl et al., 2004). Their spatial distribution also varies, presumably in relation to retinal high acuity specializations. As in mammals, the intrinsic choroidal neurons are also found associated with the ciliary nerves in their course to the anterior uvea (Bergua et al., 1996). As in mammals, the autonomic and sensory inputs to the intrinsic choroidal neurons, their output to the smooth muscle of choroidal blood vessels, and their production of vasodilators such as VIP and NO suggest a role in ChBF regulation driven by sensory and autonomic signals. The intrinsic choroidal neurons are also of interest for the role they may play in modulating the choroidal thickening that serves to reposition the retina to align it with the image plane (Nickla and Wallman, 2010).

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The evidence for this is the apparent innervation of the intrinsic choroidal neurons to the extravascular smooth muscle common to the choroid in birds but also seen in primates (De Stefano and Mugnaini, 1997; Poukens et al., 1998; Nickla and Wallman, 2010). The smooth muscle is thought to play a role in the filling or emptying of intrachoroidal lacunae that causes choroidal thickening or thinning, respectively (Nickla et al., 1998; Nickla and Wallman, 2010). This phenomenon is thought to play a role in refractive adjustment as a slow accommodative mechanism that supplements changes in lens shape. Choroidal thinning is for example seen in eyes subjected to hyperopic defocus, and choroidal thickening is observed in myopic eyes recovering from hyperopic defocus (Nickla and Wallman, 2010). The thinning is accompanied by reduced ChBF and the thickening by increased ChBF (Shih et al., 1993a,b; Reiner et al., 1995; Fitzgerald et al., 2002a), and it may be that activation of the intrinsic choroidal neurons drives both the flow increase and the choroidal thickening, and their inhibition the opposite.

Abnormalities in the intrinsic choroidal neurons may also play a role in retinal disease. For example, the intrinsic choroidal neurons may be vulnerable to high IOP, since they show significant loss in monkey eye after experimental induction of elevated IOP (May et al., 2006) and they are reduced in numbers in glaucomatous human eyes (May and Lütjen-Drecoll, 2004). Their loss in glaucoma may contribute to the reduction in ChBF observed in glaucoma (James and Smith, 1991; Kubota et al., 1993; Grunwald et al., 1998c). Given the contribution of the choroid to the prelaminar blood supply of the optic nerve head, this ChBF reduction could contribute to optic axon injury in glaucoma. Moreover, since the intrinsic choroidal neurons in mammals are associated with the fovea centralis (Flügel-Koch et al., 1994), they may be especially important for ChBF control in the region of the macula. This could be particularly important in central retinal decline in normal aging and in age-related macular degeneration (AMD), both of which involve reduced macular ChBF (Yoneya et al., 1995; Grunwald et al., 2005; Metelitsina et al., 2008). Further studies are needed to determine if intrinsic choroidal neurons are lost and to what extent in AMD and normal aging.

7. Choroidal blood flow in aging and disease

7.1. Role of choroid in age-related retinal decline

Choroidal blood flow and its adaptive regulation can be impaired by aging. For example, reductions in basal ChBF occur in the macula of humans as they age (Pauleikhoff et al., 1990; Ravalico et al., 1996; Grunwald et al., 1998a). These findings raise the possibility that abnormalities in macular ChBF and/or in its adaptive regulation contribute to normal age-related retinal declines. The mechanisms responsible for the reduced ChBF and impaired adaptive regulation with age are uncertain. Loss and narrowing of submacular choroidal vessels in normal aged eyes has been reported (Ramrattan et al., 1994; Spraul et al., 1996; Grunwald et al., 1998a). Such changes may contribute to the reduction in basal ChBF seen with aging. To examine the possible role of changes in neuronal control of ChBF, we used immunolabeling to assess the impact of age on parasympathetic innervation of human choroid, using VIP immunolabeling. Our results indicated a significant age-related decline in VIP-positive nerve fibers and vessel diameter in the submacular choroid in disease-free

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human donor eyes (Fig. 20) (Jablonski et al., 2007). These findings suggest that a decline in the neural control of ChBF and vessel diameter may help explain reductions in ChBF and its adaptive control observed with aging.

As part of an effort to relate the effects of age-related decline in ChBF and its regulation to retinal health, we carried out detailed studies in pigeons to: 1) determine if choroidal and outer retinal deterioration occur as a function of aging in a non-human species that possesses both rod and cone photoreceptors (i.e. pigeons), and might therefore be widespread concomitants of aging; and 2) obtain data that might shed light on the relationship between age-related retinal and choroidal changes (Fitzgerald et al., 2001, 2005). In a sample of 64 pigeons ranging in age over much of the pigeon lifespan (0.5–20 yrs), we measured several different ocular parameters by physiological or histological means, including: ChBF (by LDF); choriocapillary vessel abundance (by LM histology); acuity (by behavioral methods); and photoreceptor abundance (by LM histology). Statistical methods were used to ascertain the pattern of age-related changes and determine ages at which or by which significant changes occurred in specific parameters. In the sample of 53 birds for which we had obtained visual acuity and/or photoreceptor data, we observed a prominent stepwise decline of about 20% in photoreceptor abundance at the age of 4.7 years (Fig. 21), followed by lesser decline thereafter, and a curvilinear decline in acuity (with half the decline having occurred by the age of five years). The period of prominent photoreceptor loss (4–6 years of age) coincided with ages during which about 10% of photoreceptors appeared to be showing degenerative changes. For the sample of 45 birds for which we measured choroidal parameters, choriocapillary vessel abundance showed a highly curvilinear decline with age and at least half of this decline had occurred by the age of three years (Fig. 21). ChBF showed an abrupt decline of about 20% at four years of age and a further 20% decline thereafter. Our results clearly show that ChBF and choroidal vascularity decline significantly with age in pigeons, as do acuity and photoreceptor abundance. Our statistical analyses suggest that there is a positive relationship between choroidal and visual functions in pigeons, and that prominent choroidal vascular decline precedes visual decline as the birds age (Fig. 21). Thus, our findings are consistent with the view that age-related decline in choroidal function might contribute to age-related vision loss. Note that the possibility exists that retinal dysfunction preceding overt degeneration is the primary event and diminished ChBF the consequence of diminished retinal need. Nonetheless, outer retinal degeneration does not invariably lead to diminished ChBF, as evidenced in the Abyssinian cat (Nilsson et al., 2001).

In further studies in pigeons, we sought to determine if age-related changes in parasympathetic regulation of ChBF by the EWM-Ciliary Ganglion circuit could contribute to age-related choroid and retinal decline (Fitzgerald et al., 2005). To this end, we immunolabeled choroidal nerve fibers from the ciliary ganglion for the cholinergic marker choline acetyltransferase (ChAT) or for a neurofilament-associated antigen in fixed cryostat sections of the eye in 0.5–20 year old pigeons. Additionally, transcleral LDF was used to measure basal ChBF, light-evoked ChBF increases, and EWM-evoked ChBF increases in the superior choroid to assess the functional integrity of the vSCN-EWMCiliary Ganglion circuit. We observed a marked age-related linear decline in the ciliary ganglion innervation of the choroid (Fig. 21). Moreover, we observed pronounced declines in ciliary ganglion-

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mediated control of ChBF. The decline in EWM control of ChBF was pronounced by five years of age, and half of the functional decline occurred by about two-three years of age. Thus, significant loss in choroidal vascularity and innervation appear to lead to impaired basal and adaptive parasympathetic ChBF control early in the life span of pigeons (Fig. 21), which is consistent with the notion they contribute to agerelated vascular insufficiency and attendant age-related damage to the retina. These overall results for pigeons suggest that aging may deleteriously affect the retina, in part, by impairing ChBF and its neural control. The apparent impact of age-related loss of ciliary ganglion input to the choroid in birds is consistent with the impact we see of EWM lesions in younger birds. As we have shown that choroidal baroregulation also undergoes age-related dysfunction in aged pigeons (Fig. 22) (Reiner et al., 2003), impairment of adaptive ChBF baroregulation, presumably by the SSN-PPG circuit, also may contribute to age-related retinal declines.

We also examined if ChBF baroregulation changes with age in male Sprague-Dawley (SD) rats (120–657 days of age) as well as in males of the rapidly aging Fischer-344 (F344) rat strain (140–750 days of age), and whether any such changes were associable with changes in retinal structure and function (Fitzgerald et al., 2012a, 2016). ChBF was un-correlated with arterial BP (i.e. showed baroregulation) during its fluctuations above and below basal ABP in young (120–200 day old) SD rats, but became increasingly correlated with ABP as SD rats aged, so that by a year of age ChBF tended to change linearly with ABP, indicative of baroregulation failure. Subsequent to this, a progressive agerelated curvilinear decline in both the flash-evoked scotopic ERG b-wave amplitude and a-wave amplitude, and a thinning of the retina, particularly the inner plexiform and outer nuclear layers, was seen in SD rats, which was not evident until after 400 days of age (Fig. 23). For F344 rats, ChBF was highly correlated with ABP already in 170 day-old rats – i.e. baroregulation was already failing in young F344 rats. Acuity, contrast sensitivity and a-wave deficits, and ONL thinning (i.e. photoreceptor loss) were already prominent by 400 days, and acuity, contrast sensitivity, a-wave and b-wave deficits, and ONL thinning were severe by 660 days (Fig. 23). Baroregulatory failure was associated with RPEBM-choriocapillaris thickening in SD and F344 rats, perhaps due to debris buildup. Müller cell GFAP immunolabeling was analyzed blinded using the same scoring system as in our SSN-Lx and SCGx studies. The old SD rats had Müller cell GFAP labeling 3.5× those in controls. Our studies indicate that ChBF baroregulation is impaired by one year of age in SD rats, and this may drive the subsequent functional and morphological decline in the retina. By contrast, impaired baroregulation is already evident in young F344 rats (6 month old), before loss in vision and ERG is seen and before retinal thinning. This early loss of baroregulation in F344 rats may be a factor in their accelerated and severe subsequent functional decline and loss of photoreceptors. Our studies thus suggest the important role age-related failure in ChBF baroregulation may play in age-related disruption in retinal health, as ChBF baroregulatory failure precedes retinal pathology and functional decline in both SD and F344 rats, despite their differing aging patterns.

7.2. Role of choroid in disease-related retinal decline

Several diseases affecting the retina have diminished ChBF and/or its neural control as concomitants, including age-related macular degeneration (AMD) (Friedman et al., 1995;

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Grunwald et al., 1998b, 2005; Pournaras et al., 2006), chronic hypertension (Tso and Jampol, 1990; Haefliger et al., 2001), insulin-dependent diabetes (Langham et al., 1991) glaucoma (James and Smith, 1991; Kubota et al., 1993; Grunwald et al., 1998c; Su et al., 2006), ischemic outer retinal disease (Gaudric et al., 1982; Blacharski, 1988), and central serous chorioretinopathy (Tittl et al., 2005). Thus, the available data are consistent with the view that disturbances in maintenance of basal neurogenic choroidal tone and/or adaptive ChBF neural control could be contributing factors to the retinal declines seen in humans and other species in hypertension, diabetes, glaucoma, ischemic retinal disease, and central serous chorioretinopathy. Clearly other mechanisms also contribute to retinal damage in these conditions, such as edema, retinal detachment and/or neovascularization (Frank, 1988; Tso, 1988; Tso and Jampol, 1990). It is also possible that in some cases the declines in ChBF and its neurogenic control are secondary to the disease and retinal degeneration. Nonetheless, it remains important to determine if impaired neural regulation of ChBF directly contributes to degeneration of retina, as well as facilitates the pathological changes in the RPE or Bruch's membrane that further edema, retinal detachment and neovascularization (Potts, 1966; Frank, 1988; Tso, 1988; Korte et al., 1989; Tso and Jampol, 1990).

The role of diminished control of ChBF in AMD is of particular interest. Consistent with an adverse effect of aging on vasodilatory control of ChBF, the parasympathetic innervation of the choroid is diminished and basal ChBF reduced in both humans and animals during normal aging, as noted above (Yoneya et al., 1995; Fitzgerald et al., 1996; Grunwald et al., 2005; Jablonski et al., 2007; Emeterio Nateras et al., 2014; Whitmore et al., 2015). Yet more profound declines in basal ChBF occur in humans with AMD, with the ChBF declines increasing in severity with AMD severity, and predicting the development of neovascularization and the abundance of foveal drusen (Ciulla et al., 2001; Grunwald et al., 2005; Metelitsina et al., 2008; Pemp and Schmetterer, 2008; Feigl, 2009; Boltz et al., 2010; Xu et al., 2010; Berenberg et al., 2012). Given that a deficiency in parasympathetic innervation of choroid has been reported in AMD (Bhutto et al., 2010), it is possible that adaptive choroidal baroregulation during low systemic blood pressure in AMD patients is defective as well. In this regard, it is noteworthy that we have observed defects in choroidal baroregulation in aged pigeons and rats during both low and high systemic blood pressure (Fitzgerald et al., 1996; Reiner et al., 2011), and others have observed them in aged humans with high BP (Dallinger et al., 1998). Sympathetic innervation of choroid is also reduced with aging (Nuzzi et al., 2010), and consistent with a possible decline in sympathetic innervation in AMD, compensatory choroidal vasoconstriction during high systemic BP in AMD patients is defective (Pournaras et al., 2006).

Recent studies by the Schmetterer group (Told et al., 2013) have shown that at least some genetic risk factors for AMD may themselves compromise autonomic sympathetic ChBF control early in life. In particular, they found that the complement factor H (CFH) risk factor for AMD (i.e. CC complement factor H haplotype) was associated with impaired baroregulation of ChBF during high systemic blood pressure in young risk-gene carriers well before AMD symptoms. A similar impairment in baroregulation of ChBF during upward fluctuation in BP has also been seen in wet AMD patients (Pournaras et al., 2006), raising the possibility that impairment in hypertensive ChBF baroregulation may persist

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throughout the lifespan of CFH AMD-risk carriers. These findings suggest a possible role of AMD risk genes in promoting impaired autonomic regulation of ChBF early in life, thereby contributing to early AMD pathogenesis. Our work showing the adverse effect of sympathetic denervation of the rat retina is consistent with the idea that chronic impairment of sympathetic regulation of ChBF, such as seems caused by the CC-CFH haplotype, could contribute to the long-term outer retinal injury that promotes AMD progression. The study of the Schmetterer group, however, only examined baroregulation during increased high systemic BP, and it thus does not rule out the possibility of defects in hypotensive baroregulation involving impaired parasympathetic mechanisms as well. The findings of the Schmetterer group raise the possibility that chronic impairment of sympathetic and/or parasympathetic regulation of ChBF beginning in early adulthood, driven by genetic AMD risk factors, may contribute to AMD. This may be aggravated by non-genetic risk factors such as smoking, since smoking has been shown to cause choroidal thinning and to impair choroidal baroregulation during high systemic BP (Wimpissinger et al., 2003).

Impaired autonomic ChBF regulation stemming from central or peripheral defects in the control circuitry (possibly early occurring in the case of those with genetic risk factors) may cause ischemic and oxidative injury to RPE cells, increased oxidation of photoreceptor lipids, and impaired transport between retina and choroid. These possibilities are consistent with the visual deficits and retinal pathology we see in pigeons and rats (which include an increase in lipofuscin granules in the RPE) over the first few months after experimental manipulations that perturb parasympathetic or sympathetic control of ChBF. Over a longer time period, it is possible that impaired ChBF regulation may lead to waste accumulation in and along Bruch's membrane resembling that seen in normal aging (Potts, 1966; Herron et al., 1969; LaVail, 1981; Tso, 1988). In more severe cases or with sufficient time, the sub-RPE debris may take the form of the basal linear deposits and drusen in Bruch's membrane seen in AMD. The accumulation of sub-RPE waste in humans is thought to trigger the complement factor-mediated inflammatory response that is the proximate cause of the severe RPE and photoreceptor death in AMD, particularly in those with a pro-AMD genetic predisposition in the alternate complement cascade or lipid metabolism (Winkler et al., 1999; Feigl, 2009; Hageman et al., 2017). Consistent with the early stages of such a process, our rats with disrupted autonomic ChBF regulation show such AMD-like inflammatory signs as increases in outer retinal microglia, and increases in choroidal mast cells and macrophages (Bhutto et al., 2016). It is possible that a defect in ChBF regulation brought on by age, smoking, or obesity-related cardiovascular problems may be a commonality that contributes to their roles as risk factors for AMD.

8. Future directions

The evidence shows that the choroidal blood flow is modulated by parasympathetic and sympathetic nerve fibers via the central nervous system in response to at least two general categories of stimuli – retinal activity and systemic blood pressure. In both cases, the neurogenic control appears to ensure that the outer retina receives the blood supply it needs for its activity state and to ensure stability of choroidal blood flow despite fluctuations in ocular perfusion pressure as affected by systemic blood pressure. The trigeminal sensory input to choroid may also play an important role in modulating ChBF as a function of

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thermal signals, and inflammation as well. These various roles of the nervous system input to choroid seem to be important, as our studies and those of others in animal models show that disturbing these circuits has adverse effects for eye health and function. Thus, direct injury to the peripheral or cranial parts of these circuits due to trauma or due to disease would be expected to yield retinal and visual decline in humans. Little information is, however, available on this, and what is available is not without confounding variables. For example, although dysfunction of sympathetic input to choroid is likely to be a part of the generalized cranial sympathetic denervation of Horner's syndrome, no information is available on how it affects ChBF. Although visual dysfunction has been described in Horner's syndrome patients, it is not clear if it stems from dysfunction of sympathetic regulation of ChBF or from the trauma that caused the Horner's syndrome (such as internal carotid artery dissection) (Hicks et al., 1994; Nagy et al., 1997; Biousse et al., 1998). Dysfunction of neurogenic control of ChBF is also likely in the genetic disorder familial dysautonomia, a recessive, infantile-onset, autonomic and sensory neuropathy caused by a mutation that results in deficiency of the IκB kinase complex-associated protein (IKAP). Although visual dysfunction and ganglion cell loss have been reported in familial dysautonomia, it is uncertain if they are a consequence of impaired neurogenic control of ChBF or a direct effect of the mutation (Mendoza-Santiesteban et al., 2012, 2014). Similarly, visual deficits have been reported in humans with genetic deficiency in DBH, and are thus unable to synthesize noradrenaline in sympathetic postganglionic terminals (Robertson et al., 1991). In this case as well, it is uncertain if the retinal abnormalities in these individuals (who rarely live past young adulthood) stem from their choroidal sympathetic defects or from defective signaling by DBH + retinal cell types.

Although little definitive information is available on how direct trauma-related or disease-related damage to autonomic or sensory circuits might affect ChBF and thereby ocular health in humans, the impact of numerous ocular and systemic diseases or conditions on ChBF has been examined in humans, such as glaucoma, diabetes, hypertension, and AMD, as noted above. Whether ChBF changes are a primary driver of retinal dysfunction and decline in these cases or a secondary effect has not yet been determined. It is also uncertain if ChBF declines stem from disturbed neural control or vascular pathology, or both. In the case of our own studies of aging at least, it is clear that neurogenic control of ChBF and its innervation decline with age, and we have some evidence that the neurogenic ChBF declines are linked to retinal decline. In AMD as well, there is evidence for declines in ChBF, ChBF control, and in choroidal innervation. One approach for assessing the possible role of innervation decline or dysfunction in age-related related retinal decline or in the etiology of AMD would be to further evaluate the long-term consequences of experimental perturbation of parasympathetic or sympathetic control of ChBF in animals, and determine if the pathology that ensues resembles that in aging or AMD. For example, it would be useful to determine if disrupted parasympathetic ChBF control and/or disrupted sympathetic ChBF control leads to the RPE cell injury, lipid peroxidation, and outer retinal waste accumulation that create the conditions necessary for outer retinal inflammation, which in humans culminates in the pathogenic inflammatory cascade of AMD. The published findings raise the possibility that chronic impairment of sympathetic and/or parasympathetic regulation of ChBF, beginning perhaps already in early adulthood when driven by genetic AMD

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risk factors, may contribute to AMD. If a role of either parasympathetic or sympathetic baroregulatory defects in age-related retinal decline and AMD were to be demonstrated, such results would suggest the merits of evaluating neurogenic choroidal baroregulation as a tool to assess AMD risk, and recommend drugs that improve neurogenic ChBF control as therapies. In our own work, we have shown that the M2 muscarinic receptor antagonist himbacine facilitates the vasodilatory action of parasympathetic terminals in choroid by blocking the cholinergic inhibition mediated via presynaptic M2 autoreceptors (Zagvazdin et al., 2000). This could represent a possible approach for improving ChBF and its adaptive regulation.

Acknowledgments

Special thanks to Rebeca-Ann Weinstock, Raven Babcock, Amanda Valencia, Aminah Henderson, Marion Joni, Ting Wong, Julia Jones, Felicia Covington, Karen Hanks, Shani Bell, Christy Loggins, Dr. Christopher Meade, Dr. Yun Jiao, and Dr. Seth Jones for assistance and/or advice during the course of our studies. Our work has been supported by NIH-EY-05298 (AR), The Methodist Hospitals Endowed Professorship in Neuroscience (AR), the University of Tennessee Neuroscience Institute (CL), and the Department of Ophthalmology of the University of Tennessee Health Science Center (MECF), and an unrestricted grant from Research to Prevent Blindness (MECF).

Glossary

A5	A5 noradrenergic group of the pons
ABP	arterial blood pressure
Ach	acetylcholine
AND	aortic depressor nerve
AP	area pretectalis (in bird only)
AMD	age-related macular degeneration
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid type glutamate receptor
ANOVA	analysis of variance
B3	B3 serotonergic cell group of the pons
BM	Bruch’s membrane of retina
CFH	complement factor H
BP	blood pressure
CG	ciliary ganglion
CGRP	calcitonin gene-related peptide
ChAT	choline acetyl transferase
ChBF	choroidal blood flow

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ChBVel	choroidal blood velocity
ChBVol	choroidal blood volume
CO2	carbon dioxide
4DAMP	4-diphenyl-acetoxy-N-methylpiperedine
DBH	dopamine beta-hydroxylase
EM	electron microscopy
eNOS	endothelial nitric oxide synthase
GABA	gamma amino butyric acid
GAD65	glutamic acid decarboxylase MW65
ERG	electroretinogram
EW	nucleus of Edinger-Westphal
EWL	lateral subdivision of the nucleus of Edinger-Westphal (in birds only)
EWM	medial subdivision of the nucleus of Edinger-Westphal (in birds only)
F344	Fischer-344 rat
GFAP	glial fibrillary acidic protein
Hg	mercury
IBA1	Ionized calcium binding adaptor molecule 1
IOP	intraocular pressure
LDF	laser Doppler flowmetry
LM	light microscopy
7NI	7-nitroindazole
LNA	N^G-nitro-L-arginine
LNAME	N^G-nitro-L-arginine methyl ester
M2	M2 type muscarinic receptors
M3	M3 type muscarinic receptors
M4	M4 type muscarinic receptors
MSCN	medial suprachiasmatic nucleus (in birds only)
nNOS	neuronal nitric oxide synthase
nNOS-KO	nNOS knockout

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NO	nitric oxide
NPA	Nω-propyl-l-arginine
NPY	neuropeptide Y
NTS	nucleus of the solitary tract
O2	oxygen
ONL	outer nuclear layer of retina
PRV	pseudorabies virus
PPG	pterygopalatine ganglion
PVN	paraventricular nucleus
RaM	raphe magnus
RPE	retinal pigment epithelium
RVLM	rostral ventrolateral medulla
SCG	superior cervical ganglion
SCGx	superior cervical ganglion removal
SCN	suprachiasmatic nucleus
SP	substance P
SD	Sprague-Dawley rats
SSN	superior salivatory nucleus of the facial nucleus motor complex
SSN-Lx	SSN lesion
SSN-miss	inaccurate SSN lesion
TH	tyrosine hydroxylase
TRIM	1-(2-Trifluoromethylphenyl)imidazole
TRPV1	transient receptor potential cation channel subfamily V member 1 (AKA capsaicin receptor)
TRPV2	transient receptor potential cation channel subfamily V member 2
TRPV3	transient receptor potential cation channel subfamily V member 3
TRPV4	transient receptor potential cation channel subfamily V member 4
TRPA1	transient receptor potential ankyrin 1 (AKA transient receptor potential cation channel, subfamily A, member 1)

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TRPM8	transient receptor potential cation channel subfamily M member 8 (AKA the cold and menthol receptor 1)
VGLUT2	vesicular glutamate transporter-2
VIP	vasoactive intestinal polypeptide
VMAT2	vesicular monoamine neurotransmitter – 2
vSCN	visual suprachiasmatic nucleus (in birds only)
WT	wild-type

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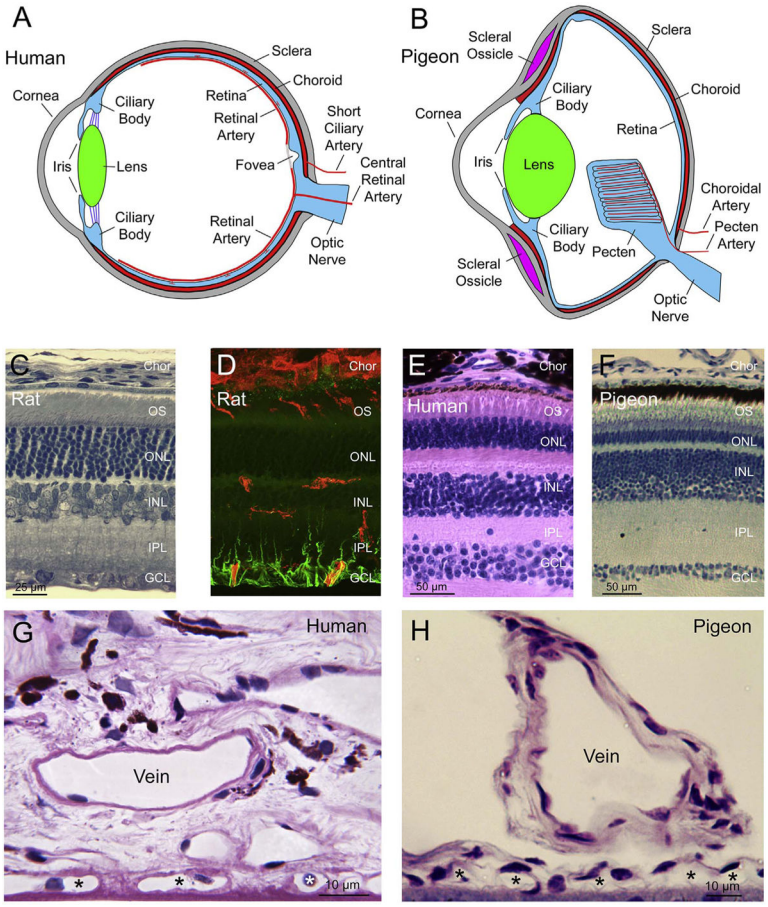

Fig. 1.

Schematics and images illustrating similarities and differences between mammalian and avian eye, using as examples of mammals the human eye (A, E, G) and the rat eye (C, D) and as an example of mammals the pigeon eye (B, F, H). Images A and B show schematics of human (A) and pigeon eye (B). The major differences are the more flattened shape of the avian eye, the presence of scleral ossicles ringing the cornea in birds, and presence of the pecten and its vasculature in birds instead of the retinal vasculature in mammals. In A and B, red is used to illustrate blood vessels or vascular layers of the eye, with small branches from the retinal arteries penetrating into the retina in the human eye. Images C–F present comparable cross-sections through rat (C, D), human (E) and pigeon (F) retina. The two images of rat retina show a transmitted light micrograph of a toluidine blue-stained plastic-embedded section showing the retinal layers (C) juxtaposed to a confocal laser scanning microscope image of a comparable view of a section through rat retina in which blood vessels are labeled red with the lectin IB4 and Müller cell endfeet and processes in the inner retina are immunolabeled green for GFAP (D). The two images show the location of the retinal vessels, fragments of which are seen in the nerve fiber layer and ganglion cell layer (GCL), in the inner plexiform layer (IPL), and in the outer plexiform layer between the INL and outer nuclear layer (ONL). Choroid (Chor) and outer segments (OS) are also present in the field of view. Note that some outer segments label with IB4. Note also that the GCL and INL are relatively thicker and the ONL relatively

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thinner in human (E) and pigeon (F) retina than in rat retina (C), but lamination is otherwise comparable. The human retinal image (E) is from the histology image bank at the online Yale University Medical School website (http://medcell.med.yale.edu/histology/). Images G and H show that human and pigeon choriocapillaris (asterisks) show close resemblance, but human (and in general mammalian choroid) is richer in connective tissue surrounding the choroidal vessels. Avian choroid by contrast is characterized by lucunar spaces around blood vessels. Pigeon choroid is from a 1-μm plastic embedded section of inferior retina. The human image shows submacular choroid in a paraffin embedded section from a 71-year old normal, caucasian male, and is stained with PAS and hematoxylin. The human section and image is unpublished and was prepared and kindly provided by D. Scott McLeod and Dr. Gerard Lutty of Johns Hopkins University (MD).

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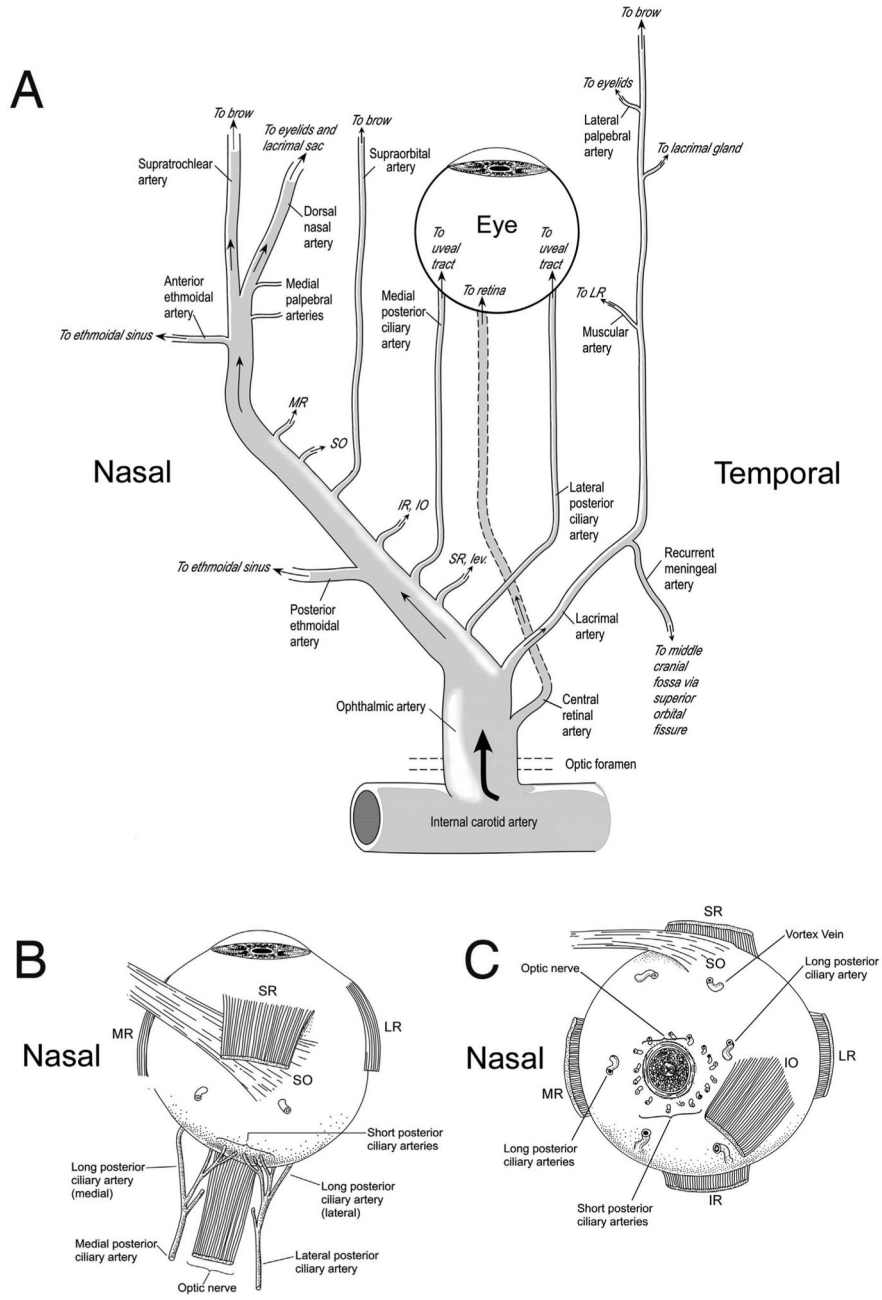

Fig. 2.

Schematic of ophthalmic artery in human and its arterial branches for the right eye. Schematic **A** is adapted from Oyster Fig. 6.8 (1999) and shows the various orbital branches of the ophthalmic artery. Blood is supplied to the eye by the central retinal artery, the posterior ciliary arteries, and the rectus muscle branches that give rise to the ciliary arteries. Other orbital arteries pass through the orbit to supply structures outside the orbit, some of which also give rise to intraorbital branches in passing (such as glandular branches). Schematics **B** and **C** are redrawn from Oyster Fig. 6.15 (1999) by N. Guley and show the posterior ciliary artery and its branches, as viewed from the top of the

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eye (**B**) and back of the eye (**C**). A medial and a lateral posterior ciliary artery typically arise from the ophthalmic artery. The small branches from the posterior ciliary arteries that penetrate the sclera around the optic nerve are the short posterior ciliary arteries, while the main posterior ciliary arteries continue as the medial and lateral long posterior ciliary arteries. Abbreviations: MR-medial rectus; SO-superior oblique; IR-inferior rectus; IO-inferior oblique; SR-superior rectus; LR-lateral rectus.

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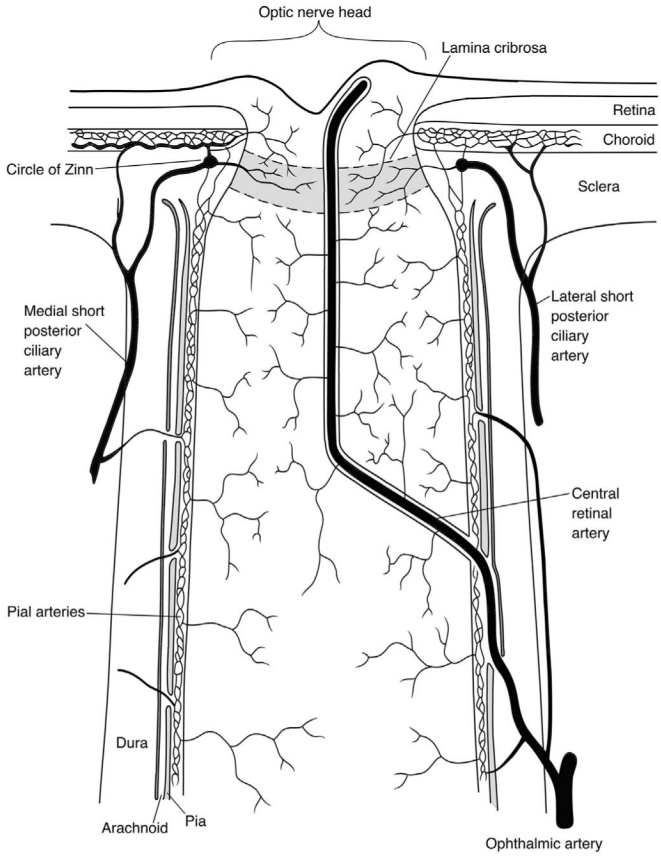

Fig. 3.

Schematic of the central retinal artery, and the optic nerve and nerve head blood supply in human, redrawn from Oyster Fig. 6.23 (1999) by N. Guley. The central core of the optic nerve is supplied and drained by the central retinal artery and vein, respectively. The more peripheral portions of the nerve and nerve head are supplied by the short posterior ciliary arteries and their branches. The laminar part of the optic nerve is also supplied from the central retinal artery and the short posterior ciliary arteries. By contrast, the prelaminar part of the optic nerve head is supplied from the central retinal artery and the choroid.

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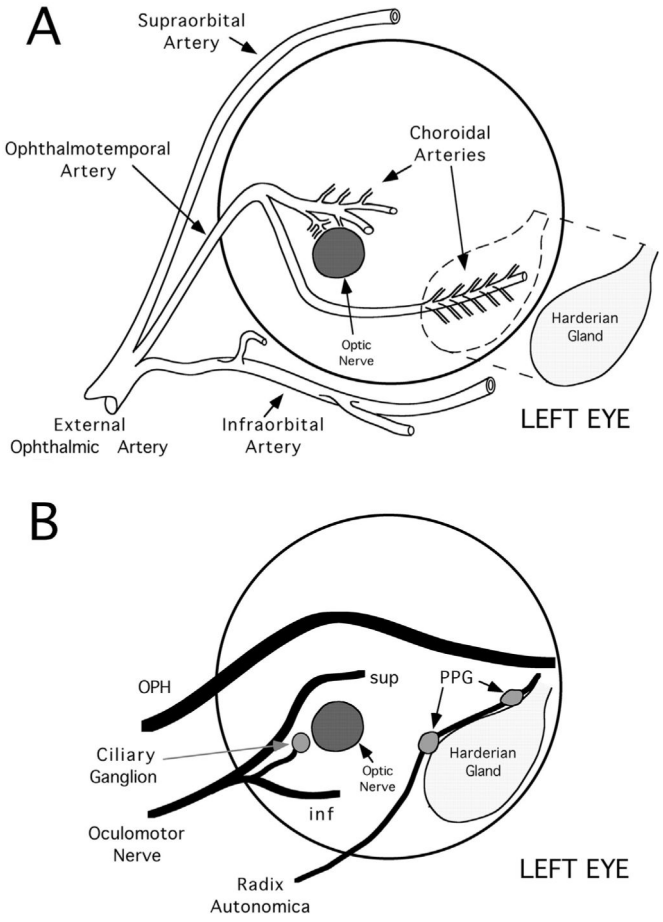

Fig. 4.

Schematic llustrations of the major ocular vessels (**A**) and nerves (**B**) and their relationship to the Harderian gland in birds, as viewed from the posterior aspect of the left eye. Schematic **A** illustrates the origin of the ophthalmotemporal artery from the external ophthalmic artery (which is itself a branch of the internal carotid) and its ocular course along the left eye. Note the course of the ophthalmotemporal artery along the temporal, posterior, and nasal poles of the eye, and note that it gives rise to choroidal arteries throughout its course. It also gives rise to additional muscular and glandular branches. The ophthalmotemporal artery is accompanied by a vein of the same name whose major branches are somewhat different from those of the artery. Schematic **B** shows the course and relative locations of several major orbital nerves, as well as the locations of the ciliary ganglion (CG) and a simplified version of the PPG system of microganglia. Superior is to the top and nasal to the right in both schematics. Abbreviations: inf - inferior branch of oculomotor nerve; OPH - ophthalmic nerve; sup - superior branch of oculomotor nerve.

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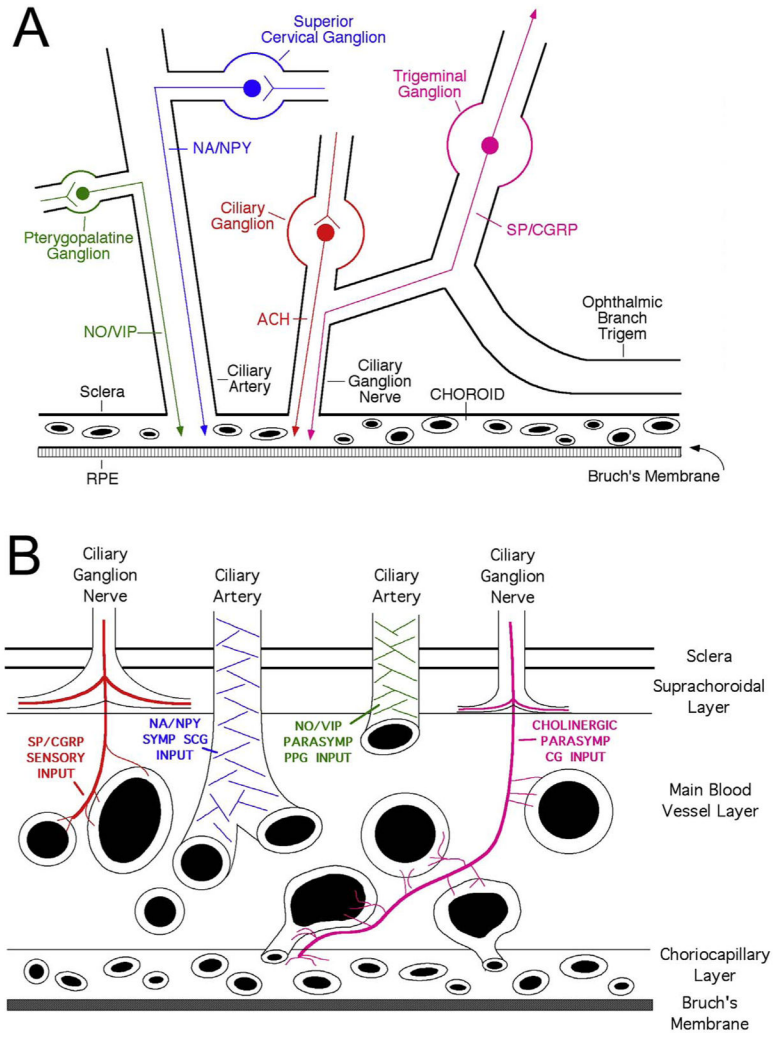

Fig. 5.

Schematics illustrating the ganglia innervating choroid (**A**) and the route fibers from each ganglion take to enter the choroid (**B**). This organization and neurochemistry for the choroidal innervation shown is true of both birds and mammals.

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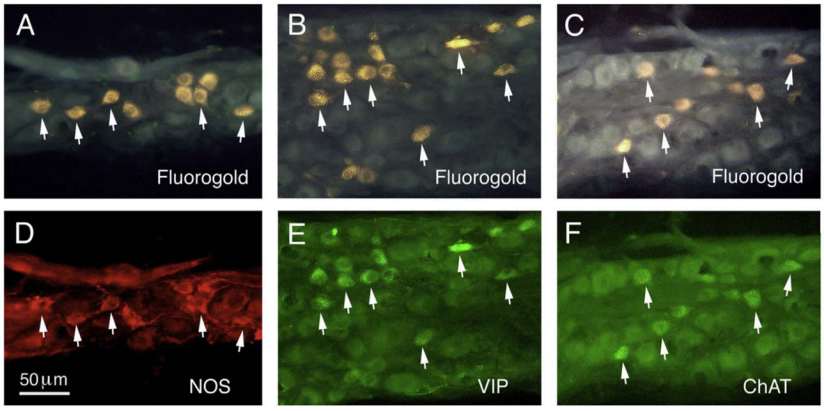

Fig. 6.

Series of images of fluorescent labeling showing that neurons of the rat PPG that project to the choroid contain NOS, VIP, and ChAT. Image **A** shows neurons of the PPG that had been retrogradely labeled by intrachoroidal fluorogold (FG) injection into the temporal sector of the choroid, and image **D** shows NOS immunolabeling in this same field of view. NOS is present in most of the FG-labeled PPG neurons, as indicated by arrows for some of the double-labeled neurons. Image **B** shows neurons of the PPG that were retrogradely labeled by the same intrachoroidal FG injection, and image **E** shows VIP + neurons in this same field. Note that VIP is also present in most of the FG-labeled PPG neurons (some indicated by arrows). Image **C** shows neurons of the PPG that had been retrogradely labeled by the same intrachoroidal FG injection as in **A** and **B**, while image **F** shows ChAT immunolabeling in the same field. Note that ChAT is also present in most of the FG-labeled PPG neurons (some indicated by arrows). All images are at the same magnification.

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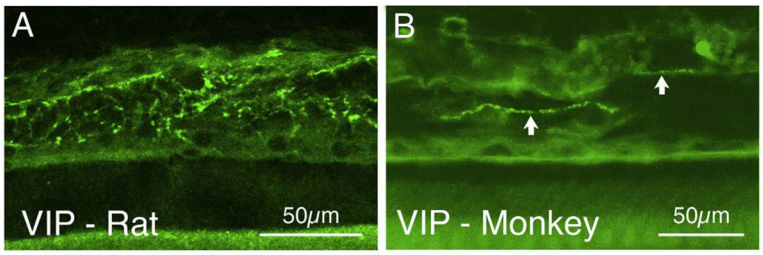

Fig. 7.

Images showing VIP + nerve fibers of presumptive PPG origin in choroid in rats (**A**) and rhesus monkey (**B**) (colorized from Stone, 1986). Arrows indicate nerve fibers in monkey choroid.

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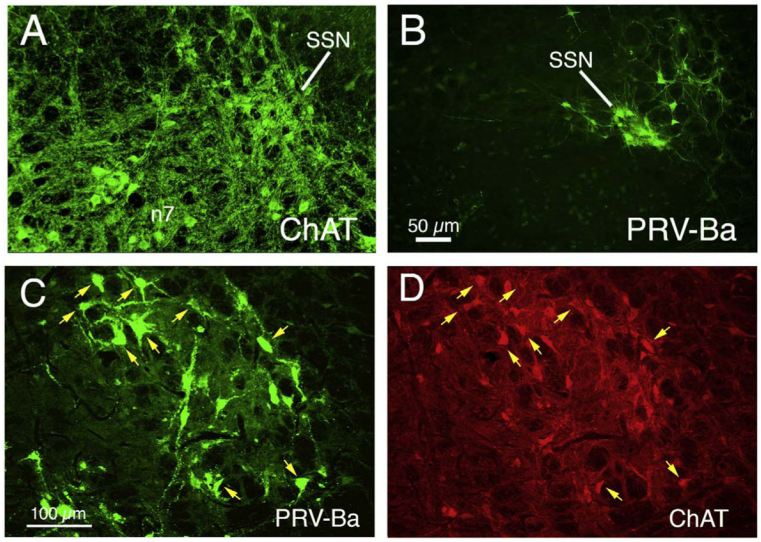

Fig. 8.

A pair of images of transverse sections of the SSN at its rostral level (**A, B**), showing neurons in rat SSN that are preganglionic to choroidal neurons of the PPG. Image **A** shows a section from a normal rat immunolabeled for choline acetyltransferase (ChAT), a marker of cholinergic neurons, while image **B** shows neurons in SSN transneuronally labeled 63 h after a pseudorabies injection into the choroid. The pair of images shows that SSN and facial motor nucleus (n7) neurons are ChAT+, and neurons regulating choroid are restricted to more ventromedial SSN. The magnification is the same in **A** and **B**. Images **C** and **D** show a single field of view of the SSN from tissue double-labeled by immunofluorescence for pseudorabies (**A**) and ChAT (**B**), from an animal that survived 65 h after ipsilateral virus injection into the choroid. The arrows indicate neurons within the SSN that were labeled for PRV from choroid and were cholinergic. These results show that the PRV + neurons within the SSN transneuronally labeled from the choroid were cholinergic preganglionic neurons, which represent a subset of SSN neurons. Images **C** and **D** are at the same magnification. All four fields are of the right side of the brain, with medial to the left and dorsal to the top.

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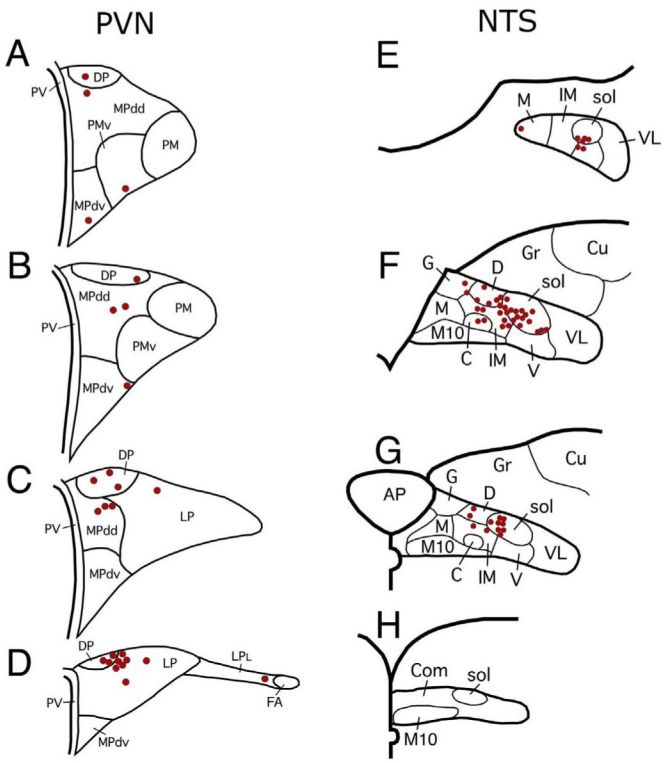

Fig. 9.

Schematic distribution of PRV + neurons in PVN, representing a composite of sections from two rats with minute intrachoroidal injection of PRV (**A–D**). Note that the majority of PRV + neurons are localized in the parvocellular subdivisions of PVN. Schematic distribution of PRV + neurons in NTS (**E–H**). Note that the majority of PRV + neurons are localized to the dorsal, intermediate and solitary tract subdivisions of NTS. PVN Abbreviations: DP - dorsal parvocellular subdivision of PVN; FA - fornical area of PVN; LP - lateral parvocellular subdivision of PVN; LP_L - lateral part of the lateral parvocellular subdivision of PVN; MPdd - dorsal part of the dorsal medial parvocellular subdivision of PVN; MPdv - ventral part of the dorsal medial parvocellular subdivision of PVN; PM - posterior magnocellular division of PVN; PMv - ventral part of the posterior magnocellular division of PVN; PV - periventricular region of hypothalamus. NTS Abbreviations: AP – area postrema; C - central subnucleus of NTS; Com - commissural subnucleus of NTS; Cu - cuneate nucleus; D - dorsal subnucleus of NTS; G - gelatinous subnucleus of NTS; Gr – gracile nucleus; IM - intermediate subnucleus of NTS; M - medial subnucleus of NTS; M10 - dorsal vagal motor nucleus; sol - solitary tract and subnucleus of NTS; V - ventral subnucleus of NTS; VL - ventrolateral subnucleus of NTS.

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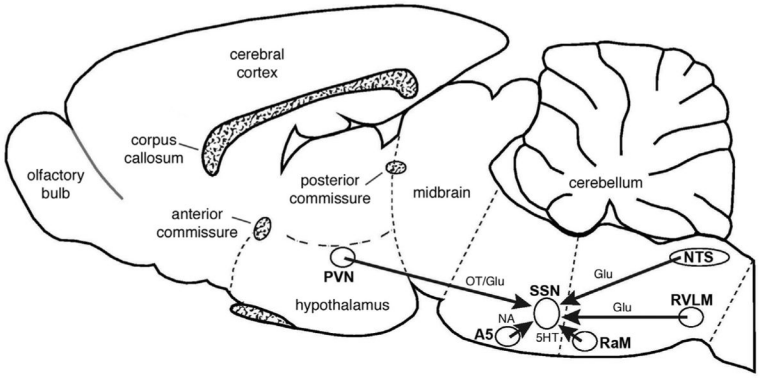

Fig. 10.

Schematic summarizing the major inputs to SSN and their neurotransmitters, as discussed in Li et al. (2015a). Abbreviations: Glu – glutamate; NA – noradrenaline; OT – oxytocin; 5HT – serotonin.

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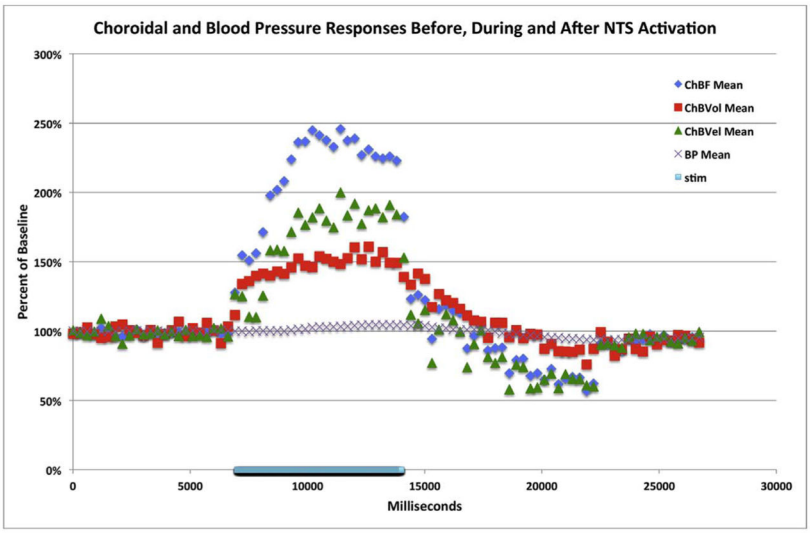

Fig. 11.

Graph showing the time course of the mean ChBF, ChBVol, ChBVel and ABP responses to stimulation at effective anodal NTS sites. The blue bar marks the stimulation period. Each data point is the mean for a 333 ms interval, and ChBF, ChBVol, ChBVel and ABP responses are all expressed as percent of basal. The rapid ChBF increases are driven by rapid increases in both ChBVel and ChBVol.

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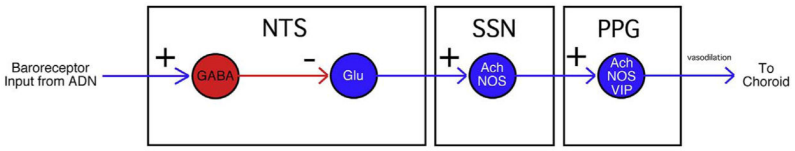

Fig. 12.

Schematic illustrating the hypothetical circuit by which baroreceptive input to the NTS may mediate disinhibitory control of choroidal vasodilation via the SSN.

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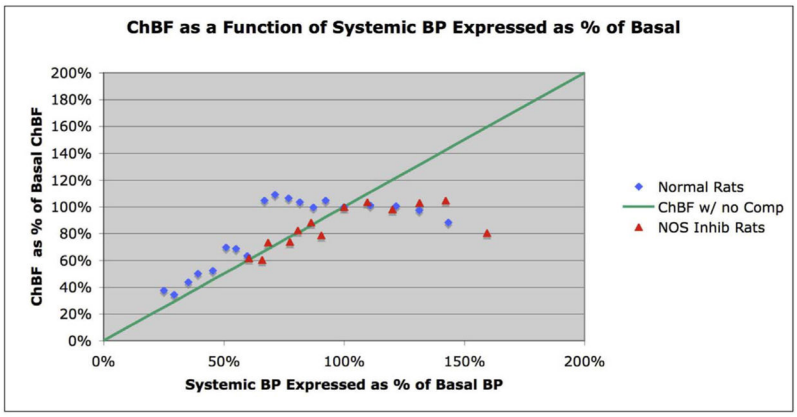

Fig. 13.

Baroregulation in rat choroid in which arterial BP was allowed to fluctuate spontaneously. Over a range of about 35% above and below basal blood pressure (BP), ChBF remains stable at about 100% of basal ChBF. Inhibition of neuronal NOS prevents ChBF baroregulation to low BP but not to high BP. Since the parasympathetic input to choroid in mammals from the PPG uses NO as a vasodilator, these data suggest that compensatory vasodilation of choroid to low BP is mediated by parasympathetic circuitry.

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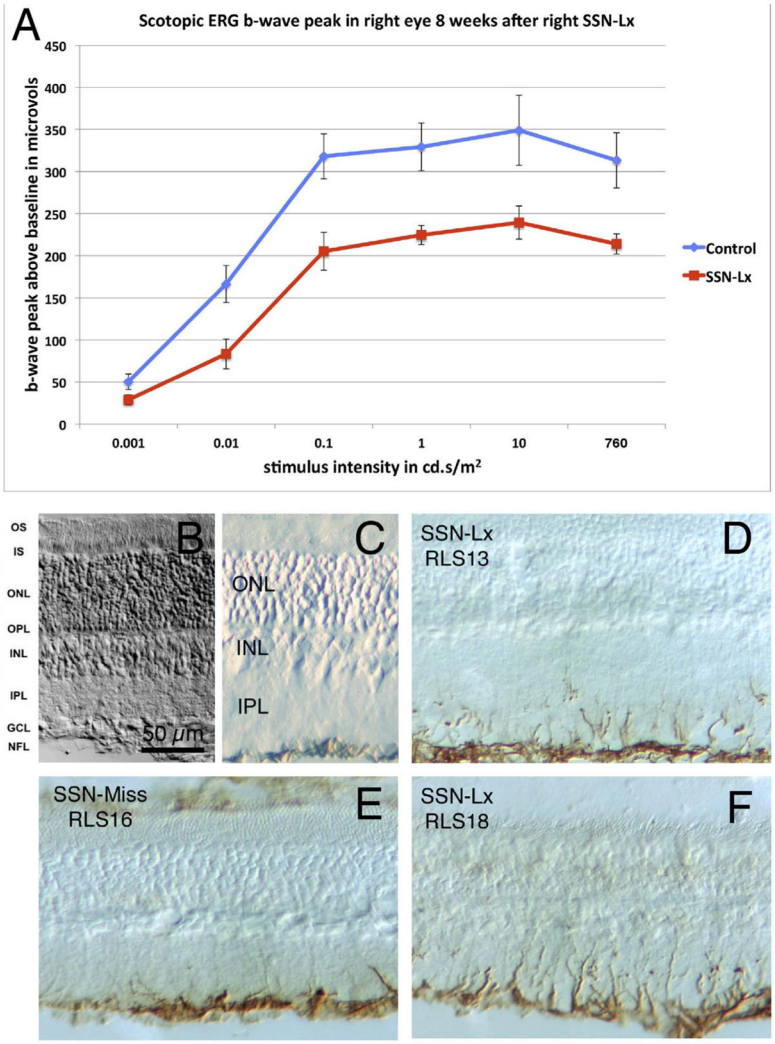

Fig. 14.

The effects of choroidal SSN destruction on retinal function and structure. At 8 weeks post-lesion, the flash-evoked scotopic b-wave ERG peak was significantly reduced for the right eye in right SSN-Lx rats (n = 10) compared to control rats (n = 9) for all light intensities (**A**). Intense GFAP immunolabeling was seen in Müller cell processes in the IPL of the right eye after right choroidal SSN destruction (**D**, **F**), but not in control retinas, which included SSN-miss cases (**E**) and normal control rats. GFAP upregulation occurred throughout the topographic extent of the retina (above images show superior retina). Magnification the same in B–F.

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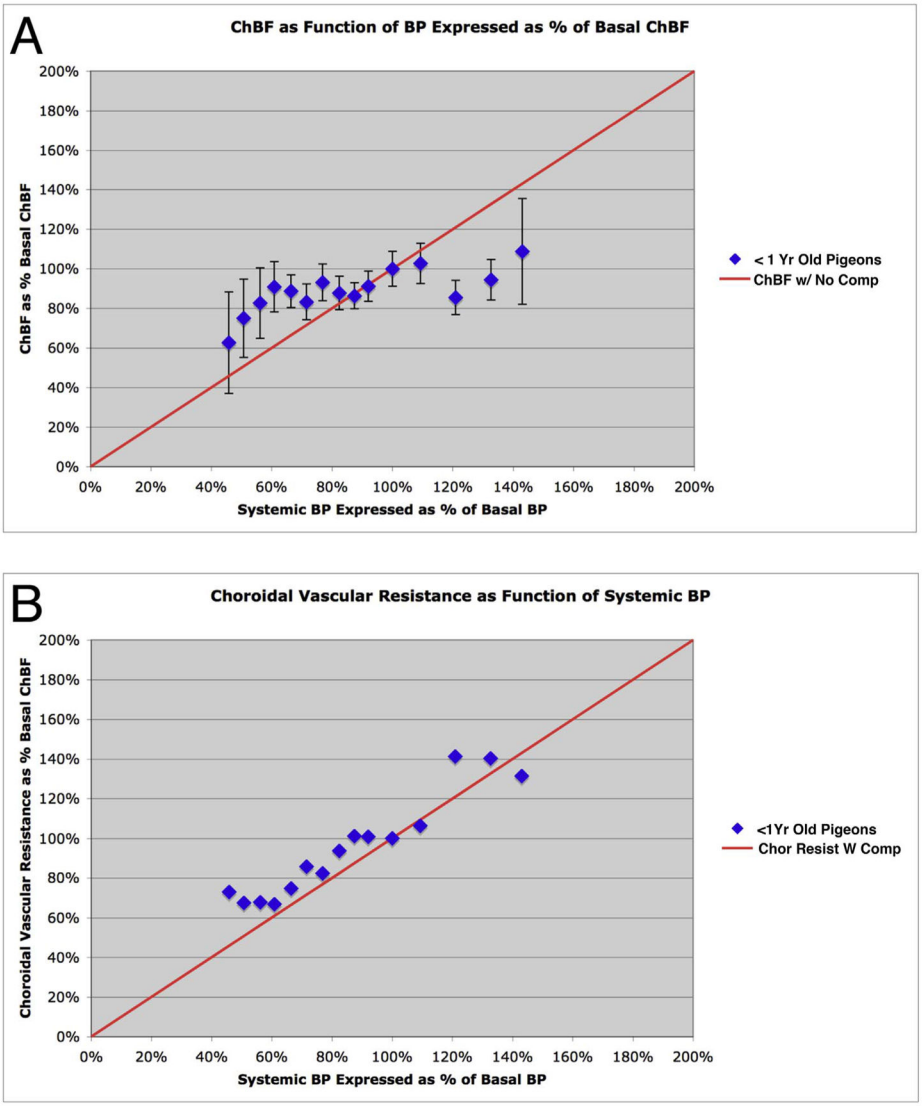

Fig. 15.

Graphs showing baroregulation in normal young pigeons. The graphs show mean ChBF (A) and choroidal resistance (B) (±SEM) plotted as a function of the corresponding ABP, over an ABP range of 40 mmHg–135 mmHg (45%–145% of basal ABP) in 50 normal young pigeons under a year of age. The mean ChBF and ABP are graphed as a percent of basal ChBF and ABP for the 50 < 1 year old pigeons. The red line in A shows ChBF as it would be if it linearly followed ABP, that is, with no compensation. Note that from about 30% above and 40% below basal ABP, ChBF remains between 80% and 100% of basal. The red line in B shows choroidal resistance as it would be if it linearly followed ABP, that is, with baroregulatory compensation. Note that choroidal resistance in the young pigeons decreased linearly as ABP declined from 40% above to 50% below basal ABP.

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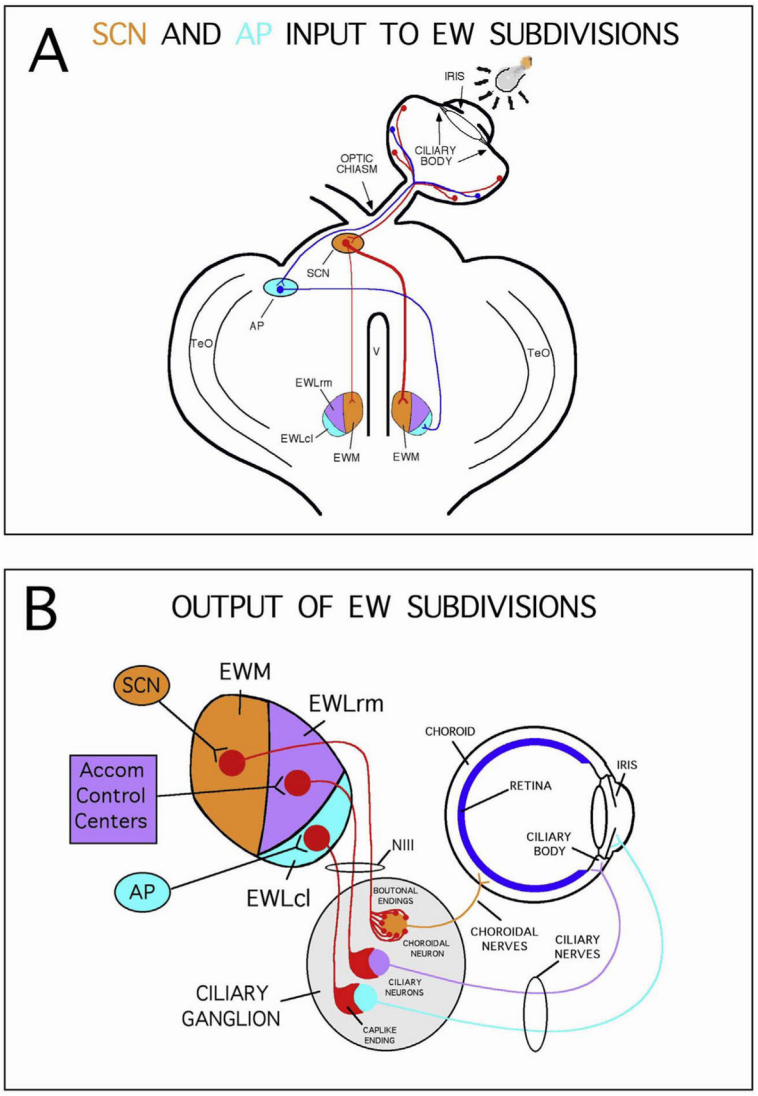

Fig. 16.

Schematized horizontal views of the midbrain and the eye (**A**) and of the nucleus of Edinger-Westphal (EW), the ciliary ganglion and the eye (**B**) showing the circuitry in pigeon for the bisynaptic retinal pathways to the nucleus of EW that drive ChBF increases and pupil constriction. The pathway shown in **A** with red lines depicts the crossed projection from retinal ganglion cells to the suprachiasmatic nucleus (SCN) that, in turn, has a bilateral (but greater contralateral than ipsilateral) projection to medial EW (EWM), which controls ChBF via its ipsilateral projection to choroidal neurons of the ciliary ganglion, as depicted in (**B**). The pathway depicted with blue lines in (**A**) shows a crossed projection from retinal ganglion cells to area pretectalis (AP), which then projects to the contralateral caudolateral part of lateral EW (EWLcl), which controls the pupillary light reflex (PLR) via an ipsilateral projection to pupilloconstrictive neurons of the ciliary ganglion, as depicted in **B**. The lower schematic (B) details the peripheral circuitry controlling ChBF and PLR, with EW, the ciliary ganglion (CG), and the eye, all in horizontal view. The EW projects ipsilaterally

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via the oculomotor nerve to the CG, where EWM input terminates with boutonal endings on choroidal neurons that project to choroidal blood vessels. Projections from both the rostromedial part of lateral EW (EWLrm) and from EWLcl terminate with cap-like endings on ciliary neurons that project to the ciliary body and the iris, and control accommodation and the PLR, respectively. The subdivisions of EW are color-coded in (**A**) and (**B**), and the projections of each to the eye via the ciliary ganglion in **B** follow the same color code. Other abbreviations: EWL - lateral subdivision of the nucleus of Edinger-Westphal; TeO - optic tectum.

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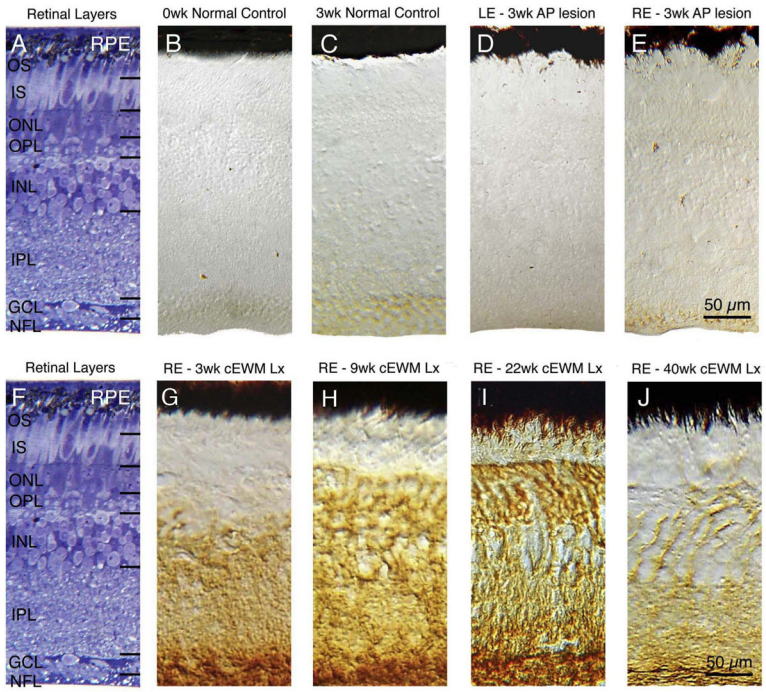

Fig. 17.

Images of representative GFAP-immunolabeled sections through the superior - central retina of normal pigeons (**B**, **C**), a pigeon with a left area pretectalis (AP) lesion (**D**, **E**), a pigeon that survived for 3 weeks with a complete right EW lesion (**G**), a pigeon that survived for 9 weeks with a complete right EW lesion (**H**), a pigeon that survived for 22 weeks with a complete right EW lesion (**I**), and a pigeon that survived for 40 weeks with a complete right EW lesion (**J**), all housed in a 12 h moderate light/12 h dark cycle. Images (**A**) and (**F**) show a 1 mm thick toluidine blue-stained plastic-embedded section of pigeon retina, with the different retinal layers delimited by hash marks, and the hash mark between the outer nuclear layer and the inner segment layer located at the outer limiting membrane. Retinal sections from a normal pigeon never housed in an individual cage, show no GFAP immunolabeling (**B**), while the retinal section from a normal pigeon housed for 3 weeks in an individual cage shows slight GFAP-immunostaining of the nerve fiber layer (NFL) and ganglion cell layer (GCL) (**C**). The lesion of left AP eliminated the pupil light reflex and chronically dilated the pupil of the right eye. In the left eye, GFAP-immunolabeling is weak and does not extend beyond the NFL (**D**), while GFAP-immunolabeling in the right eye fills Müller cell processes into the GCL (**E**). The image of the right eye (**G**) of a bird 3 weeks after a complete lesion of both the right EWM and EWL shows GFAP immunolabeling fills Müller cell processes into the inner nuclear layer (INL). The image of the right eye (**H**) of a bird 9 weeks after a complete lesion of right EWM and EWL shows GFAP-immunolabeling extends through the outer plexiform layer (OPL). The image of the right (**I**) eye of a bird 22 weeks after a complete lesion of EWM and EWL shows GFAP-immunolabeling extends through the outer plexiform layer to the outer limiting membrane (OLM). Finally, the image of the right eye (**J**) of a pigeon 40 weeks after a complete lesion of EWM and EWL shows GFAP-immunolabeling filled the Müller cell processes through the INL. Abbreviations:

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GCL - ganglion cell layer; INL - inner nuclear layer; IPL, inner plexiform layer; IS - inner segment layer; NFL - nerve fiber layer; OLM - outer limiting membrane; OS - outer segment; and RPE - retinal pigment epithelium. Magnification the same in all images.

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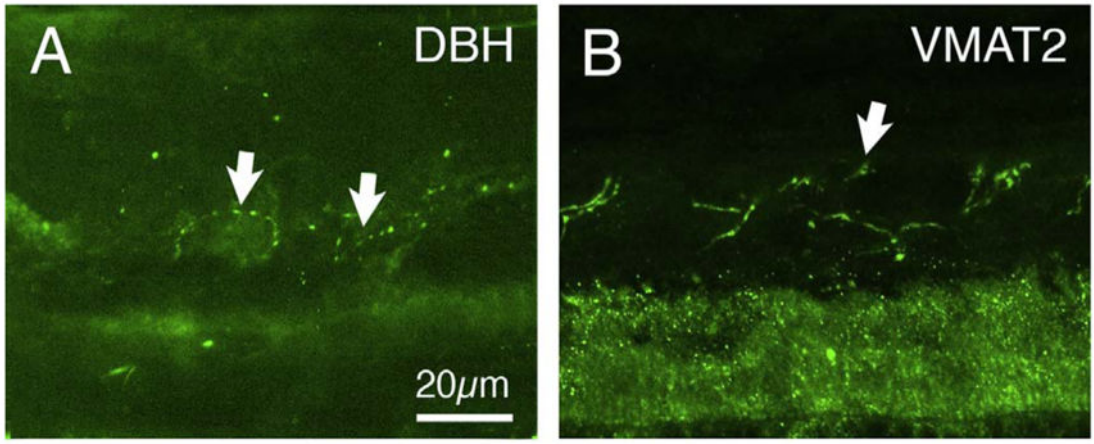

Fig. 18.

Images **A** and **B** show sympathetic nerve fibers with varicosities in rat central choroid immunolabeled for dopamine beta-hydroxylase (DBH) and vesicular monoamine transporter-2 (VMAT2). Both images are at the same magnification.

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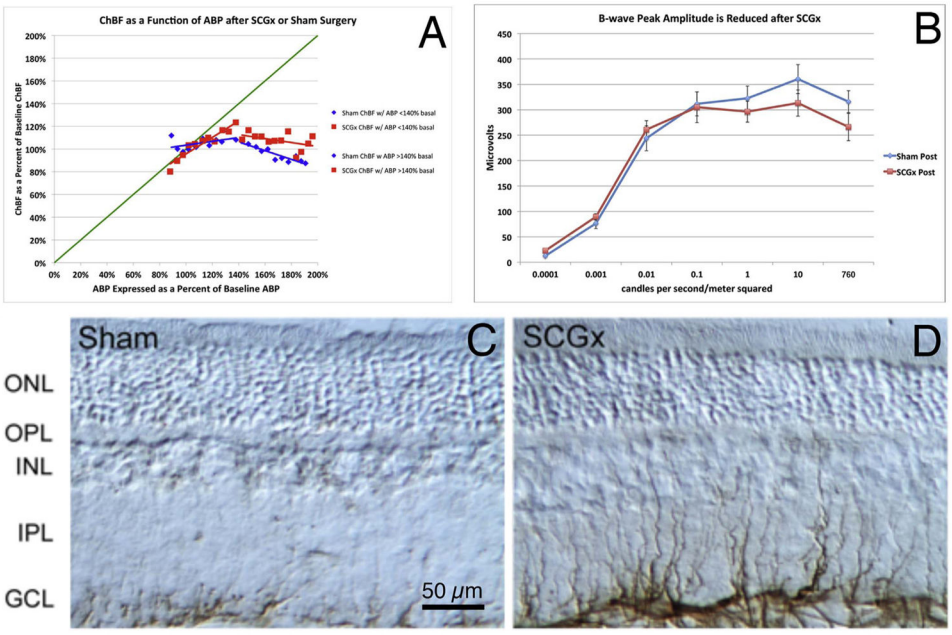

Fig. 19.

At 2–3 months post-SCGx, choroidal baroregulation during high arterial BP was impaired. Image **A** shows a plot of ChBF as function of arterial BP for sham rat eyes (n = 14) and SCGx rat eyes (n = 14). As ABP rapidly rose above baseline after LNAME administration, ChBF in sham eyes remained relatively stable but followed ABP in SCGx eyes. After ABP had stabilized at an elevated level, ChBF in SCGx eyes declined toward baseline but remained elevated compared to sham eyes. The impairment in ChBF baroregulation was associated with a deficit in the flash-evoked scotopic b-wave ERG peak, which was significantly reduced for SCGx eyes compared to control eyes (**B**). Additionally, GFAP immunolabeling of Müller cells was increased in retina by 3 weeks after SCGx (**C**, **D**). The immunolabeled Müller cell processes in SCGx eyes traversed the IPL and some extended into the INL (**C**). By contrast, in control retinas, GFAP labeling of Müller cell processes did not extend much beyond the GCL (**D**).

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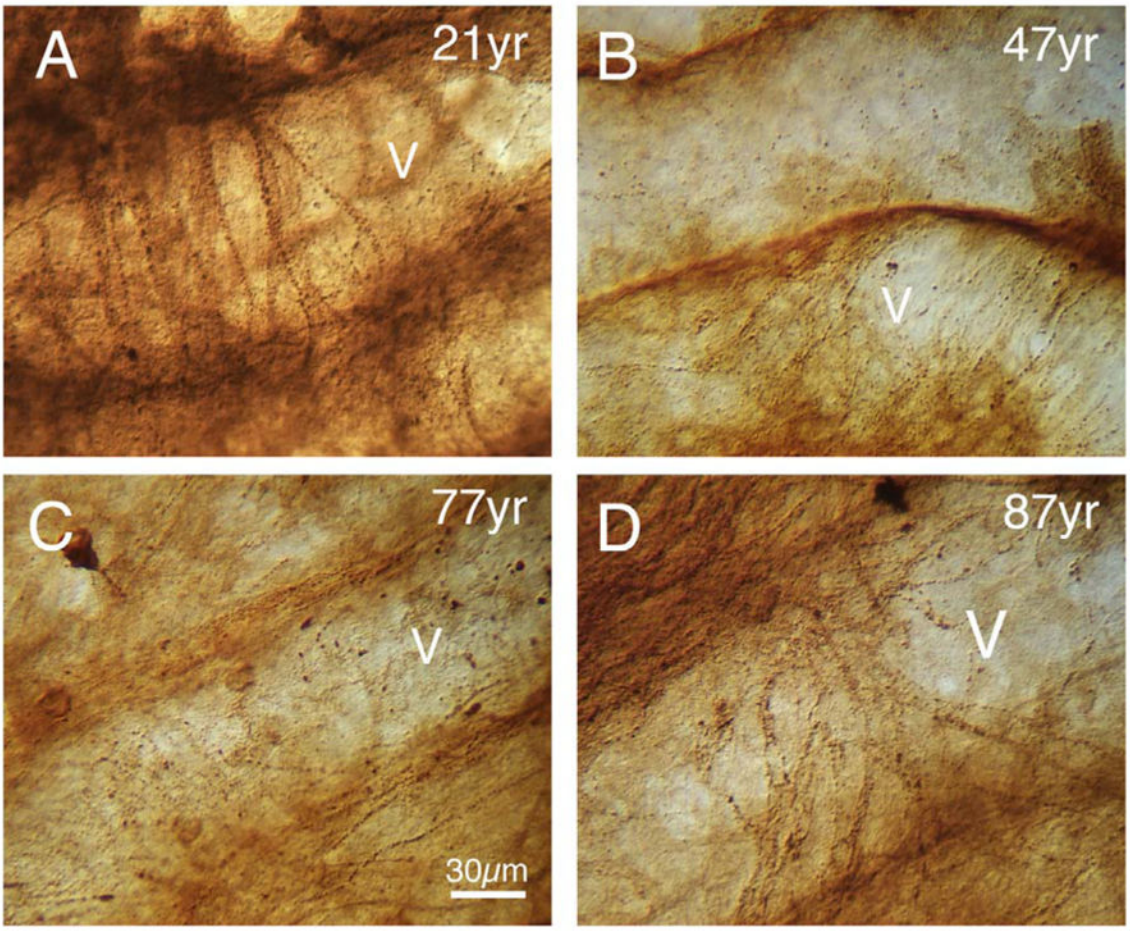

Fig. 20.

Examples of VIP immunolabeling of nerve fibers on macular choroidal vessels (V) in humans of differing age. The abundant beaded striae running across the vessel lumens are the VIP-positive fibers. Note the generally lesser amount of VIP-positive fibers in elderly compared with young eyes. Note also the VIP + intrachoroidal neuron in **C**. Magnification is the same in all images.

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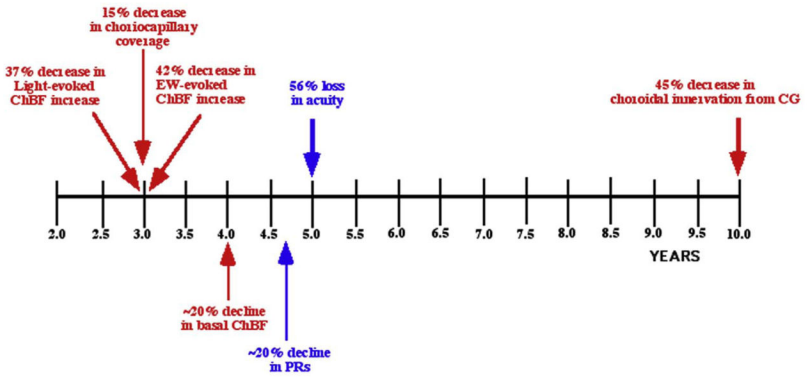

Fig. 21.

A time line summarizing the ages by which or at which significant change in visual and choroidal parameters occurred in our sample of pigeons. The age by which 50% loss had occurred for the retinal illumination and EWM-evoked ChBF responses was around three years (2.9 and 3.16, respectively). Despite the early decline in the function of the EWM-ciliary ganglion circuit, the age by which 50% of the loss in the ciliary ganglion innervation of the choroid was not until 10 years. These results suggest that the functional decline in this circuit precedes the actual intrachoroidal fiber loss. These findings are compared with findings for basal ChBF, choriocapillary vessel abundance, photoreceptor loss and acuity decline, and choroidal parameters. The half loss point for choriocapillary vessels was also at 3 years, but the significant drop in photoreceptor abundance and the 50% loss for acuity did not occur until after the major declines in the various choroidal vascular and functional parameters.

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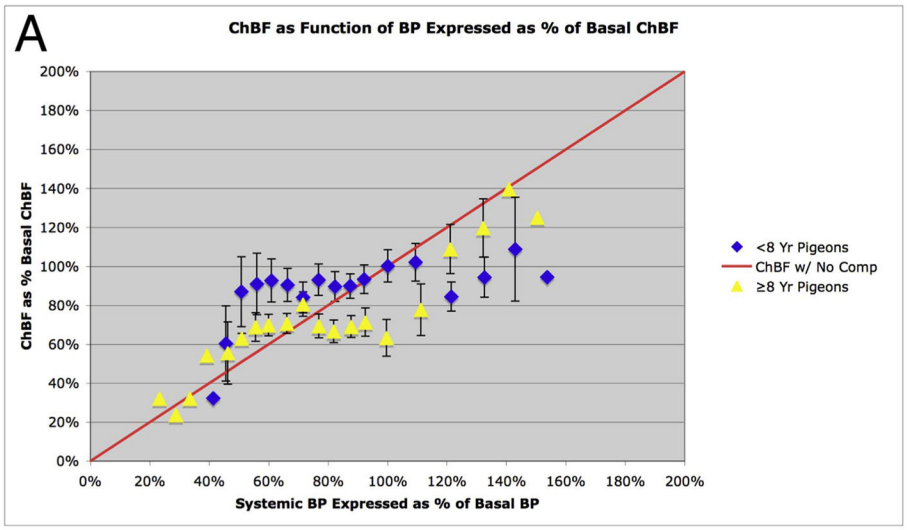

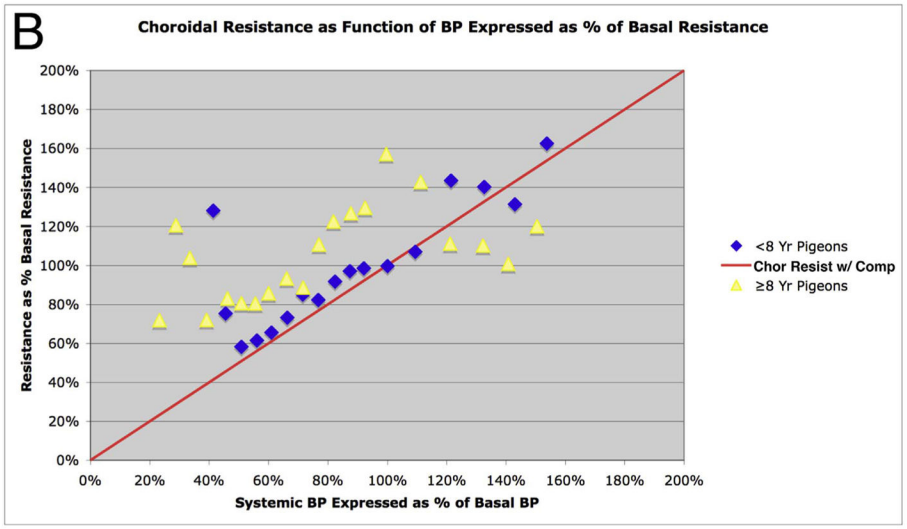

Fig. 22.

Graphs comparing ChBF (**A**) and choroidal resistance (**B**) (±SEM) as a function of corresponding ABP in young pigeons (< 8 years) and old pigeons (< 8 years) over an ABP range of 20–150 mm Hg (20%–150% of basal ABP). The blue diamonds show the ChBF for young pigeons, whereas the yellow triangles show the ChBF for the old pigeons. The mean ChBF and ABP are graphed as a percentage of basal ChBF and ABP for all 59<8-year-old pigeons. The red line in the graph in **A** shows ChBF as it would be if it linearly followed ABP (i.e., with no compensation), whereas the red line in B shows choroidal resistance as it would be if it linearly followed ABP (i.e., with baroregulatory compensation). As detailed in the text, ChBF and choroidal resistance with ABP differed significantly in the old pigeons from that in the young pigeons, and baroregulation was clearly impaired.

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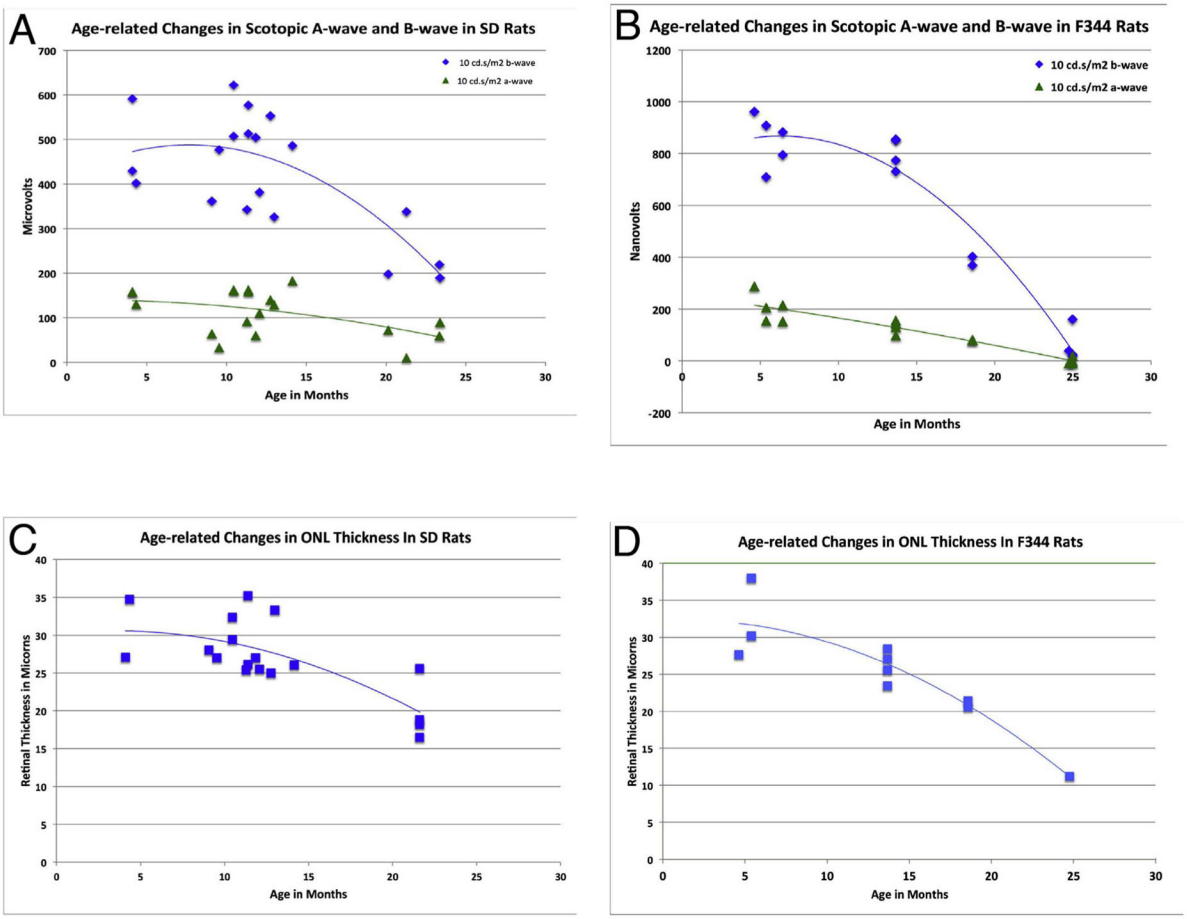

Fig. 23.

Retinal declines were more rapid and severe in Fischer-344 (F344) rats than in Sprague-Dawley (SD) rats. The flash-evoked scotopic b-wave peak and inverted a-wave peak showed significant curvilinear age-related declines (r = 0.743 and r = 0.507, respectively) in SD rats (**A**). The flash-evoked scotopic b-wave peak and inverted a-wave peak showed significant curvilinear age-related declines (r = 0.967 and r = 0.938, respectively) in F344 rats (**B**). ONL thickness measured using Neurolucida showed a significant curvilinear age-related decline (r = 0.747) in SD rats (**C**). ONL thickness measured using Neurolucida also showed a significant curvilinear age-related decline (r = 0.912) in F344 rats (**D**).

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Table 1

Listing the sources of neural input to the choroid, the vasoactive substances the inputs use, the higher central circuits having input to them, and whether or not the circuit has been demonstrated in mammals and/or birds.

Type of Input to Choroid	Ganglion of Origin for Input to Choroid	Neurotransmitters Utilized by Input to Choroid	Preganglionic Input to Ganglion having Input to Choroid	Brain Input to Preganglionic Neurons	Is Circuit Present in Mammals	Is Circuit Present in Birds
Parasympathetic	PPG	Ach, VIP, NO	SSN	PVN, NTS, RaM	Yes	Yes
Sympathetic	SCG	NA, NPY	C8/T1 intermediolateral column - spinal cord	unknown	Yes	Yes
Sensory	Trigeminal	SP, CGRP	None	none	Yes	Yes
Parasympathetic	CG	Ach	EW	medial SCN	Uncertain	Yes
Intrinsic	None	VIP, NO	unknown	unknown	Fovea in Primates	Yes