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Mechanisms of Adaptation to Mean Light Intensity in the Chick Retina

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Mechanisms of Adaptation to Mean Light Intensity in the Chick Retina

by

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Abstract

Through adaptation, animals can function visually under an enormous range of light intensities. Adaptation to changes in light intensity takes place early, in the retina.

One role of adaptation is to regulate the spatiotemporal tuning of retinal outputs via retinal ganglion cells. In my project, I used the optokinetic response (OKR) to characterize contrast sensitivity (CS) in the chick retina as a function of spatial frequency and temporal frequency at different mean light intensities. I first found that adaptation from light to dark caused a shift in tuning preference of CS (Chapter 2). In the daytime, photopic CS of the chick was tuned to spatial frequency; whereas in the nighttime, scotopic CS was tuned to temporal frequency. Later, I explored mechanisms that modulated spatiotemporal CS under different adaptational conditions (Chapter 3). Since dopamine (DA) and nitric oxide (NO) are putative light-adaptation messengers in the retina, I injected agents affecting DA and NO actions and gap junction coupling into the eyes (vitreous bodies) of dark- or light-adapted chicks. Finally, I investigated the role of cell-cell coupling, a downstream mediator of DA or NO actions in the retina, in adaptation. I demonstrated that the chick uses a similar strategy to that used by mammals, to adapt to ambient illumination; and that DA, NO, as well as cell-cell coupling are adaptation-sensitive modulators of spatiotemporal visual processing in the retina.

Optokinetic CS is a rapid and noninvasive method for assessing retinal function, which can be manipulated rapidly, conditionally and reversibly by intravitreal injection of specific pharmacological agents. The chick's large eyes, and the similarities of control of light adaptation in chick and other species (e. g., mouse), make the chick a powerful new model for retinal research.

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The help I got to get this thesis finished. Chapters 1 and 4 were written entirely by me, with limited comments and suggestions by Dr. Stell. Chapters 2 and 3 were written collaboratively with my co-author, Dr. Stell. Chapter 2 has been published (PLoS ONE 8(9):e75375) and benefited from the helpful comments of the PLoS ONE editor (Dr. W. Rowland Taylor) and reviewers (Dr. Eduardo Solessio and an anonymous reviewer). PLoS ONE is an open journal that permits republication here without requiring permission. Chapter 3 has been submitted for publication to the Journal of Neuroscience; an editorial decision and reviews have not been received at the time of submitting this PhD Thesis to my Examining Committee. I performed all of the experiments described in Chapters 2 and 3 entirely on my own. Regardless of the contributions of others, I take full responsibility for the design and conduct of the experiments, and for the resulting data and my interpretations of them, as presented in this Thesis.

Family and friends. I would like to thank my loving and supportive parents and grandparents, for letting me leave them and do what I wanted to do. I also want to thank my kind and generous friends in Calgary that made my adaptation to a completely new place much easier.

To “Dr. B”

If I were to go through this journey again, I would still choose you over a two-photon microscope.

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List of Figures and Illustrations

List of Abbreviations and Symbols

AC	Amacrine cell
AOS	Accessory optic system
BC	Bipolar cell
Cd/m^2	Candela per meter squared
CS	Contrast sensitivity
Cx	Connexin
Cyc/deg	Cycles per degree
Cyc/s	Cycles per second
D	Dark
DA	Dopamine
DMSO	Dimethyl sulfoxide
DSGC	Direction-selective ganglion cell
D-NMMA	D-N^G-monomethyl arginine acetate
D1R	D1 dopamine receptor
D2R	D2 dopamine receptor
GCL	Ganglion cell layer
HC	Horizontal cell
INL	Inner nuclear layer
IPL	Inner plexiform layer
L	Light
L-NIO	L-N5-(-1-Iminoethyl)ornithine hydrochloride
L-NMMA	L-N^G-monomethyl arginine acetate
MFA	Meclofenamic acid
MFQ	Mefloquine
nBOR	Nucleus of basal optic root
ND	Neutral density
NO	Nitric oxide
NOS	Nitric oxide synthase
OKR	Optokinetic response

ONL	Outer nuclear layer
OPL	Outer plexiform layer
RGC	Retinal ganglion cells
SD	Standard deviation
SF	Spatial frequency
sGC	Soluble guanylyl cyclase
SNAP	S-nitroso-N-acetyl-DL-penicillamine
SNP	Sodium nitroprusside
TF	Temporal frequency
TH	Tyrosine hydroxylase
V	Velocity

Chapter 1: Introduction

In the ever-changing world, animals self adjust when moving from one environment into another. This self-adjustment process is known as adaptation. Visual adaptation includes many aspects – mean light intensity, color, contrast, etc. – of which adaptation to mean light intensity is extremely crucial, since we can experience a factor of 10^9 change in ambient illumination in a single day, a range so enormous that it can potentially exhaust our visual system.

Visual adaptation occurs at every stage of the visual system (for review, see Kohn, 2007), starting in the retina where vision begins. The retina is an elegantly structured neural tissue (Figure 1.1) that comprises three cellular layers interleaved with two synaptic layers, the outer plexiform layer (OPL) and the inner plexiform layer (IPL), where retinal neurons interact with each other. Visual processing begins when photoreceptors transduce light into electrical signals and relay light information to bipolar cells (BCs), which then pass signals to retinal ganglion cells (RGCs), the final output of the retina. The photoreceptor-BC-RGC flow of information forms the vertical conduit in the retina, although rod pathways of the mammalian retina rewire according to ambient lighting conditions (for review, see Bloomfield and Dacheux, 2001). From the very first synapse in the outer retina, i. e., the photoreceptor-BC synapse, retinal circuits are split into the ON- and OFF pathways, which upon light stimulation code increase and decrease in illumination, respectively. The vertical pathway is finely shaped by inputs from horizontal cells (HCs) in the outer retina and by those from amacrine cells (ACs) in the inner retina. Therefore, the retina is not simply a light transducer; it conducts a variety of computation and processing before sending signals to higher visual centers (Gollisch and Meister, 2010). Retinal adaptation to mean light intensity can be categorized into photoreceptoral (receptoral) adaptation and post-

receptoral, or network adaptation, depending on where it takes place. The work presented below is focused on retinal network adaptation.

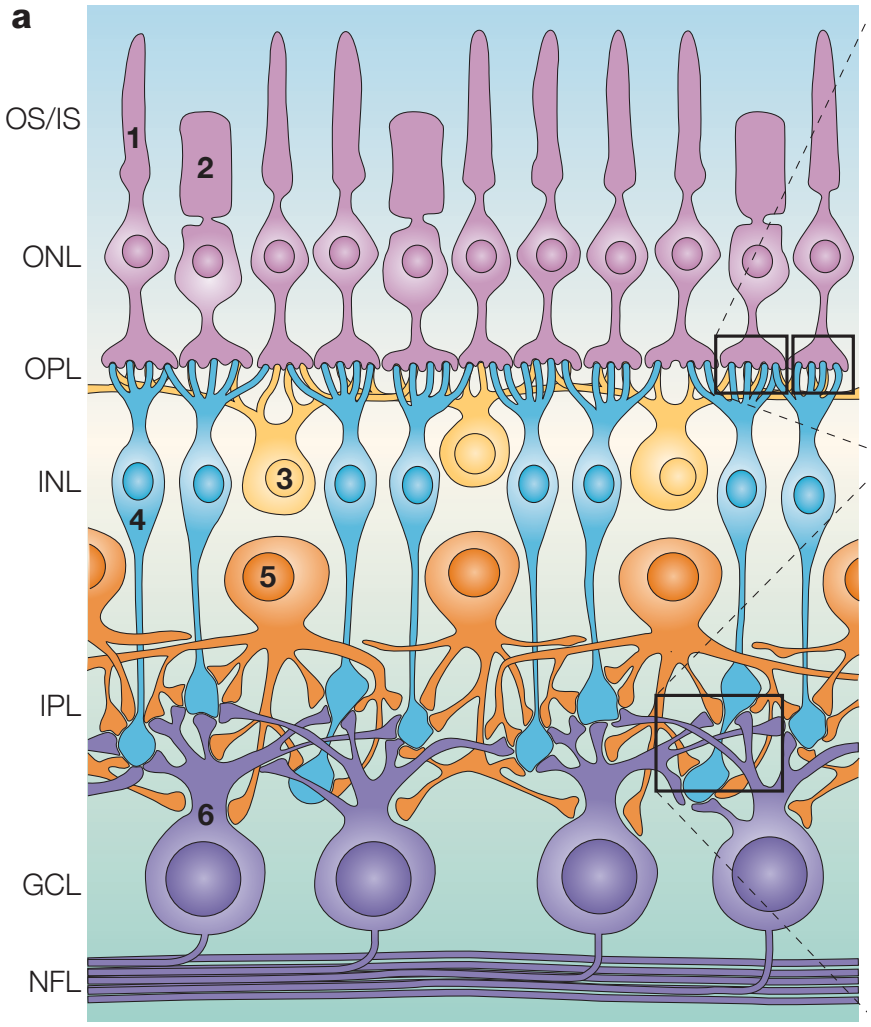

Figure 1.1 Layers of the retina. OS/IS, outer and inner segments of rods and cones; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; NFL, optic nerve fiber layer. Reproduced from Wässle, 2004, with permission of the publisher.

Contrast Detection and Sensitivity

"Mean light intensity" is not simply a property of a single pixel of an image. Instead, it influences spatial and temporal integration by neurons at each level of the visual hierarchy. Spatial and temporal integration depends on the receptive field of the cells involved. Except for photoreceptors, whose receptive center is their own outer segments, the rest of retinal neurons of the vertical pathway – BCs, RGCs – have center-surround receptive field. The center of a retinal cell's receptive field is generally its dendritic field that receives inputs directly from the presynaptic cell, which is either a photoreceptor or a BC. The surround part of the receptive field, on the other hand, is typically the input from interneurons, namely, HCs and ACs. In other words, the ultimate input a retinal neuron receives is a combination of direct synaptic or primary sensory signals from vertical pathway, and indirect lateral signals from lateral interneurons, the latter of which often attenuate the strength of the former. The significance of the center-surround antagonism can be illustrated in the example below: for an ON-type RGC, a stimulus that contains a brighter center and a darker surround elicits larger response, for the darker periphery inhibits the inhibitory component. In contrast, a uniformly bright stimulus of the same size yields a weaker response because the inhibition caused by the bright surround counteracts the center response (Figure 1.2). As a result, the center-surround antagonism makes the retina more of a contrast detector than a uniform-stimuli detector, and contrast detection has long been used as a reliable visual indicator.

Contrast detection is affected by ambient illumination. Human psychophysical studies (Campbell and Robson, 1968; Pasternak and Merigan, 1981) showed that under reduced luminance, subjects' peak contrast sensitivity (CS) and CS at higher SF decreased compared to that tested at

higher luminance (Figure 1.4). Similar observations were made in cats and mice (Pasternak and Merigan, 1981; Umino et al., 2008). In my thesis, CS was used as a measure of retinal function.

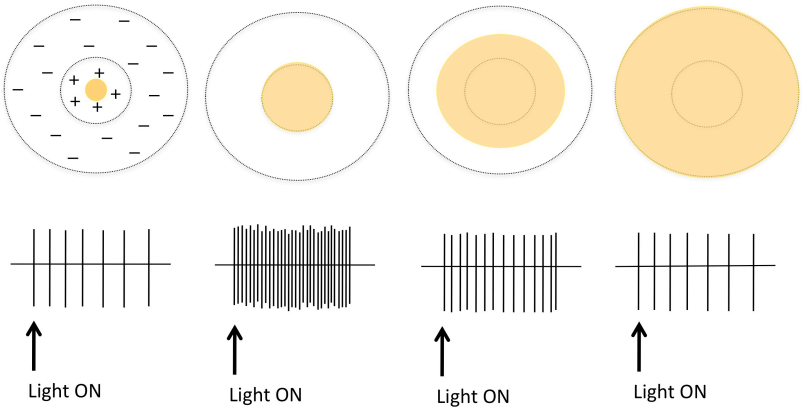

Figure 1.2 Center-surround antagonistic receptive field of an ON-center RGC. Arrow indicates onset of light stimulus.

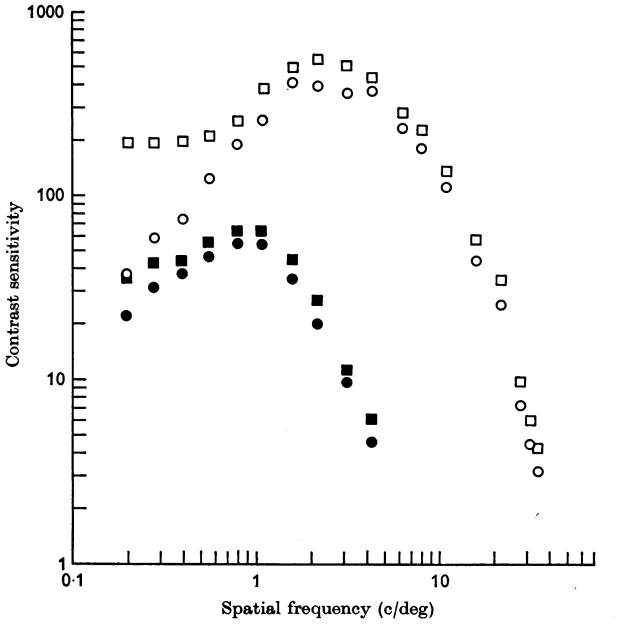

Figure 1.3 Contrast sensitivity for sine-wave gratings (circles) is compared with that for square-wave gratings (squares) for subject FWC. Upper pairs of curves for luminance of 500 cd/m^2. Lower pair of curves for luminance of 0.05 cd/m^2. Figure from Campbell and Robson (1968), used with publisher's permission.

The Optokinetic Response (OKR), Accessory Optic System (AOS), and Direction-selective Ganglion Cells (DSGCs)

Retinal behaviors can be studied in many ways. The electroretinogram, for example, features mainly in noninvasively recording field potentials from populations of cells in the outer-middle retina, photoreceptors and BCs, for example, whereas it cannot rigorously examine properties of the inner retina, e. g., responses of ACs and RGCs. *In vitro* methods, which can target on retinal responses more specifically to a certain type of cell, such as patch clamping, sharp-electrode single cell recording, multielectrode array (MEA), often require dissecting the eye, isolating the retina, and sometimes cutting the retina into thin sections. These techniques, however, also have their limitations: 1) isolated retinas, especially those from mammals, need careful maintenance for long-term recording; 2) patch-clamping electrodes cannot go too deep into tissue due to potential contamination of their relatively large tips, this requires experimenters either to study cells easily approachable, or to use retinal sections, which will truncate processes of cells with large dendritic tree, such as HCs, wide-field ACs and RGCs, and thereby influence their physiology; 3) MEA, although it can gather a huge number of data simultaneously, a) can only measure responses of cells on the surface of the tissue- the RGCs in the retina; whereas a special type of RGCs – the displaced RGCs, whose somata reside at the border between inner nuclear layer (INL) and IPL in certain species – can hardly be recorded using the MEA; b) requires massive post-recording analysis, and c) one can only start data analyzing – e. g., looking at responses of certain type of cells – after the entire recording session has been completed; 4) removing the optic system of the eye can change the visual environment or expose the retina to something completely different than the natural scene. Psychophysical testing such as two-alternative forced choice task, on the other hand, usually involves computation or inputs from

higher nervous centers, and thereby cannot serve as a good representative of retinal properties. Moreover, long-term training of the animals in many psychophysical studies can be laborious and more importantly, visual properties during early development cannot be easily obtained before learned behaviors have been established. Training certain species, such as turtle, fish, frog, etc., can be simply challenging, even though their retinas have served as good models in retinal research for quite long due to 1) their good color vision (turtle and fish), 2) the presence of certain motion-detecting RGCs (the "fly detector") in frog (Grüsser et al., 1968), 3) the large size of their cells; hence, ease of single-cell recording, and 4) ease of maintenance of their retinas *ex vivo*.

In my project, a noninvasive, fast, reflexive, visually guided behavior – the optokinetic response (OKR) – was used to assess retinal function. The visual world is constantly changing, because of self movement and/or environmental movement, resulting in relative movement of the image on our retina. The OKR is one of the two strategies animals use to stabilize slipping image on the retina; the other is the vestibulo-ocular response, which is a reflex compensatory to the OKR, driven by inputs from the vestibular system and will not be discussed further here. The OKR refers to the turning movement animals make when attempting to track the moving scene. It can be either an eye movement (the optokinetic nystagmus) or a head movement (the optocollic response), or both, with the ultimate goal of fixating image on the retina. The OKR is the behavioral readout of the accessory optic system (AOS). The retinal input to the AOS comes from a specific type of RGC- the direction-selective (DS) RGCs, or DSGCs. They respond robustly to visual stimulus moving in a certain direction (preferred direction) whereas exhibit little or no responsiveness to stimulus moving in the opposite direction (null direction) (Barlow

and levick, 1965). So far, ON, ON-OFF, and OFF DSGCs have been described in mammals (for review, see Vaney et al., 2012). The ON-OFF type of DSGCs have been well characterized in the rabbit retina (Barlow and Hill, 1963), the four subtypes of which code motion in four cardinal directions- superior, inferior, anterior, and posterior (Oyster and Barlow, 1967). These cells have been reported to provide input to subcortical visual pathways in mammals (Huberman et al., 2009; Kay et al., 2011). The OKR-driving DSGCs are the ON-type DSGCs. The ON DSGCs code directions in three ocular axes- inferior, superior-temporal, and superior-dorsal (Oyster and Barlow, 1967), aligned with the three vestibular axes. The OKR-mediating DSGCs project mainly to the medial terminal nucleus (MTN) of the AOS in mammals, or nucleus of the basal optic root (nBOR) in nonmammalian species. In birds, the displaced RGCs of Dogiel – a special subtype of ON DSGCs that reside between INL and IPL – have been shown to be the exclusive retinal source of the nBOR (pigeon: Karten et al., 1977; chick: Mey and Johann, 2001). The nBOR projects to the oculomotor nuclei and a few other areas in the brain (Brecha and Karten, 1979) (Figure 1.3). The oculomotor nuclei then innervate ocular muscles, forming the motor limb of the reflex. In mammals studied so far, the AOS nuclei do not project directly to the oculomotor nucleus, but to the accessory oculomotor nucleus, which innervates neck muscles for optokinetic head movement (Giolli et al., 2006). In this sense, the OKR is the behavioral readout of the responses of ON DSGCs or displaced RGCs, and can be used to study the properties of these cells.

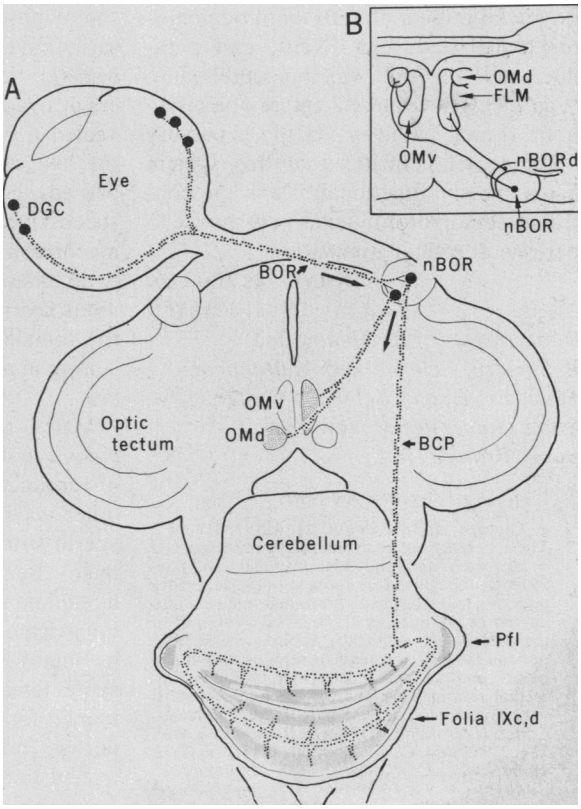

Figure 1.4 A schematic of the displaced RGC projection onto the nBOR complex and the nBOR complex projections onto the oculomotor nuclear complex and the vestibulocerebellum. DGC, displaced ganglion cell; BCP, brachium conjunctivum; BOR, basal optic root; nBOR, nucleus of the basal optic root; nBORd, nucleus of the basal optic root pars dorsalis; Pfl, paraflocculus. Reproduced with publisher's permission.

The OKR has served as a method in retina research since decades ago. In the lab, it is often evoked using a horizontally rotating cylindrical drum or arc whose inner surface was painted with black and white bars. Whereas the OKR is a "simple" behavioral test to perform, these "drifting drums" were very limited in the stimuli they could provide, and the drifting speed could not be easily manipulated. Fortunately, Prusky and colleagues (2004) revolutionized OKR testing by designing a virtual-reality optomotor system – OptoMotry® – which uses computer monitors as a display of visual stimuli and a camera to capture the behavior of the animal being tested. With the OptoMotry®, we can easily alter spatial frequency (SF), velocity (V), temporal

frequency (TF), and contrast (defined as Michelson contrast= I_{max}-I_{min}/I_{max}+I_{min}, where I_{max} and I_{min} represent maximum and minimum intensity), to study the contrast sensitivity (CS) function against these parameters. Furthermore, mean luminance of the OKR environment can be easily altered by inserting neutral density (ND) filters inside OptoMotry®. Contrast sensitivity is defined as the reciprocal of the threshold contrast, the lowest contrast at which OKR can be evoked. Additionally, the axis of the rotation of the virtual cylinder can be shifted to coincide with the center of animal's head, so that the spatial frequency will not decrease as the animal moves toward the screen or increase as the animal moves away from it – a common problem encountered when the "OKR cylinder" was being used.

Retinal Dopamine, Nitric Oxide, and Cell-cell Coupling

Some retinal neural messengers act as mediators of light adaptation. Dopamine (DA), for example, has been well known to mediate light-adapted retinal function (Witkovsky, 2004). Retinal DA is synthesized upon light stimulation (Godley and Wurtman, 1988; Boatright et al., 1989; Kirsch and Wagner, 1989), by DAergic ACs or interplexiform cells. In these cells, tyrosine hydroxylase – the rate-limiting enzyme in DA synthesis – converts tyrosine, the precursor for DA, into 3, 4- dihydroxyphenylalanine (L-DOPA), which in turn is converted into dopamine by DOPA decarboxylase. Dopamine exerts its effect through two main groups of DA receptors, D1-like receptors and D2-like receptors. D1-like receptors include D1R and D5R, whereas D2-like receptors include D2R, D3R, and D4R. Both groups of receptors are G-protein coupled receptors; they differ in that upon binding DA, D1-like receptors activate adenylate cyclase and cause an increase in rate of cAMP synthesis, whereas D2-like receptors do the opposite. Both rods and cones express D2-like receptors (Muresan and Besharse, 1993). D2R is also present in

DAergic cells themselves (Veruki, 1997), serving as so-called autoreceptors that suppress DA release when activated (Dubocovich and Weiner, 1985). D1R, on the other hand, is found in BCs, HCs, ACs, and RGCs (Veruki and Wässle, 1996; Nguyen-Legros et al., 1997). Aside from retinal neurons, the retinal pigmented epithelium contains D5R (Versaux-Botteri et al., 1997); Müller cells, the main type of glia in the retina, possess D2R (Biedermann et al., 1995). In the chick retina, there has been anatomical evidence for both D2R and D4R. Rohrer and Stell (1995) found that antibody against D2R labeled photoreceptor inner segments, inner and outer plexiform layers (IPL, OPL), and ganglion cell layer (GCL) of the chick retina. Using in situ hybridization, they found the mRNA of D4R to be mainly present in cell bodies in the proximal one third of INL and in GCL (Rohrer and Stell, 1995).

Dopamine mediates a number of light-adapted properties in the retina. Retinal regulation of eye growth, for example, requires the retina to be well illuminated. In myopia models produced in the laboratory, exposing the previously induced myopic eye to bright light for periods of normal vision can halt excessive eye growth (Napper et al., 1997). Intravitreal injection of DA agonists has been found to mimic the light-induced effect (Feldkaemper and Schaeffel, 2013; Stone et al., 2013; Cohen et al., 2012). Another light-induced characteristic of vision is Purkinje shift, a shift in the peak spectral sensitivity from relatively long wavelength in the daylight to shorter wavelength at dusk (Wald, 1945). Purkinje shift reflects a shift from cone- to rod-dominated vision (or the other way around). In the Japanese quail, applying D2R agonist in dark-adapted eye shifted the peak spectral sensitivity from 500 nm to 550 nm (Manglapus et al., 1999), a normally light-driven phenomenon.

Additionally, retinal cell-cell coupling via gap junctions is also an adaptation indicator. Gap junctions are channels between abutting cells, and allow direct passage of currents as well as small molecules (< 1000 kD), such as metabolites, between the coupled cells. In the retina, coupling is not only found in cells of same types (between HC-HC, AC-AC, for example); it also exists between different cell types, such as between rods and cones (Raviola and Gilula, 1975), and between ACs and BCs (Famiglietti and Kolb, 1975; Mills and Massey, 1995). The molecular mechanism of uncoupling of gap junctions has been suggested to involve phosphorylation of certain serine residues of connexin (Cx), the family of proteins that comprises gap junctions (Ouyang et al., 2005, Kothmann et al., 2007), although dephosphorylation of Cx36, the Cx that conducts signals from AII ACs to cone BCs, between photoreceptors, and between AII-AII, has also been hypothesized (Kothmann et al., 2009) to mediate uncoupling of gap junctions in the rabbit retina. Coupling between retinal cells is plastic, being modulated by changes in light intensity. For example, in the fish and rabbit retina, rods and cones were more intensely coupled at night or in the dark, whereas little coupling was observed during the day, or in the light (Wang and Mangel, 1996; Ribelayga and Mangel, 2010). This is supported by the detection of stronger rod-mediated signals in cone-exclusive HCs (of the goldfish retina) at night or in the dark, whereas little or no rod-driven responses were detected during the day or under light adaptation. It has been shown that the light-induced uncoupling of rods and cones is likely mediated via activation of D2R (Ribelayga et al., 2008). Additionally, intracellular injection of Neurobiotin, a tracer small enough to traverse gap junctions, shows that coupling between AII cells undergoes a "triphasic" plasticity; under low and high light levels, a small number of AII cells are coupled, whereas under their normal operating range, much larger networks are formed by AII cells (Bloomfield et al., 1997). The significance of this is its correlation with computational models

(Smith and Vardi, 1995) that show AII-AII coupling helps to preserve high sensitivity of rod-dependent signaling in the inner retina. Similarly, studies of HCs in the rabbit retina show that HC networks undergo an uncoupling-coupling-uncoupling change with increments in luminance (Xin and Bloomfield, 1999). Aside from the abovementioned observation from the rabbit retina, controversial findings of HC coupling in teleost fish retina have been reported. For example, it has been reported by some that size of the receptive field of cone-HCs is decreased under prolonged dark adaptation (Mangel and Dowling, 1985; Tornqvist et al., 1988), whereas others report that light adaptation causes uncoupling (Baldridge and Ball, 1991; Umino et al., 1991). The latter is also consistent with studies of the amphibian retina, in which light-adaptation causes reduced HC coupling (Witkovsky and Shi, 1990; Dong and McReynolds, 1991). Coupling through gap junctions is also seen in the innermost retinal population- the RGCs. This is supported by physiological studies that show concerted spiking activity of certain types of neighboring RGCs (Arnet and Spraker, 1981; Mastronarde 1983; Meister et al., 1995; Brivanlou et al., 1998), as well as tracer-coupling of alpha RGCs in mammalian retina (Stewart, 1981; Vaney, 1991; Dacey and Brace, 1992; Xin and Bloomfield, 1997). Also, RGCs, including ON-type DSGCs (Ackert et al., 2006) and intrinsically photosensitive RGCs (Schmidt and Kofuji, 2009; Müller et al., 2010), have been shown to be electrically coupled indirectly through subtypes of ACs. Not all RGC types are coupled or show strong concerted firing; for example, although in the cat and primate retinas both ON- and OFF-type RGCs show concerted spiking activities and coupling (Mastronarde, 1983a, b; Dacey and Brace, 1992), in the salamander and rabbit retina only OFF-type RGCs have been reported so far to show such features (Meister et al., 1995; Hu and Bloomfield, 2003). Both tracer labeling and physiological recording show that coupling between RGCs, unlike that among other retinal cell types, is largely increased under

light adaptation but reduced in the dark (Hu et al., 2010). In contrast with coupling between other cell types – such as HC-HC coupling, which mainly serves to sum limited information – coupling of RGCs has a different function: since the receptive field of paired RGCs that have synchronous firing is smaller than that of individual RGCs, synchronization helps to preserve spatial resolution (Meister et al., 1995; Meister, 1996) and hence to maximize the encoding ability with limited capacity of the optic nerve (Meister and Berry, 1999).

It has been shown that DA agents can mimic light-induced uncoupling between retinal neurons. In a study in which HCs were isolated from perch retina, electrical conductance between HCs, measured through voltage and current clamping, was reduced after application of DA or of 8-bromo-cyclic AMP, a membrane-permeable cAMP equivalent, indicating the direct uncoupling effect of DA on HCs (Lasater and Dowling, 1985). In the mudpuppy retina, exogenous DA reduces coupling between HCs, an effect also mimicked by applying D1R agonist SKF38393 (Dong and McReynolds, 1991). In contrast, coupling is enhanced by D1R antagonist SCH23390, suggesting a role of D1R in inhibiting HC-HC conduction (mudpuppy: Dong and McReynolds, 1991; mice: He et al., 2000; primate: Zhang et al., 2011). Similar to the findings of HC network, exogenous DA has been shown to inhibit AII-AII coupling (Hampson et al., 1992; Kothmann et al., 2009) in the rabbit retina. Additionally, rod-cone coupling is also under the regulation of DA (goldfish and mouse: Ribelayga et al., 2008), possibly through D2R at the terminals of rods and cones.

Besides DA, another important light-adaptation indicator/modulator is nitric oxide (NO), an atypical neurotransmitter. In the retina, the neuronal NO synthase (nNOS) oxidizes L-arginine

and produces NO and L-citrulline (Alderton et al., 2001). The majority of NO-producing cells that are identified by observing nNOS are present in the inner retina, presumably in ACs (Dawson et al., 1992; Yamamoto et al., 1993; Shin et al., 1999; Neufeld et al., 2000). In the chick retina, it has been found that NO is produced in a variety of cell types including certain subtypes of ACs, some RGCs, and glial cells, indicated by double-labeling with NOS and NADPH-diaphorase, a presumed marker of NOS (Fischer and Stell, 1999; Wilson et al., 2011; Tekmen-Clark and Gleason, 2013). Unlike DA, NO acts on its target, soluble guanylate cyclase (sGC), by diffusion through plasma membrane. Once activated, sGC converts GTP to cGMP. Expression of sGC is distributed in multiple retinal cell types; for example, in rat retina- sGC is immunolabeled in cone BCs and ACs, and also to a lesser degree, in rod BCs and RGCs (Ding and Weinberg, 2007).

The synthesis and liberation of retinal NO, like those of DA, are upregulated by light adaptation (Donati et al., 1995; Neal et al., 1998). It has been shown in the mouse retina that light stimulation increases NOS expression in immunocytochemistry and a NO-sensitive dye labeling (Giove et al., 2009). Similarly, NO has also been shown to modulate retinal functions as light does. In myopia research, for example, NOS activity is required for myopia prevention (Nickla et al., 2006). The adaptational role of NO is more often seen in cell-cell coupling studies. Like DA, it uncouples gap junction-mediated retinal networks. Nitric oxide donors, cGMP analog 8-bromo-cGMP, and NO precursor L-arginine, have all shown uncoupling effect in HCs (Miyachi et al., 1990; Pottek et al., 1997; Lu and McMahon, 1997; Xin and Bloomfield, 2000; Daniels and Baldridge, 2011). Interestingly, NO is able to uncouple the heterotypic gap junctions between AII cells and cone BCs, which are unresponsive to DA agents (Mills and Massey, 1995; Xia and

Mills, 2004). In a study on two morphological indicators of light adaptation – cone contraction and HC-spinule formation – Haamedi and Djamgoz (2002) found that DA- and NO-driven effects take place in series rather than in parallel, that is, light increases the release of retinal DA, which then stimulates NO production, which in turn induces cone contraction and formation of spinules on HCs. Coincidentally, this "light→DA→NO→effect" sequence has been found in our lab (Moinul et al., ARVO 2012) and by Nickla and colleagues (Nickla et al., 2013) in myopia studies in the chick.

In addition to DA and NO, another light-adaptation marker is retinoic acid, which in the chick eye is produced in certain types of ACs, retinal pigmented epithelium, choroid, as well as sclera (Fischer et al., 1999). In both rabbit and mouse retina, retinoic acid has been shown to completely block coupling between HCs (for review, see Weiler et al., 2000). The roles of retinoic acid in retinal circuits and function are largely unknown, and the work presented in this thesis will only focus on the mechanisms of DA and NO system in adaptation.

In the mouse, using the same device as ours (Chapters 2 and 3, Materials and Methods), Umino and colleagues (2008) reported that photopic optokinetic CS was tuned to SF and velocity, whereas scotopic CS was tuned to SF and TF (Umino et al., 2008) – a drastic shift in tuning preference between cone- and rod-dominated signaling. Here, I characterized adaptation-induced modulation of spatiotemporal processing of the chick retina, under photopic and scotopic conditions (Chapter 2). Furthermore, I explored the role of DA, NO, and cell-cell coupling in modulating retinal function by pharmacology (Chapter 3).

Chapter 2: *Die Fledermaus*: Regarding Optokinetic Contrast Sensitivity and Light-Adaptation, Chicks Are Mice with Wings

ABSTRACT

Background: Through adaptation, animals can function visually under an extremely broad range of light intensities. Light adaptation starts in the retina, through shifts in photoreceptor sensitivity and kinetics plus modulation of visual processing in retinal circuits. Although considerable research has been conducted on retinal adaptation in nocturnal species with rod-dominated retinas, such as the mouse, little is known about how cone-dominated avian retinas adapt to changes in mean light intensity.

Methodology/Principal Findings: We used the optokinetic response to characterize contrast sensitivity (CS) in the chick retina as a function of spatial frequency and temporal frequency at different mean light intensities. We found that: 1) daytime, cone-driven CS was tuned to spatial frequency; 2) nighttime, presumably rod-driven CS was tuned to *temporal frequency* and spatial frequency; 3) daytime, presumably cone-driven CS at threshold intensity was invariant with temporal and spatial frequency; and 4) daytime photopic CS was invariant with clock time.

Conclusion/Significance: Light- and dark-adaptational changes in CS were investigated comprehensively for the first time in the cone-dominated retina of an avian, diurnal species. The chick retina, like the mouse retina, adapts by using a "day/night" or "cone/rod" switch in tuning preference during changes in lighting conditions. The chick optokinetic response is an attractive model for noninvasive, behavioral studies of adaptation in retinal circuitry in health and disease.

INTRODUCTION

Vision functions over a vast range of light intensities, as much as 14 $\log_{10}$ units (Stockman and Sharpe, 2006). For species survival, it is advantageous to use as much as possible of the available intensity range for vision. Animals are able to do this – to see effectively over a range of light intensities, from weak starlight to brilliant sunshine – because visual sensitivity and gain can adjust automatically to ambient light intensity, thus optimizing visual function under widely varying conditions. This property of vision is called *adaptation*. In the retina, adaptation depends in part on the duality of photoreceptor systems – rod photoreceptors, which mediate vision in relatively low-intensity (*scotopic*) conditions, and cone photoreceptors, which mediate vision in relatively high-intensity (*photopic*) conditions, with the two functioning together in an intermediate (*mesopic*) range. However, retinal circuitry also changes functionally – from high sensitivity and low acuity at low intensity, to low sensitivity and high acuity at high intensity. This switching between rod- and cone-driven retinal circuits, and the adjusting of the sensitivity and gain of both photoreceptor responses and post-receptoral circuits, are the main factors that make useful vision possible over such a wide range of light intensities.

In the present study, we have investigated the effects of light- and dark-adaptation on visual processing in the chick retina, using the optokinetic response (OKR). In animals with laterally placed eyes, such as mice and chickens, the OKR is a simple, unlearned reflex turning of the head and neck (therefore also called the "optocollic" response) to follow the rotation of a global visual pattern in the horizontal plane. It is quite simple to determine the minimum contrast (threshold) at which the animal can follow a vertical stripe pattern (grating) of known spatial frequency, contrast and velocity, on a cylindrical surface rotating around it. Although the OKR

is modulated by connections within the brain, especially from the vestibulocerebellum (Cohen et al., 1992), its contrast sensitivity (CS), gain, and response to the direction and speed of image movement are determined largely by the function of a single class of directionally selective retinal ganglion cells (DS-RGC) (Yonehara et al., 2009). In the horizontal OKR, these cells respond preferentially to object movement in the temporonasal direction, and when they are activated in one eye, they are silent in the other. Therefore, one can test alternately the retinal function in each eye independently, simply by reversing the direction of movement (Douglas et al., 2005). The recent introduction of a "virtual optomotor system" (OptoMotry®) – in which spatial frequency, contrast, velocity, and intensity of a computer-generated drifting grating can be changed instantly and continuously over a wide range – makes it possible to measure optokinetic CS as a function of these parameters, rapidly and easily (Prusky et al., 2004). As a result, this method has become standard for characterizing normal and experimentally altered retinal function in small animals – in particular, mouse (Umino et al., 2008; Doering et al., 2008; Lodha et al., 2010; Cantrup et al., 2012; Jackson et al., 2012; Burroughs et al., 2011; Ecker et al., 2010; Umino and Solessio, 2013), rat (Douglas et al., 2005; McGill et al., 2007; McGill et al., 2012), Nile grass rat (Gaillard et al., 2008), and even zebrafish (Tappeiner et al., 2012).

Using the OKR, Umino et al. (2008) have found that CS of the light-adapted mouse is tuned to velocity, whereas CS of the dark-adapted mouse is tuned to temporal frequency (and both, to spatial frequency). That is, *the tuning preference of the mouse retina switches under different adaptational states*. In extremely dim-light conditions, rod-dependent signals in a typical mammalian retina are relayed indirectly: rods → rod bipolar cells (BCs) → AII amacrine cells → cone BCs → RGCs; in contrast, cone-dependent signals go through a more direct pathway: cones

→ cone BCs → RGCs. It is tempting to imagine that this switch of tuning preference between light- and dark-adaptation involves the unique rod-specific pathway of the mammalian retina – comprising rod bipolar cells and their AII-amacrine cell relay – under scotopic conditions, and its removal in favor of cone pathways under photopic conditions. Certainly, dark-adaptation brings dramatic changes in cell activity and cell-cell coupling in the rod-specific pathway (Bloomfield et al., 1997), which should alter the way visual information (such as spatial contrast and detail) is processed in the inner-retinal circuits that control DS-RGCs. However, while much is known about the neural circuitry and function of DS-RGCs under light-adapted conditions (Vaney et al., 2012), effectively nothing is known about how they change with dark-adaptation.

The chick is an attractive alternative to mice and other common laboratory mammals for studying such mechanisms. Chickens (as opposed to the ubiquitous laboratory mouse and rat models) have excellent cone-based vision from the time of hatching, and their retinal function as revealed by the OKR reaches a stable adult level by 5-7 days after hatching (P5-P7) (Schmid and Wildsoet, 1998). These young chicks are small, docile, and very suitable for testing in OptoMotry. While most neurobiological studies of retinal mechanisms for adaptation have been carried out in animals having rod-dominated retinas, it is of biological interest also to know how retinal functions adapt in cone-dominated retinas. The chicken retina is cone-dominated, with cones in some strains comprising ~86% of photoreceptors in the center and ~70% in the periphery of the retina (Morris, 1970). Furthermore, while powerful methods for manipulating gene expression (as in mice) are not yet available for birds, the large size of chick eyes makes it easy to manipulate retinal function – *one eye at a time, independently*– by the equally powerful

method of delivering pharmacological agents preferentially to the retina by intravitreal injection (Fischer et al., 1998; Vessey et al., 2005).

The spatial visual function of birds, assessed as CS, has been documented by electrophysiological (Ghim and Hodos, 2006) as well as behavioral methods (Schmid and Wildsoet, 1998; Gover et al., 2009). All of these studies have reported that the photopic CS in birds is tuned to spatial frequency (SF) – although the optimum SF and CS vary from study to study, possibly in part because of differences in (e.g.) methods (ERG vs. learned vs. innate behaviors), species being tested (e. g., barn owls vs. quails and pigeons), and ambient light intensity. In one study (Gover et al., 2009), *visual acuity* of the chicken was tested under five different luminances (from 0.06 to 57.35 cd/m^2) using a classically conditioned, task-performance method, and was found to increase as light intensity was increased. However, how *spatial and temporal CS* change under different lighting conditions – that is, how spatiotemporal signaling adapts to maximize vision under different adaptational states – has not yet been studied in any avian species. Furthermore, although they differ from mice in having a strongly cone-dominated retina (Morris, 1970), the eyes of diurnal birds do have well documented rod function, which predominates at night; this has been detected by electroretinography, in the chicken (Schaeffel et al., 1991) and the closely related Japanese quail (Manglapus et al., 1998). Finally, vision in chicks is also of special interest because chicks have served for decades as the most-studied model of myopia (Wallman et al., 1978; Wallman and Winawer, 2004). The chick is thus a perfect subject for further studies of these fundamental visual functions.

The observations reported here show that the functional strategy for optimizing visual function over a wide range of light intensities in the chick is remarkably similar to that in the mouse. Specifically, contrast sensitivity is high and tuned preferentially to fine detail (high SF) in the daytime, when environmental light is abundant, but switches to lower CS and tuning to coarser detail in the nighttime, when light is scarce. Some of these findings have been reported previously in abstract form (Shi et al., 2012).

MATERIALS AND METHODS

Animals

White Leghorn cockerels (*Gallus gallus domesticus*) were purchased from Canadian hatcheries, delivered to us on post-hatching day 1 (P1), and tested on days P5-P13. For reasons of cost and availability, at various times we used chicks of 2 different strains: Lohmann (Pacific Pride Chicks, Ltd, Abbotsford, BC) and Bovan (Rochester Hatchery, Westlock, AB). Chicks were kept at 26^{o}C on a 12:12 hr light-dark cycle (light on at 06:00 am) and had unlimited access to food and water.

Ethics Statement

Animal use protocols were approved by the Health Sciences Animal Care Committee of the University of Calgary (Protocol #M10008), and complied with the CCAC Guide to the Care and Use of Experimental Animals as well as the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Testing the Optokinetic Response (OKR)

Setup: OptoMotry®

The OKR was tested using a computer-operated virtual optomotor cylinder, OptoMotry® (Cerebral Mechanics, Lethbridge, AB, Canada), which generates horizontally drifting vertical gratings on the walls of a square enclosure formed by four 17-inch-diagonal flat-panel color LCD monitors (model 1703FP; Dell, Phoenix, AZ). The grating waveform (sinusoidal in our experiments), horizontal drift velocity (V, deg/sec) and spatial frequency (SF, cycles/deg) – and thereby the temporal frequency (TF [= SF x V], cycles/sec) – were controlled by the experimenter in software. Because of aliasing and other technical limitations, the upper limits of test parameters were SF = 1.0 cyc/deg and V = 50 deg/sec. Luminance was measured with a photometer (Minolta LS-110 Luminance Meter, operating in spot mode with a 1 degree acceptance angle), aimed horizontally in the place of an animal being tested. According to these measurements, in our experiments the maximum luminance of the light bars was 195 cd/m^2, the minimum luminance of the dark bars was 2.91 cd/m^2, and the mean luminance (of the entire grating) was 95 cd/m^2, or 1.98 log cd/m^2. This is about 4 log units below the luminance of bright sunlight (Stockman and Sharpe, 2006). However, it has been shown that at this luminance, chickens are strongly sensitive to long-wavelength light far beyond the rhodopsin spectral range (Prescott and Wathes, 1999), and therefore that this level of illumination is photopic for chickens. For testing at lower mean luminance, neutral-density (ND) filters (Lee Filters, Toronto, ON, Canada) in increments of ND = 0.5 or 0.9 were placed inside a transparent, cylindrical holder (inner diameter = 19.5 cm, outer diameter = 20.3 cm) between the monitors and the stand for the animal. The transparent cylinder alone had no measurable effect on CS function.

The OKR Testing Procedure

The OKR was tested under three light intensities: (i) in the day, at unattenuated mean luminance (I_{mean} = 1.98 log cd/m^2), under which the OKR was driven by cones (defined as "photopic" condition); (ii) at night, at the lowest luminance under which an OKR could be elicited (defined as "scotopic condition", or "nighttime threshold luminance"); and (iii) in the day, at the lowest luminance under which an OKR can be produced (defined as "daytime threshold luminance"). For scotopic testing, the chick was first dark-adapted in a dark room for at least 1.5 hours, then covered with an opaque black cloth and transferred quickly into the OptoMotry chamber. The scotopic OKR was viewed from above with an infrared-sensitive CMOS night-vision mini-camera (Model CM900, Clover Electronics, Cerritos, CA, USA), with infrared LEDs emitting outside the visible range (λ_{max}=950 nm, no detectable emission at λ<800 nm), and the infrared LEDs were covered with 10 filters of ND=0.9 each, to block completely any illumination of the chick by light within its visible range. The infrared camera was inserted through the lid of the OptoMotry chamber. Layers of black cloth were wrapped around all contacts between the filter cylinder and the testing chamber, to prevent illumination of the chick except by light passing through the ND filters; nevertheless, it is possible that a small amount of light leaked through, which could be detected by the chick's retina but not by our instruments.

To determine the threshold luminance (for eliciting an OKR) under the above-described conditions (ii) and (iii), ND filters were inserted until the OKR to gratings of 100% contrast could not be elicited any more. Filters were then removed in 0.5 ND steps until the OKR reappeared, and the I_{mean} at which that occurred was defined as "threshold".

The grating contrast was set initially at 100% and then lowered in a stepwise manner, that is, 100% → 50% → 25% → … $(100/2^n)$%, holding SF and V constant, until the chick failed to respond. The chick was tested five times under the same stimulus conditions, and the response was accepted as reliable if the OKR was elicited in four of the five trials. Contrast was then reduced by one step, and the test procedure was repeated until the response rate failed to reach the 4-of-5 criterion. The lowest contrast at which the chick reliably produced an OKR was defined as threshold contrast, whose reciprocal (100 / $\%contrast_{thr}$) is CS. We chose not to test threshold also with contrast ascending from near zero, or varying randomly, because we obtained results reliably and much more rapidly using the method just described, and because the determination of absolute threshold was not our objective. Contrast sensitivity was tested further, as just described, at a number of SFs and temporal frequencies (TFs), the latter of which was derived by calculating TF = SF x V.

Testing for Circadian Regulation – Effect of Time of Testing on Daytime Contrast Sensitivity

Previous studies have shown that avian light sensitivity varies with clock time, that is, that light-sensitivity oscillates within the 24-hour cycle without an external cue such as light (Schaeffel et al., 1991; Manglapus et al., 1998). Therefore, before spatial and temporal CS were characterized, CS was tested at different times of day to determine (i) whether there were circadian rhythms of CS in the chick, and (ii) whether we needed to test CS at a specific time of day. The CS of the OKR was tested under daytime photopic and threshold luminances, at SF = 0.5 cyc/deg and velocity (V) = 9 deg/sec, on the same chicks at 8:00-8:30 am, 12:00-12:30 pm, 4:00-4:30 pm, and 8:00-8:30 pm. To minimize disturbance of their regular light-dark cycle, chicks were fetched

from the holding room 5-10 minutes before 6:00 pm (their regular light-off time), left in the dark until testing, and put back in the dark immediately after the test run was completed.

Data Analysis

CS was obtained by calculating the reciprocal of the threshold contrast (100 / %contrast$_{thr}$). The error bars in all graphs represent the standard deviations of the means. One-way ANOVA was performed to assess the significance of differences ($p<0.05$) among values at different independent variables (SF or TF) in each spatial and temporal CS function. Statistical analyses and graphing were performed using InStat^{TM} version 3.1a and Prism^{TM} version 5.0a, respectively, for Macintosh (GraphPad Software, Inc., LaJolla, CA, USA). The validity of parametric statistics was confirmed by testing for normal distribution of data, using InStat.

RESULTS

Daytime, Cone-driven Contrast Sensitivity Function

Photopic experiments were all performed in the daytime, from clock time 9 am to 3 pm, at 1.98 log cd/m^2 (mean unattenuated luminance), contrast sensitivity (CS) was tested under a series of TFs (TF=0.9, 1.8, 3.6, 4.5, 6.0 cyc/sec). In all chicks tested, regardless of strain (Bovan or Lohmann), the *spatial* CS function (CS *vs* SF) showed an inverted "U" shape (bandpass characteristic). Typical curves from each strain are shown in Figures 2.1A and 2.1B. This bandpass characteristic was seen at nearly all TFs tested (data not shown). Contrast sensitivity peaked at an intermediate SF, ca. 0.5 cyc/deg, where $CS_{max} = 13.2 \pm 2.8$ in Bovan chicks ($n = 8$) and 19.1 ± 5.2 in Lohmann chicks ($n = 8$). Our findings suggest that the photopic, or cone-driven, CS of the chick was tuned to SF, and the optimum SF was about 0.5 cyc/deg. In Figure

2.1C, spatial acuity, the highest SF at which an OKR could be elicited under a specific light intensity, was estimated by curve-fitting, using the continuous function for CS vs SF of the quail's pattern ERG (Ghim and Hodos, 2006); this function was fitted by eye to the chick data, by adjusting position along the x-axis and adjusting x- and y-dimensions. CS in this range of SFs could not be tested using our setup, because of aliasing at SF >1.0 cyc/deg. While this method lacks quantitative rigor, it satisfies the need to visualize what the complete CS function might be and to estimate the spatial acuity of the photopic OKR. We estimated the acuity at maximum luminance to be about 2 cyc/deg in Bovan chicks (Fig. 2.1C), but data from Lohmann (Fig. 2.1B) and other chick strains (e.g., HyLine; data not shown) suggested that it could be as high as 6-8 cyc/deg.

In contrast, photopic CS was not consistently tuned to *temporal* frequency (TF), in either Lohmann (Figure 2.2A) or Bovan (Figure 2.2B) chicks. For example, in Figure 2.2B, at SF=0.1 and 0.5 cyc/deg, the CS function appears to be almost high-pass. At SF=0.5 cyc/deg, difference in CS between the three highest TFs was insignificant (Figure 2.2B, between 1.8 and 3.6 cyc/s, $p<0.05$; between 1.8 and 4.5 cyc/s, $p<0.01$, one-way ANOVA). At SF=0.2 and 0.32 cyc/deg, CS is more band-pass ($p<0.05$, one-way ANOVA).

Chicks Function Visually Over A ≥6-Log cd/m^2 Range of Light Intensity

Since previous ERG studies of the chicken (Schaeffel et al., 1991) and quail (Manglapus et al., 1998) showed that rod functions predominated at threshold intensity in the nighttime, we determined the lowest intensity at which the OKR of the chick could be evoked at night. We found that the nighttime OKR could be elicited at -4.32 log cd/m^2 (in Bovan chicks); this was 6.3

log cd/m^2 lower than the maximum photopic luminance, 1.98 log cd/m^2. It is of importance to note that 1.98 log cd/m^2, the highest light intensity in our study, was far from that of the natural environment for diurnal birds such as chickens, being about 4 log units lower than that of full sunlight. Assuming that birds can see at that luminance, the real range of light intensities over which an OKR can be elicited would be >10 log units.

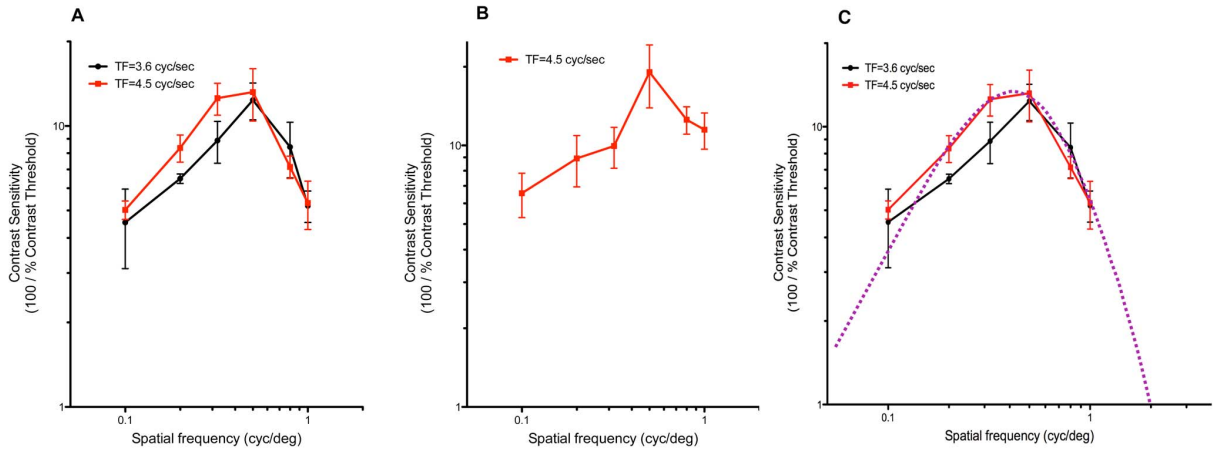

Figure 2.1 Examples of daytime, photopic spatial CS functions. (A) Bovan chicks (n = 6 8), (B) Lohmann chicks (n = 8). Contrast sensitivity peaks at about 0.5 cyc/deg. (C) Contrast sensitivity function for quail pERG (purple line; Ghim and Hodos, 2006) scaled and fitted by eye to CS function of Bovan chicks (n = 6-8). Unattenuated mean luminance (I_{mean} = 1.98 log cd/m^2) in all cases; mean±SD. Peak CS is 13.2±2.8 in Bovan chicks (A, n = 8) and 19.1±5.2 in Lohmann chicks (B, n = 8), at ~0.5 cyc/deg, and estimated SF_{max} (acuity) is ≥2 cyc/deg. TF, temporal frequency. doi:10.1371/journal.pone.0075375.g001

Does Photopic Contrast Sensitivity Vary with Clock Time During the Day?

We wished to know whether CS of the chick varies in circadian fashion, as spectral sensitivity was found to do in previous studies (Schaeffel et al., 1991; Manglapus et al., 1998), and to determine whether our daytime experiments had to be performed in a short time period close to mid-day. For this, we determined the light-adapted, photopic CS of a single group of chicks at four times of day, that is, at 8:00 am, 12:00 pm, 4:00 pm, and 8:00 pm. The chicks were light-

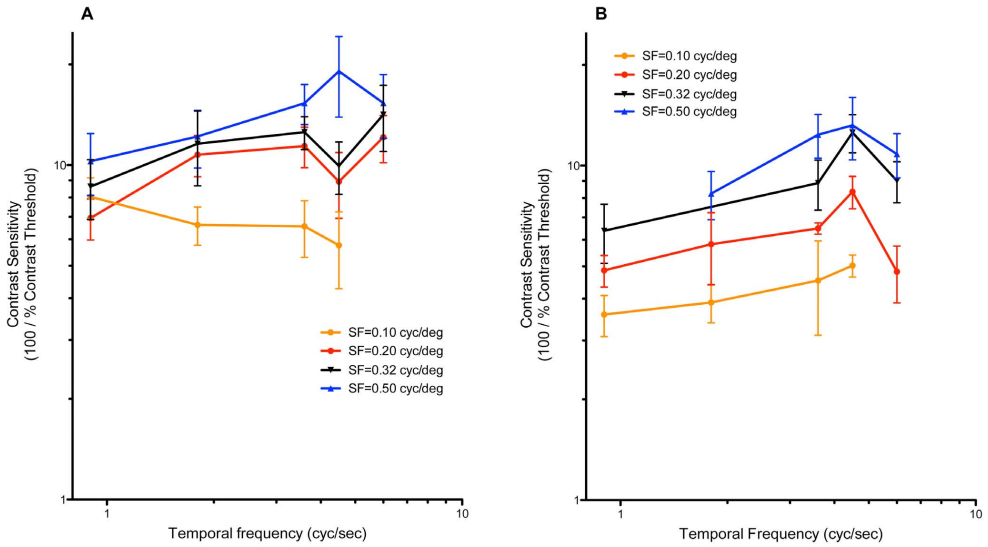

Figure 2.2 Daytime, photopic temporal CS functions. (A) Lohmann chicks (n = 6-8) and (B) Bovan chicks (n = 7-8), at unattenuated luminance (I_{mean} = 1.98 log cd/m^2); mean±SD. The CS functions of Lohmann chicks showed no statistically significant preference for any temporal frequency (A). In Bovan chicks, at SF = 0.2 and 0.32 cyc/deg, CS appeared to be bandpass, whereas at SF = 0.1 and 0.5 cyc/deg, they appeared to be more high-pass (at SF = 0.5 cyc/deg, difference in CS between the three highest TFs was insignificant, one-way ANOVA). SF, spatial frequency. doi:10.1371/journal.pone.0075375.g002

adapted for 1.5 hours before testing and then tested at *maximum* luminance. In contrast to light-sensitivity, the maximal photopic CS at SF = 0.5 cyc/deg was invariant with clock time (data not shown). To confirm further the lack of circadian rhythms in CS, we replicated the experiments under daytime *threshold* luminance at 8:00 am, 12:00 pm, and 4:00 pm. Chicks being tested were dark-adapted for 1.5 hours prior to experiments. Contrast sensitivity was not tested at 8:00 pm or later, because the OKR at this luminance in the nighttime would likely be driven by both rods and cones, if not by rods exclusively, and we did not want rod activity to influence the results. Again, no rhythms in CS were observed at different times (data not shown). Therefore, CS of the

chick's daytime OKR was not under circadian regulation, and we did not have to confine subsequent experiments to a short mid-day testing period.

Nighttime Contrast Sensitivity Function

The nighttime threshold luminance was determined by adding ND filters (see Methods) and observing the chicks' responses in infrared (IR) light. In the nighttime (from 9:00 pm to 12:00 midnight), the threshold luminance for peak CS of the OKR was 3.6 log cd/m^2 lower than that in daytime for the Lohmann chicks, and 6.3 log cd/m^2 lower than that in daytime for the Bovan chicks; this indicated a large *increase in light-sensitivity* at night. Another difference from the daytime OKR was that at night, CS was tuned to TF but not to SF (Figures 2.3A and 2.3B). We present these data only for Lohmann chicks, even though their range of light-sensitivity was lower, because the nighttime OKR of the Lohmann chicks could be elicited over a wider range of SFs and TFs than that of the Bovan chicks. Contrast sensitivities at various SFs (tested at SF=0.08, 0.1, 0.2, 0.32, 0.5, 0.8 cyc/deg) all peaked at a low-to-medium TF, 1.8 cyc/sec (Figure 2.3A), whereas CS did not vary consistently with SF at most SFs (Figure 2.3B). This clear bandpass characteristic was not seen in the daytime photopic CS function (Figures 2.2A and 2.2B). Additionally, the maximum CS was much lower in the nighttime (7.32 ± 0.80) than in the daytime (19.1 ± 5.16) (p=0.0004, unpaired *t*-test). Moreover, the peak nighttime CS was found at a significantly lower SF (0.32 cyc/deg) than the peak daytime CS (0.5 cyc/deg), demonstrating a shift in spatial resolution (loss of CS at higher SFs) with adaptation from day to night. Finally, temporal acuity (a measure of highest temporal resolution), the highest TF at which an OKR could be elicited at a given light intensity, was estimated by curve-fitting (as with SF under photopic conditions; see above). While it could not be determined directly at the highest TFs,

using our setup, in this way we estimated temporal acuity for the chick's scotopic, rod-dominated OKR to be in the range of 10-20 Hz (Figure 2.3C).

Daytime, Threshold-luminance Contrast Sensitivity

To determine whether rod-driven function was also not detectable in the daytime OKR, as suggested by the earlier ERG studies (Schaeffel et al., 1991; Manglapus et al., 1998), we determined the CS function at "threshold luminance" in the daytime. This threshold luminance (for the Bovan chicks) was -2.12 log cd/m^2, that is, 4.1 log units lower than the unattenuated photopic luminance (1.98 log cd/m^2). Interestingly, in these conditions CS did not vary consistently with either SF or TF, appearing to be tuned to SF at some TFs but not others (Figure 2.4A), and to TF at some SFs but not others (Figure 2.4B). At relatively high TFs – for example, 3.6, 4.5 or 6 cyc/sec – the shape of the CS-*vs*-SF curve suggested a low-pass characteristic (Figure 2.4A, $p<0.0001$, comparison between mean CSs at each SF tested, ordinary ANOVA); at TF = 1.8 cyc/sec, however, the curve showed a typical inverted U-shape or band-pass characteristic ($P=0.0015$, Figure 2.4A), as did the photopic spatial CSF. At TF = 0.9 cyc/sec, CS increased monotonically as SF increased (Figure 2.4A). SF varies inversely with V; therefore, at TF = 0.9 cyc/sec, SF could be increased only by making the velocity too low for the chick to follow, and so CS could not be tested at still higher SFs.

These results led us to question whether rod-dependent functions were detectable only at night, because the daytime CS function at threshold luminance was clearly not like that which had been observed at either photopic or scotopic luminance. Either rod-dependent mechanisms had started to contribute to the OKR, or cone-dependent functions had been modified to a large degree,

under this extremely low (yet higher-than-nighttime) threshold luminance. Further research will be needed to determine whether CS at daytime threshold luminance is driven by cones exclusively, or by a combination of cones and rods.

To summarize, we found that both temporal and spatial contrast functions were modulated in different states of adaptation. For example, temporal CS exhibited a bandpass characteristic under scotopic conditions at night (Figure 2.5A) – but not in the daytime, whether light-adapted or dark-adapted. In contrast, spatial CS showed a bandpass characteristic only under photopic conditions in the daytime (Figure 2.5B) – but not under dark-adapted conditions, whether at night or in the daytime.

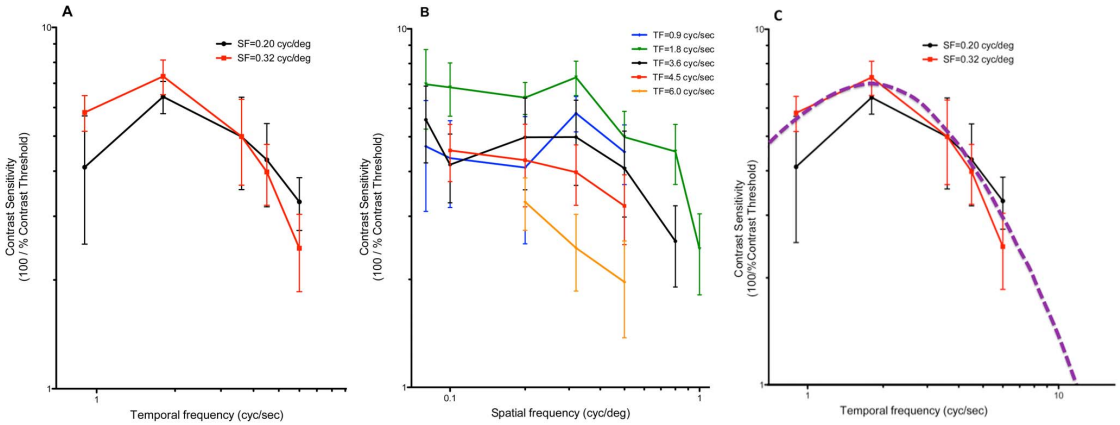

Figure 2.3 Nighttime, scotopic CS function of Lohmann chicks at minimal mean luminance (I_{mean}= -1.62 log cd/m^2). (A) At the two spatial frequencies to which chicks were most sensitive, CS was clearly tuned to TF, with maximum CS = 7.32±0.804 at about 1.8 cyc/sec (n = 8-10). (B) In contrast, over a wide range of temporal frequencies, CS was poorly tuned to SF, with no significant dependence upon SF at any TF (n = 7-10). (C) Contrast sensitivity function for quail pERG (purple line; Ghim and Hodos, 2006) scaled and fitted by eye to CS function of Lohmann chicks (n = 8-10). Estimated temporal acuity is 10-20 Hz. doi:10.1371/journal.pone.0075375.g003

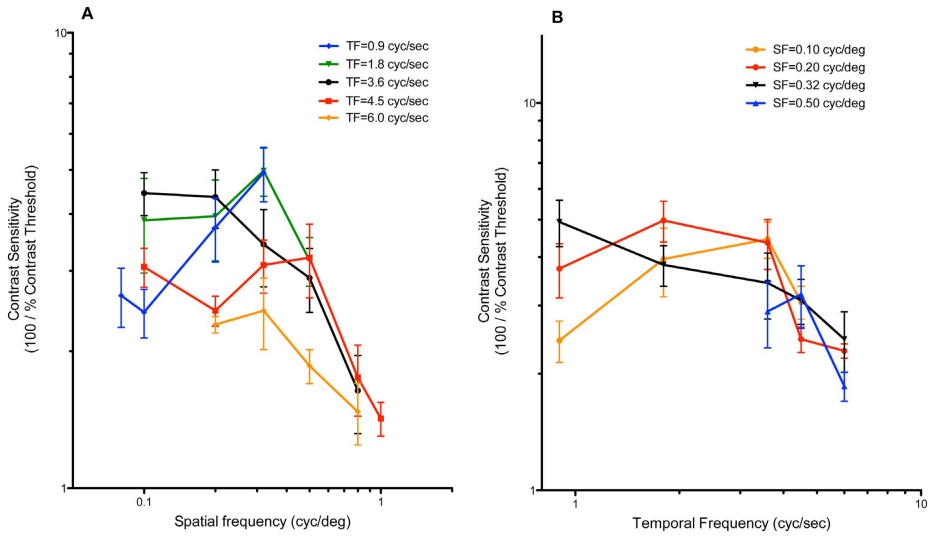

Figure 2.4 Daytime, scotopic CS of Bovan chicks (n = 6-7) at minimal mean luminance (I_{mean}= -2.12). No unique tuning characteristic could be discerned at this luminance, as low-pass, bandpass, and high-pass characteristics were seen in the spatial CS functions (A), and both bandpass and low-pass characteristics were seen in the temporal CS functions (B). doi:10.1371/journal.pone.0075375.g004

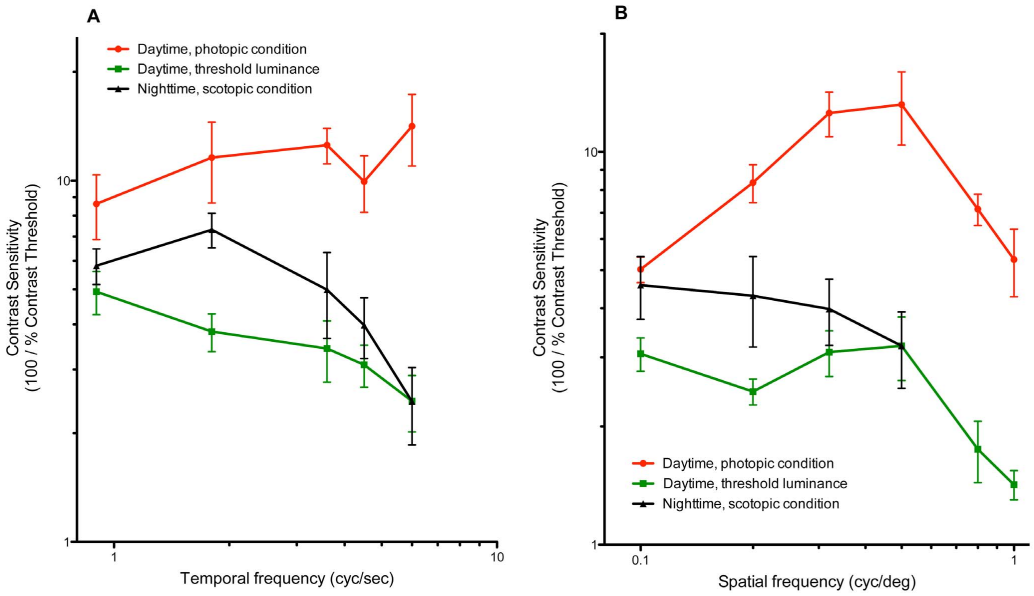

Figure 2.5 Contrast sensitivity functions under three conditions of adaptation and day-night cycle. (A) Temporal CS function at a specific SF (SF = 0.5 cyc/deg), under (i) daytime, photopic, (ii) daytime, threshold luminance, and (iii) nighttime, scotopic conditions. (B) Spatial CS functions at a specific TF (TF = 4.5 cyc/s), under the same three conditions as in (A). doi:10.1371/journal.pone.0075375.g005

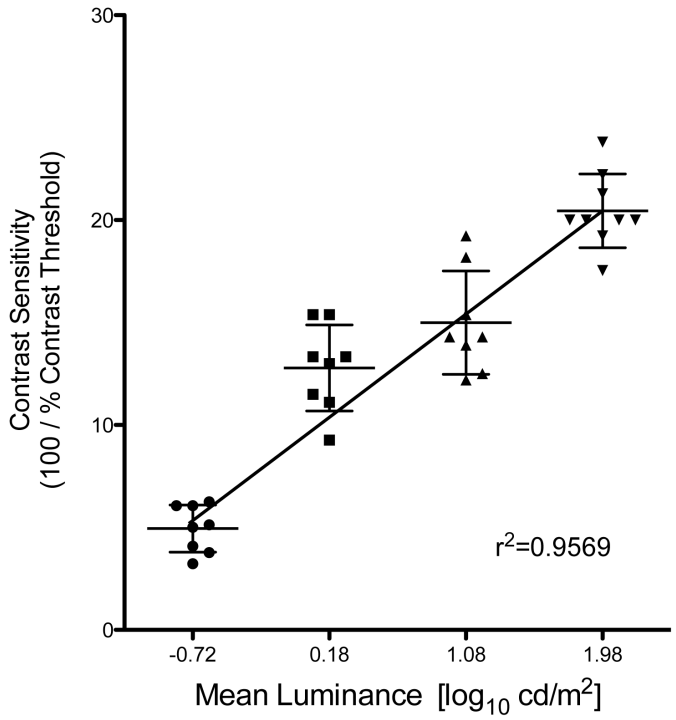

Figure 2.6 Photopic OKR vs mean luminance: In light- adapted chicks, with contrast sensitivity adaptated to steady state at test luminance for 30-60 min, CS declined linearly with log10 mean luminance (Weber's Law). The critical threshold luminances, below which the photopic OKR was undetectable (in light-adapted chicks) and above which a scotopic OKR was elicited (in dark-adapted chicks), were statistically indistinguishable (P = 0.55) at -1.86 $\log_{10}$ cd/m^2. Thus, rods and rod circuitry made no detectable contribution to even the scotopic OKR, under these daytime conditions. doi:10.1371/journal.pone.0075375.g006

DISCUSSION

Importance of Adaptation to Changes in Light Intensity

Through adaptation, animals are enabled to optimize their ability to survive and thrive in constantly fluctuating sensory environments. In the visual world, the most fundamental form of adaptation is to light intensity, which permits effective vision over a wide range of intensities. The retina uses enormous functional plasticity, dictated by ambient light levels, to extract the

most useful information from visual images while discarding less useful information; the goal ultimately is to match visual function optimally to the needs- and opportunities! – imposed by the visual environment.

While the CS functions of various species have been explored in the light, only a few studies, e. g., psychophysics in human (Campbell and Robson, 1968; Lavois et al., 1974) and macaque (Lavois et al., 1974), and murine OKR (Umino et al., 2008), have investigated how it is affected by mean levels of illumination. Here we have used a rapid and reliable indicator of inner-retinal circuit function, the OKR, to test the CS function in the retina over a 6 $\log_{10}$ range of mean intensities. Interestingly, we have found that the scotopic OKR, tested at night, is tuned to TF, rather than to SF as when tested under photopic conditions during the day. A change in tuning preference at night indicates that the function of neural circuits in the retina has undergone major reorganization. In the retina of eutherian mammals, such as mice, rod signals are relayed in large part via a separate pathway that involves rod-only ON-bipolar and AII-amacrine cells (Kolb and Famiglietti, 1974; Crooks and Kolb, 1992; Kolb and Nelson, 1983; Raviola and Gilula, 1973); this is bypassed when rods are inactive, under photopic conditions. Before undertaking the present studies, we assumed that switching to this rod-only pathway might account for the change in tuning of the OKR, from spatial to temporal frequency, which was observed in the mouse (Umino et al., 2008). This led to the hypothesis that a comparable shift in tuning would *not* take place in the chick, because avian equivalents to the mammalian rod-only bipolar and AII-amacrine cells have not been identified so far (Cajal, 1892; Quesada et al., 1988). However, to our surprise the changes to spatiotemporal tuning of CS of the OKR with dark-adaptation in

the chick were as profound as, and rather similar to, those in the mouse – and hence, the tongue-in-cheek title of this article.

Different Strains of White Leghorns Can Be Very Different

We have noticed striking differences in the two strains of White Leghorn chicks used in the present study, such as absolute values of CS, behavior, etc. A major difference is that the Bovan chicks are much more sensitive to dim light at night than are the Lohmann chicks (a 2.7 log cd/m^2 difference). One possible reason for this is that the Bovan chicks may have a higher rod:cone ratio than Lohmanns, since different rod:cone ratios have been reported previously in other strains (Morris, 1970; Meyer and May, 1973). Different strains of chickens also were found to respond differently in myopia studies (Troilo et al., 1995), indicating that processing of visual information in the retina may be expected to differ among strains, possibly as an inadvertent result of selection for other traits.

Inter-strain differences in pupil size also might contribute to differences in sensitivity However, since the difference in light sensitivity was found under extremely low light levels, this explanation would require the maximum pupillary area of the two strains to differ by ~1000 times for the pupil-size theory to hold. Therefore, pupil size alone is very unlikely to explain such drastic difference.

A Comparison with Results from Other Avian Studies

Previous studies from our lab have produced similar findings to these; in 6-12 day-old male White Leghorn (HyLine strain) chicks, optimal OKR CS was ~10 (threshold 9.9%) at SF = 0.5

cyc/deg, with I_{mean} = 55 cd/m^2 and V = 12 deg/sec (Bonfield et al., 2008; Bonfield, 2009). Schmid and Wildsoet (1998) also have shown that the optokinetic CS of chicks ≤8 days old is tuned to SF, under conditions not very different from those in the present study. They reported that CS peaked at 1.2 cyc/deg; they likely missed the true maximum CS, which we have found to occur at about 0.5 cyc/deg, because their apparatus was not set up to test at any SF between 0.17 and 1.2 cyc/deg. In their study, the drift velocity was 6 deg/sec, the mean light intensity (I_{max}) was 29 cd/m^2, the contrast range was 4-78%, and the grating waveforms were sinusoidal from 0.12-1.7 cyc/deg. OptoMotry allowed us to test at an almost infinite number of contrasts, velocities and SFs, so that we could define the optimal parameters with greater precision than by any other method; however, our inability to test at SF >1 cyc/deg allowed us only to *estimate* spatial "acuity", which could be defined with greater precision and tested at higher light intensities with a mechanical optokinetic cylinder such as that used by Schmid and Wildsoet, or a projected grating image such as that used by Schaeffel and colleagues (Diether et al., 2001). Similarly, we estimate temporal "acuity", the upper limit of responsiveness to temporal frequency, as 10-20 Hz (cyc/sec) – under nighttime scotopic conditions, the only ones in which tuning to TF was observed. This may seem rather low, since in another study (Lisney et al., 2012) that used the flicker fusion frequency of the ERG and learned behavioral discriminations, the upper limit of flicker frequency perceived by Lohmann chickens was almost 120 Hz at high mean luminance (2740 cd/m^2); however, at lower photopic luminance (0.7 cd/m^2), the ERG flicker fusion frequency was 20 Hz (Lisney et al., 2012). Thus the estimated 10-20 Hz temporal acuity for the scotopic OKR observed in the present experiments (Figure 2.3C) seems plausible or even higher than might be expected, given that it was observed under presumably rod-

dominant conditions, at approximately 1/4,000 the minimum luminance that was tested in the flicker-ERG experiments.

All things considered, despite the technical limitations and differences in apparatus used in these and other other studies, there is substantial agreement on fundamental properties: *at I_{mean} = 29-98 cd/m^2 and V = 6-12 deg/sec, in 8-13-day-old male chicks of several White Leghorn strains, maximum contrast sensitivity is ca. 10-13, at SF = 0.5 cyc/deg, and spatial acuity is about 2-8 cyc/deg.*

Contrast sensitivity has been studied in several other species of birds, by a variety of methods. Ghim (2003) and Ghim and Hodos (2006), using threshold of the pattern ERG (pERG), reported the photopic CS of six species of birds; in the species most closely-related to chicken, Japanese quail, the peak CS was 9.85 at 1.05 cyc/deg. The difference in CS functions between their study and ours could not be explained simply by the difference in light intensities, since the mean luminances were almost the same (94 vs 98 cd/m^2). A number of factors might have contributed to these differences: (1) The pERG stimuli were presented at sequentially ascending contrasts, sufficiently briefly (each one for 17 reversals at 7.5 Hz) that local contrast adaptation might have not been complete (Smirnakis et al., 1997); whereas our stimuli were presented at contrasts descending stepwise from 100%, and each contrast level was presented at least 5 times for 5 seconds, thereby creating a steady state of contrast adaptation and likely somewhat reduced CS. (2) The OKR is driven by retinal ganglion cells (ON DS-RGCs) that have rather large receptive fields and are not tuned optimally to fine detail, whereas the pERG represents the mixed responses of all retinal elements that are excited by pattern-reversal (Thompson and Drasdo,

1994). Since CS is determined by the relative strengths of center and surround processes, any elements that have a smaller receptive-field centre than those in the direction-selective pathway would have caused a shift of peak SF to the right, as seen in these pERG studies. Learned visual tasks require processing in higher visual centers that are more concerned with spatial discrimination *per se*. Thus Jarvis et al. (2009), using a learned contrast-discrimination task (involving higher-level visual processing), found that the maximum CS of year-old chickens was slightly higher than 10 – at highest luminance (16 cd/m^2) and SF = 1 cyc/deg – and that when luminance was reduced to 0.1 cd/m^2, peak CS declined to about 3-4 at 0.7 cyc/deg. Similarly, in mice, spatial acuity using the OKR (Prusky et al., 2004) is considerably lower than that using a learned discrimination task (Prusky et al., 2000).

Comparison of CS in the Chick with CS in the Mouse

In vision research, the OKR (sometimes called optokinetic nystagmus, or optomotor response) is used widely as a measure of visual function in studies of disease and development (Doering et al., 2008; Lodha et al., 2010; Fleisch and Neuhauss, 2006; Qian et al., 2005; Brockerhoff et al., 1995; Mora-Ferrer et al., 2005). It has also been employed as an indicator of drug effects in retinal pharmacology (Cahill et al., 2011; Jardon and Bonaventure, 1992; Bonaventure et al., 1992a, 1992b; Schaeffel and Howland, 1988).

However, only in the mouse has the OKR been used to characterize CS functions comprehensively under a wide range of environmental and visual stimulus conditions. The properties of CS in the chick, shown in the present report, are shared to a large extent with those of the mouse, measured by the OKR with a setup identical to ours (Umino et al., 2008); that is,

its photopic CS is tuned to SF of the visual stimulus, whereas its scotopic CS is tuned to TF. However, significant differences are also seen. For example, the peak CS under photopic conditions in the mouse is found near SF = 0.1 cyc/deg, which is much lower than that in the chick; this is likely due to the small fraction (≤5%) and sparse distribution of cones in the mouse retina, the correspondingly larger absolute diameter of receptive fields in the mouse retina, and the difference in optical magnification factors due to differences in eye size (ca. 150 μm/deg in chick: (Schaeffel and Howland, 1988; Avila and McFadden, 2010); 30 μm/deg in mouse: (Schmucker and Schaeffel, 2004)). Additionally, in the study by Umino et al. (2008), CS did not change much from the highest luminance (1.8 log cd/m^2) to the second highest luminance tested (-2.7 log cd/m^2), but changed substantially when luminance was reduced further from -2.7 to -4.5 log cd/m^2. In contrast to this, in chicks we observed that CS at 0.5 cyc/deg decreased as a linear function of log I (Figure 2.6), and when intensity was reduced by 5 log cd/m^2, the CS function ceased being similar to that at maximum luminance. Moreover, the scotopic CS of the mouse was reported to vary as a distinct low-pass function of SF, which we did not observe in chicks. These differences may stem from differences in the functional organization of the retina in the two species – specifically, the existence of a dedicated rod pathway in mouse (mentioned earlier), which has not yet been discerned in avian retinas. Moreover, rod-dependent function (ERG) in quail and chick retinas has been reported to be observable only during the night (Schaeffel et al., 1991; Manglapus et al., 1998), suggesting that a circadian/retinal clock-dependent mechanism selectively suppresses rod function in the daytime in these diurnal species. In contrast, although evidence has been reported for circadian rhythms in the retinas of mice (Cameron and Lucas, 2009; Katti et al., 2013) and several other vertebrates (Ribelayga et al.,

2008; Ribelayga and Mangel, 2010), rod-dependent functioning in the mouse retina can be evoked in the daytime simply by adaptation to lower light intensity (Umino et al., 2008).

Finally, we note that the linear increase in CS with log I, up to the maximum intensity at which we could test, suggests that CS might continue to increase substantially with further increases in intensity. Since the absolute nighttime scotopic threshold I of the chick OKR is several log units higher than that of the mouse, it is logical to suppose that CS in birds is so remarkably low as emphasized by Ghim & Hodos (2006) simply because the entire operating range of cone-dominated, diurnal avian vision is shifted several log units towards higher intensities compared to that of rod-dominated, nocturnal mammals such as the mouse. We predict, therefore, that testing of the chick's CS at mean luminances on the order of 1,000-100,000 cd/m^2 would reveal substantially greater maximum contrast sensitivities, comparable to those of many mammals.

Is Nighttime Contrast Sensitivity Driven by Rods in the Chick?

Since it is practically impossible to determine whether the sustained scotopic luminance in the present study corresponds to the scotopic range for the flash ERG (Manglapus et al., 1998), because the units of light measurement in the two studies are not readily interconvertible, we could not definitely prove that the nighttime, threshold OKR was driven by rods. Moreover, the absolute scotopic range varies between different strains of a single species (see, e.g., our data for Bovan and Lohmann chicks).

However, such a correlation can be made between the mouse OKR and ERG studies. After converting light intensity to "retinal illuminance", the scotopic range for the mouse OKR (Umino

et al., 2008) correlates with that of the scotopic threshold response (STR) in an ERG study using the same strain of mice (Saszik et al., 2002). The STR, originating proximal to where the ERG b-wave is generated (Sieving et al., 1986; Frishman and Steinberg, 1989; Naarendorp and Sieving, 1991), appears at much weaker flash intensity than does the b-wave. Therefore, the rod-driven OKR of the mouse is produced *at light intensities below the b-wave threshold*. The findings from the ERG study of quail (Manglapus et al., 1998) were obtained under different light conditions, including those higher than required for the negative STR (see their Figures 2 and 5) – such as at b-wave levels. If the chick scotopic OKR was evoked at a light level equivalent to that which evokes the STR, as seen in the mouse (Umino et al., 2008; Saszik et al., 2002), then our "scotopic" light intensity was not higher than the equivalent flash intensities in the chick and quail ERG studies; therefore, the nighttime threshold OKR is very likely to be driven by rods in the chick. To put this conclusion in context, one needs to bear in mind that the AII-amacrine cells in mammalian retinas are strongly coupled under very low light intensities at which rods function (Bloomfield et al., 1997). The high degree of spatial summation resulting from this coupling is thought to increase sensitivity and reduce noise at low light-levels, thus accounting for the low intensity threshold of the STR in the mammalian retina; but again, a comparable pathway or mechanism for increasing scotopic sensitivity has not yet been identified, and may not exist, in diurnal avian retinas.

Contrast Sensitivity of Chick OKR Does Not Vary with Clock Hour During Daytime

It has been shown that photoreceptor responses, post-receptoral responses, and spectral sensitivity of Japanese quail varied with time of day (Manglapus et al., 1998) – even when they were being dark-adapted over days, indicating an endogenous circadian clock. This led us to

wonder whether similar behavior could be seen in OKR contrast sensitivity in chicks. In fact, since CS is a measure of the ability to detect details or patterns, rather than mere light sensitivity, we had assumed that CS would be high in the daytime and low in the nighttime – with the peak at noon and the trough at midnight. This hypothesis was teleological or intuitive: in the daytime, when light is abundant, the retina needs to be less sensitive to light but can be more sensitive to small changes over space, time, and wavelength, facilitating more precise visual tasks such as spatial and temporal contrast and hue-discrimination; whereas at night, when photons are scarce, the most primitive function of the retina – simply to detect light with the highest sensitivity possible – outweighs all other demands, at the expense of those more photon-demanding photopic functions. However, we observed no variation of CS under constant lighting conditions during the daytime. This was not unexpected, as we tested throughout the daytime light phase at maximum luminance; and since light can override circadian rhythms (Figure 9 of (Manglapus et al., 1998); Figures 1 and 3 of (Ribelayga et al., 2008)), testing in the light-adapted, photopic state might have masked any regulation of optokinetic CS by an endogenous rhythm. To address this question, it will be necessary to keep the animals in constant darkness.

Significance for Retinal Control of Ocular Growth and Myopia

It has been established that light controls eye growth through retinal signaling, and that only a few hours' daily exposure to intense light, or to visual scenes rich in spatial detail (high SFs), prevents the induction of myopia (for data and review, see Ashby et al., 2009). The primary mechanisms for the visual prevention of myopia reside in the retina, where a major role is played by amacrine cells (Wallman, 1990; Wallman, 1993). The effects of dark-adaptation on functional organization of the ON-center DS-RGCs appear not to have been studied (Vaney et al., 2012),

and how the avian retina adjusts physiologically during light- and dark-adaptation remains almost unknown, except for the ERG studies in chick (Schaeffel et al., 1991) and quail (Manglapus et al., 1998). This is a significant gap in knowledge, because of the major role of the chick as a model for human myopia plus the well-known importance of lighting, contrast and spatiotemporal processing in the cause and prevention of myopia (Wallman and Winawer, 2004). Therefore, the present study adds further understanding of how light-adaptation alters retinal circuit functions, and it may direct our thinking into new areas of knowledge that are critical for preventing and treating myopia in the future.

Conclusions

In the present study we used a rapid, noninvasive behavioral measure of visual function – the optokinetic response – to characterize retinal contrast sensitivity, under various light intensities and at different times of day and night. We found that the chick retina, like the mouse retina, showed a "day→night" or "cone→rod" switch in tuning preference, when adapting to the change from light to dark. This kind of change helps to optimize retinal functions under different lighting conditions. Finally, our study showed that all retinas, although different from species to species, might use simple and very similar mechanisms for light/dark adaptation. A better understanding of these conserved mechanisms awaits further exploration.

Acknowledgements

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OptoMotry. We thank Dr. Yves Sauvé, University of Alberta, and Dr. Narsis Daftarian and Brittany J Carr, University of Calgary, for helpful comments on the manuscript.

Chapter 3: Light Adaptation in the Chick Retina: Dopamine, Nitric Oxide, and Cell-Cell Coupling Modulate Spatiotemporal Contrast Sensitivity

ABSTRACT

Adaptation to changes in light intensity, which is essential for vision as we know it, begins in the retina, regulating the sensitivity and spatiotemporal tuning of retinal outputs via ganglion cells (RGCs). In strong illumination, the spatial contrast sensitivity (CS) of RGCs is bandpass; in weak illumination, CS decreases, especially at high spatial frequencies (SFs). Retinal dopamine (DA), nitric oxide (NO), and cell-cell coupling (via gap junctions) play key roles in light adaptation. Here we describe how DA, NO and gap-junction coupling regulate spatial tuning of displaced RGCs, in the chick retina *in vivo*.

We used the optokinetic response (OKR) to measure spatial CS in light- and dark-adapted chick retinas after intraocular injection of agents affecting DA and NO actions and gap junction coupling. We found that: 1) DA agonists mimicked light-adaptation of CS functions in dark-adapted chicks; 2) DA antagonists or NO synthase inhibitors mimicked dark-adaptation of CS functions in light-adapted chicks; and 3) a gap-junction blocker, meclofenamic acid, increased CS – in light-adapted, but not in dark-adapted chicks.

This work shows that NO and DA are adaptation-linked modulators of spatiotemporal processing in the retina, possibly via gap-junctions. Optokinetic CS is a noninvasive measure of retinal function, which can be manipulated rapidly, conditionally, and reversibly by intravitreal injection of pharmacological agents. The chick's large eyes, and the similarities between processes of light

adaptation in chick and other species (e. g., mouse), make them a powerful new model for retinal research.

INTRODUCTION

In the ever-changing visual world, seeing effectively over a wide range of intensities is made possible by matching function optimally to ambient light levels. Such *adaptation* begins in the retinal photoreceptors, and continues in post-receptor neural networks in the retina (Rieke and Rudd, 2009).

Spatial tuning of retinal ganglion cell (RGC) receptive fields is one of many properties that are modified by ambient light levels (Barlow et al., 1957; Enroth-Cugell and Shapley, 1973). Spatial tuning functions of RGCs are bandpass – peaking at intermediate spatial frequencies (SFs), but decreasing at lower and higher SFs – as might be expected from the typically center-surround opponent organization of their receptive fields (Barlow, 1953; Kuffler, 1953). As light dims, the sensitivity and/or responsiveness of RGCs to higher SFs decreases, and the optimal SF becomes lower than at higher intensities (Barlow et al., 1957; Campbell and Robson, 1968), suggesting that the resolution of fine details is compromised in dim light, in the retina as in our own visual experience. But how does this happen?

The synthesis and release of dopamine (DA) and nitric oxide (NO) in the retina increase in the light and decrease in the dark; thus they are thought to mediate changes in retinal function during light adaptation (e.g., Jackson et al., 2012). In other words, retinal function may reflect the low levels of DA and/or NO in dim light, and their higher levels in intense light; e.g., DA shifts the dark-adapted, nighttime spectral sensitivity of quail retina towards longer wavelengths, as if it were adapted to daylight (Manglapus et al, 1999). Similarly, inhibition of NO production – either genetically (Wang et al., 2007) or pharmacologically (Nemargut and Wang, 2009) – decreases

the responsiveness of RGCs to light. Finally, both modulators mimic light adaptation by decreasing coupling between retinal cells in dim light (Teranishi et al., 1983; Pottek et al., 1997).

We have investigated the mechanisms of retinal light adaptation in the chick, a diurnal animal whose retina is heavily cone-dominated (Morris, 1970). Its good vision, large eyes, and ease of optokinetic testing make it ideal for rapid, inexpensive, reversible, mechanism- and time-specific ("conditional"!) pharmacological manipulation of retinal function. Furthermore, its adaptational mechanisms may be very similar to those in the mouse, a nocturnal species whose retina is rod-dominated (Shi and Stell, 2013). We investigated the roles of DA, NO, and cell-cell coupling in light-adaptive modulation of spatiotemporal contrast sensitivity (CS), using a rapid, noninvasive test – the optokinetic response (OKR) (Shi and Stell, 2013) – to indicate how CS was affected by drugs that mimic or inhibit DA and NO signaling, or that block gap junctions. We confirmed that DA is critical in modulating CS during light-adaptation, as reported previously in mice (Jackson et al., 2012); and we showed, for the first time, that NO plays a role similar to that of DA, and that uncoupling in retinal networks enhances CS selectively at photopic intensity. Some of these results have been reported in abstract form (Shi and Stell, 2013, *Invest Ophthal Vis Sci* (*ARVO Abstracts),* 54:3427).

MATERIALS AND METHODS

Animals

White Leghorn male chicks (*Gallus gallus domesticus;* strains: Bovan, Shaver) were purchased from a local hatchery (Rochester Hatchery, Westlock, AB), shipped to us on post-hatching day 1

(P1), and tested on days P7-P11. Chicks were kept on a 12:12 hr light-dark cycle under fluorescent room lights (I_{mean}= 35-41 cd/m^2, provided by Ecoflux F34CW-RS-WM-ECO, Cool White 34W USA; measured by Minolta LS-110 Luminance Meter, in spot mode with 1 degree acceptance angle) at 26^{o}C, light on at 06:00 am, and had unlimited access to food and water.

Ethics Statement

Experimental protocols were approved by the Health Sciences Animal Care Committee of the University of Calgary (Protocol #M10008), and complied with the CCAC Guide to the Care and Use of Experimental Animals as well as the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Testing the Optokinetic Response (OKR)

Setup: OptoMotry®

The OKR testing setup and procedures used in this study have been described in detail in a previous paper (Shi and Stell, 2013). We tested the OKR with a computer-operated virtual optomotor cylinder, OptoMotry® (Cerebral Mechanics, Lethbridge, AB, Canada); it displays horizontally drifting vertical gratings on a square box of four 17-inch-diagonal flat-panel color LCD monitors (model 1703FP; Dell, Phoenix, AZ), creating a virtual cylinder of uniform vertical stripes of horizontally sine-wave-modulated intensity through software. Intensities of the monitors were measured with the same photometer as for room lights (above). The maximum

luminance (center of bright bars) was 201.6 cd/m^2, and the minimum luminance (center of dark bars) was 1.6 cd/m^2; thus the mean luminance of the entire grating was 101.6 cd/m^2, or ~2.0 log cd/m^2. The maximum mean intensity in our setup is almost 4 log units lower than that of the strongest sunlight (Stockman and Sharpe, 2006). At this luminance, however, chickens are strongly sensitive to light of long wavelengths, far beyond the spectral range of chicken rhodopsin (Prescott and Wathes, 1999); therefore, this level of illumination is photopic, and we are investigating primarily cone-driven vision in these experiments, even under 'dark' conditions.

To attenuate light intensity, we inserted neutral density (ND) filters (Lee Filters, Toronto, ON, Canada) in increments of ND=0.9 inside the OptoMotry chamber. They were held in place by a transparent plastic cylinder (inner diameter = 19.5 cm, outer diameter = 20.3 cm), which alone had no measurable effect on the OKR. The OKR was tested from 9:00 am to 3:00 pm, under one of two light intensities: (i) unattenuated (I_{mean} = 2.0 log cd/m^2), defined as light-adapted ("light" condition); or (ii) attenuated by 2.7 log units, using three 0.9 ND filters (I_{mean}= -0.7 log cd/m^2), defined as dark-adapted ("dark" condition). The latter was chosen because we previously documented an effect of dark adaptation on the OKR contrast-sensitivity function at this luminance (Figure 6 in Shi and Stell, 2013). Note that in the present study, the OKR was always generated by cone-dependent mechanisms; therefore, the term "dark adaptation" as used here does not imply a shift from cone- to rod-dominant functioning, which in galliform birds (chicken, quail) seems to occur only at night (Schaeffel et al., 1991; Manglapus et al., 1998; Shi and Stell, 2013).

The OKR Testing Procedure

Procedures for photopic testing of the OKR were described in detail previously (Shi and Stell, 2013). For experiments under reduced light intensity, chicks were first kept in complete darkness (all lights off, in a covered holding box) for at least one hour, then covered with an opaque black cloth and transferred quickly into the OptoMotry chamber. The dark-adapted OKR was viewed from above with an infrared night-vision mini-camera (Vision Nocturna, Model CM900), with 6 infrared LEDs emitting outside the visible range (l_{max}=950 nm; no detectable emission at l<800 nm), and the infrared LEDs were covered with 10 filters of ND=0.9 each, to further reduce any possible illumination from the LEDs at visible wavelengths. The infrared camera was inserted through an opening in the lid of the OptoMotry chamber. All contacts between the filter cylinder and the testing chamber were wrapped with layers of black cloth – to keep light from leaking around the filters, and thus ensure that the chick saw only light transmitted from the monitors via the ND filters, during testing.

Grating contrast was set at 100% initially, and then decreased in a stepwise manner, that is, 100% → 50% → 25% → … $(100/2^n)$%, with constant SF and V, until the chick could not respond. The chick was tested five times under the same stimulus conditions, and the response was considered reliable if the OKR was elicited in four of the five trials. Contrast was then reduced step by step, until the chick's response failed to meet the 4-of-5 reliability criterion. The lowest contrast at which the chick responded reliably was defined as threshold contrast, the reciprocal of which (100 / $\%contrast_{thr}$) is contrast sensitivity (CS). Threshold contrast was determined further, as just described, at a series of spatial frequencies (SFs): 0.1, 0.2, 0.32, 0.5,

0.8, and 1.0 cyc/deg. Only spatial CS (at constant, optimal drift velocity [9 deg/sec]) was studied in this work, because photopic CS in the chick is known to be tuned to SF at constant velocity (Shi and Stell, 2013).

Intraocular Injection

Before injection of drugs, chicks were anaesthetized with 2.0% isoflurane in 100% oxygen. The upper eyelid of the eye being injected was cleaned with 70% ethanol immediately prior to injection. Drugs, in 20 µL of vehicle, were injected into one eye using a 25-µL Hamilton syringe and 26-gauge needle. Effects and pharmacological specificities of some dopamine agents have been documented in the chick model (Zawilska and Iuvone, 1990; Zawilska and Iuvone, 1992). Strongly hydrophobic drugs were dissolved in dimethyl sulfoxide (DMSO, Sigma, Lot#SHBB9317V) and subsequently diluted to a final vehicle concentration of 0.2% or 0.5% DMSO; DMSO concentrations as high as 3% were found to have no significant effect on chicks' retinal histology, ocular growth, form-deprivation myopia or OKR (B. Carr, K. Quach, Q. Shi, M. Teves; unpublished results, 2014). The dopamine D1 receptor (D1R) agonist, SKF38393, is susceptible to oxidation and degradation by light, and was therefore dissolved in 0.1 mM L-ascorbic acid (Sigma, Lot#031M0164V) as anti-oxidant and protected from light with aluminum foil. For dark-adaptation experiments, injections were made swiftly under dim room light (I_{mean}= 0.61 log cd/m^2), and the chick was moved back into darkness immediately after the injection. Before characterizing drug-treated CS function, the effective dose of each drug was found by starting with a published working concentration and testing dilutions over a range of ~3 log units, in 0.5-1.0 log steps; this screening was done at SF = 0.5 cyc/deg and V = 9 deg/sec, the

conditions found previously to be optimal at full intensity and at which a significant decrease in CS was induced by dark adaptation (Shi and Stell, 2013). The OKR of the same chick was then tested repeatedly at different times (e. g., 30 min, 1 hr, 2 hrs) after injecting the drug. The OKR was tested only ≥30 min after injection, to permit recovery from pain and anesthesia and to allow the drug's concentration in the retina to reach a steady state; our own data (see below) indicated that the effect had *not* already reached optimum and begun to decline at that time. The effects of each drug and concentration on CS were then plotted, to identify the optimal concentration and post-injection testing time, and definitive tests were done using these optimal parameters. All concentrations are given as concentrations in the syringe. As a guideline, we estimate that the maximum concentration of the injected drugs at targets in the retina is ≤10% of their concentration in the syringe, assuming that the 20μL injection volume is distributed rapidly and uniformly in ≥200μL vitreous humor volume; in reality, however, for many reasons even the maximum concentrations in the retina are probably considerably less than that.

Control Experiments

Since all drug administration in this study was through intraocular (intravitreal) injection, we performed two sets of control experiments. In the first set we tested whether injection of vehicle alone had any effect on the OKR. In these experiments, the photopic OKR at maximal CS (SF=0.5 cyc/deg, V=9 deg/s) was tested: before, 30 min after, and 60 min after injection. Three vehicles were tested: distilled water, 0.1 mM L-ascorbic acid, and 0.5% DMSO. None of them altered the results statistically significantly (distilled water: p=0.8764, N=9; 0.1 mM ascorbic acid: p=0.2385, N=9; 0.5% DMSO: p=0.7716, N=9; repeated-measures ANOVA) (data not

shown). In the second set of controls we determined whether the brief exposure to dim light (I_{mean}= 0.61 log cd/m^2) during injections influenced the results. Since the earliest time a drug effect was tested was 30 min after injection, we dark-adapted chicks for one hour, tested the OKR, injected distilled water, kept them in the dark immediately after injection, and tested the OKR again after 30 minutes in the dark. No detectable difference was observed between pre- and post-injection CS in the same animals (p=0.8133, N=10, paired *t*-test).

Data Analysis

Contrast sensitivity was obtained by calculating the reciprocal of the threshold contrast (100 / % $contrast_{thr}$). The error bars in all graphs represent the standard deviations of the means. The significance of differences ($p<0.05$) between CS values, before and after injection, was determined by paired and independent-sample *t*-tests (when distributions were normal) and by a non-parametric test (Mann-Whitney U-test) when they were not. Statistical analyses and graphing were performed using InStat™ version 3.1a and Prism™ version 5.0a, respectively, for Macintosh (GraphPad Software, Inc., LaJolla, CA, USA).

RESULTS

Dark Adaptation Reduced Contrast Sensitivity at High Spatial Frequencies

The light-adapted spatial CS function of the chick's OKR exhibited a bandpass characteristic, as reported previously (Shi and Stell, 2013). The optimal or peak CS was observed at about 0.5 cyc/deg (Figure 3.1). Dark adaptation at I_{mean}= -0.7 log cd/m^2 caused a decrease in CS at that spatial frequency (SF) (Figure 1: $p<0.001$, unpaired *t*-test, n=5-7). Because dark adaptation

decreased CS preferentially at higher SFs, the optimal SF shifted downward in the dark-adapted state, from 0.5 cyc/deg (light) to 0.32 cyc/deg (dark).

Dopamine Agonists Mimicked Light Adaptation in Dark-Adapted Chicks

To test whether the light-induced increase in CS was due to an increase in the production and release of dopamine (DA) in the light, DA agonists were injected into the eyes of chicks that had been dark adapted for at least one hour. In a pilot study, we showed that the CS of dark-adapted chicks was increased, preferentially at higher SFs, by a nonspecific dopamine-receptor agonist, (±)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) (Shi and Stell, 2013, *Invest Ophthal Vis Sci* (*ARVO Abstracts*), 54:3427). In the present study, more specific dopamine-receptor ligands were tested.

A D2R-selective agonist, quinpirole (10 mM), increased dark-adapted CS at higher SFs compared to the dark-adapted CS before injection (Figure 3.2A: $p<0.0001$ at SF=0.5 cyc/deg; $p<0.01$ at SF=0.8 cyc/deg; $p<0.05$ at SF=1.0 cyc/deg; n=6-7, unpaired *t*-test). It also slightly but significantly *de*creased CS at SF=0.1 cyc/deg (Figure 3.2A; $p<0.05$, n=6, unpaired *t*-test). A D4R agonist, PD168077 (1.0 mM), significantly increased the dark-adapted CS at all SFs tested (Figure 3.2B: $p<0.01$ at SF=0.1 cyc/deg; $p<0.001$ at all other SFs; unpaired *t*-test, n=6-7).

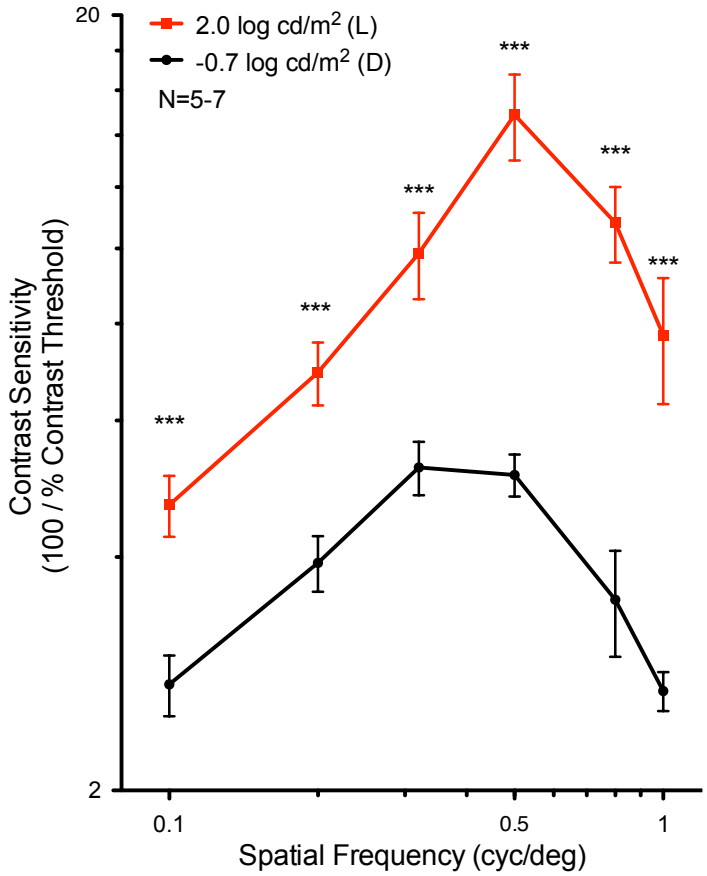

Figure 3.1 Spatial contrast sensitivity (CS) of the chicks under light- and dark adaptation. At every spatial frequency (SF) tested, dark adaptation significantly decreased CS (***$p<0.001$, N=5-7, unpaired t-test). In the light (I_{mean}= 2.0 log cd/m^2), the CS function peaked at about 0.5 cyc/deg. Under dark adaptation (I_{mean}= -0.7 log cd/m^2), this peak shifted leftward on the SF axis. Data are presented as mean ± SD.

Given that both D2R and D4R are in the D2-like receptor family, we asked next whether activation of D1 receptors would have a similar effect on CS. However, SKF38393, a D1R agonist, did not alter CS – not at any concentration, nor at any time tested.

Dopamine Antagonist Mimicked Dark Adaptation in Light-Adapted Chicks

To confirm that DA was an essential messenger in light-adaptation of CS, we injected DA receptor antagonists into the eyes of light-adapted chicks – in principle, to simulate the dark-adapted, low-DA state by blocking the action of light-released endogenous DA at those receptors. We observed that a D2R antagonist, spiperone (200 μM in 0.2% DMSO), increased CS at all SFs tested except the two lowest (Figure 3.2C: $p<0.01$ at SF=0.32 cyc/deg; $p<0.001$ at SF=0.5; 0.8 cyc/deg, $p<0.05$ at SF=1.0 cyc/deg; paired *t*-test, n=8-10). The D1R antagonist SCH23390, on the other hand, had no effect at any concentration tested, nor at any time after injection ($p=0.053$ at 0.1 mM; $p=0.4166$ at 0.316 mM; $p=0.6334$ at 1.0 mM; $p=0.3831$ at 10 mM; repeated-measures ANOVA, N=9).

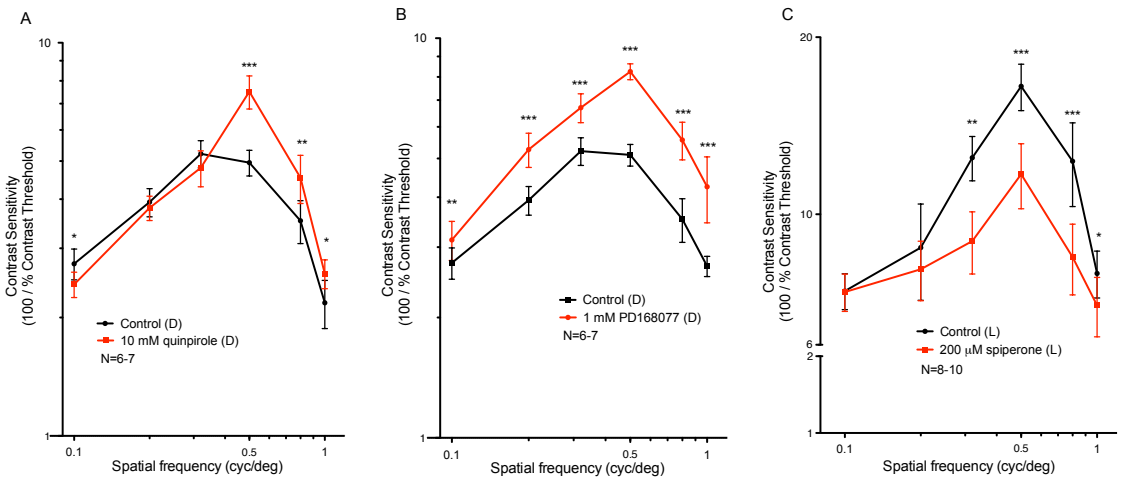

Figure 3.2 Dopamine agents mimicked the effects of light- and dark adaptation upon CS. (A) The D2R agonist, quinpirole (10 mM), increased CS of previously dark-adapted chicks selectively at higher SFs (***$p<0.0001$ at SF=0.5 cyc/deg; **$p<0.01$ at SF=0.8 cyc/deg; $p<0.05$ at SF=1.0 cyc/deg; N=6-7, unpaired *t*-test). It also *de*creased CS at SF=0.1 cyc/deg (*$p<0.05$, N=6, unpaired *t*-test). (B) The D4R agonist, PD168077 (1mM), increased CS of dark-adapted chicks at all SFs tested (**$p<0.01$ at SF=0.1 cyc/deg; ***$p<0.001$ at all other SFs; N=6-7, unpaired *t*-test). (C) The D2R antagonist, spiperone (200 μM), decreased CS of light-adapted chicks at all but the lowest two SFs (**$p<0.01$ at SF=0.32 cyc/deg; ***$p<0.001$ at SF=0.5; 0.8 cyc/deg; *$p<0.05$ at SF=1.0 cyc/deg; N=8-10, paired *t*-test). Data are presented as mean ± SD.

Nitric Oxide Synthase Inhibitors Mimicked Dark-Adaptation in the Light

A parallel strategy was employed to test for involvement of nitric oxide (NO) in light adaptation of optokinetic CS. We first simulated dark adaptation in the light by blocking production of NO with L-N^5-(1-Iminoethyl)ornithine hydrochloride (L-NIO, 5mM), a broad-spectrum NOS-inhibitor reported to be very effective in the intact chick retina (Wellard et al., 1995). L-NIO decreased light-adapted CS at all but the lowest SF tested (Figure 3.3A: $p<0.05$ at SF=0.2 and 0.32 cyc/deg; $p<0.001$ at SF=0.5 cyc/deg; $p<0.01$ at SF=0.8 and 1.0 cyc/deg; N=7-8, paired *t*-test). To increase our confidence that the effect of L-NIO was due to inhibition of NOS and not to some other action of this L-arginine-like compound (Palmer et al., 1988; Rees et al., 1989), we tested another NOS inhibitor, L-N^G-monomethyl arginine acetate (L-NMMA, 0.1 mM), also found to be very effective in the intact chick retina (Wellard et al., 1995). Again, dark-adaptation of the CS function was mimicked in light-adapted chicks by L-NMMA, which reduced CS at all SFs except the lowest (Figure 3.3B: $p<0.001$ at SF=0.2 and 1.0 cyc/deg; $p<0.05$ at SF=0.32 and 0.8 cyc/deg; $p<0.01$ at SF=0.5 cyc/deg; N=7-9, paired *t*-test). To confirm that this action of L-NMMA was due to inhibition of NOS, we tested D-NMMA, an enantiomer that is ineffective at inhibiting NOS activity (Palmer et al., 1988; Rees et al., 1989). Unlike L-NMMA, D-NMMA did not alter CS, even at concentrations 10-100 times those at which L-NMMA was effective (1.0 and 10 mM; p=0.22 and p=0.27, respectively, repeated-measures ANOVA, N=9), at any time tested. D-NMMA at 0.1 mM did have a marginally significant effect on CS at 0.5 cyc/deg (p=0.0429, repeated-measures ANOVA, N=9); however, it did not alter light-adapted CS at SF=0.2 and 0.8 cyc/deg, where L-NMMA strongly decreased CS (p=0.98 and p=0.70, respectively, paired *t*-test, N=9 for both, data not shown). Overall, therefore, D-NMMA had

virtually no effect on light-adapted CS, and the marginal effect at 0.5 cyc/deg might disappear if the sample size were larger.

Nitric Oxide Donors Did Not Mimic Light Adaptation in the Dark

To test further the role of NO in light adaptation, we injected NO donors – either S-nitroso-N-acetyl-DL-penicillamine (SNAP) or sodium nitroprusside (SNP) – into the eyes of dark-adapted chicks. However, no increase in CS was observed. In fact, a high concentration of SNAP (20 mM) *decreased* CS at higher SFs ($p=0.001$ at SF=0.8 cyc/deg, N=6; $p=0.0006$ at SF=1.0 cyc/deg, N=9; paired *t*-test), although it did not alter CS significantly at other concentrations during the period tested. Sodium nitroprusside (15.8 mM), on the other hand, caused the chicks to move their heads continuously in the direction opposite to that expected of the OKR – i.e., naso-temporally rather than temporonasally – in the absence of visual stimulation. This nonvisual movement lasted for days, suggesting an irreversible, and therefore non-pharmacological (i.e., toxic) effect of SNP at that dose. These findings, which are inconsistent with our results from the NOS inhibitors, will be considered below in the "Discussion" section.

Did DA or NO Modulate CS by Acting in Series?

It has been reported elsewhere that in DA- and/or NO-mediated light adaptation of circuit functions, NO acts downstream to the DA mechanism. For example, the effect of DA on regulating eye growth in chicks, an indicator of light adaptation, requires NOS-activation by DA (Nickla et al., 2013); and spinule formation in horizontal cell dendrites, a read-out for light

adaptation in the carp retina, cannot be mimicked using DA agonist alone while NOS is inhibited (Haamedi and Djamgoz, 2002). To find out whether this holds for optokinetic CS in the chick, we tested dark-adapted CS after injecting a cocktail of DA agonist and NOS inhibitor. As might be predicted from such findings, we found that the increase in CS in the dark, routinely elicited by D2R or D4R agonist (Figure 3.2A,B), was absent when either L-NIO or L-NMMA was added

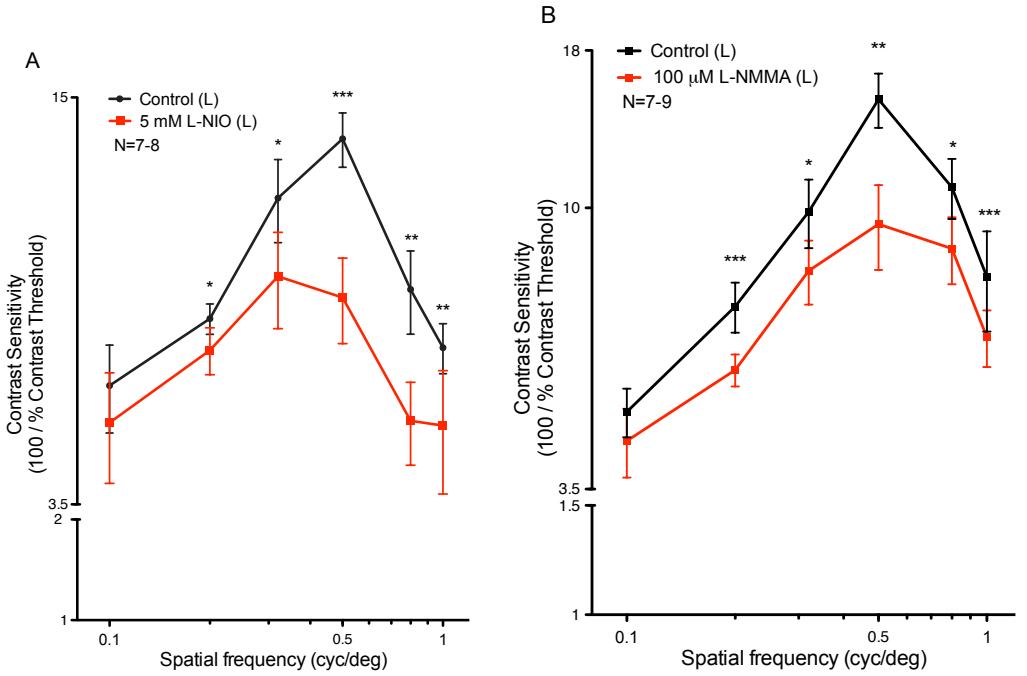

Figure 3.3 Nitric oxide synthase (NOS) inhibitors mimicked dark adaptation. (A): L-NIO (5 mM) decreased CS in light-adapted chicks at most SFs tested (*p<0.05 at SF=0.2 and 0.32 cyc/deg; ***p<0.001 at SF=0.5 cyc/deg; **p<0.01 at SF=0.8 and 1.0 cyc/deg; N=7-8, paired *t*-test). (B): L-NMMA (0.1 mM) also decreased light-adapted CS (***p<0.001 at SF=0.2 and 1.0 cyc/deg; *p<0.05 at SF=0.32 and 0.8 cyc/deg; **p<0.01 at SF=0.5 cyc/deg; N=7-9, paired *t*-test). Data are presented as mean ± SD.

to the injected solution (Figure 3.4). Indeed, NOS inhibitors even lowered *dark-adapted* CS to some degree in the presence of DA agonist (Figure 3.4).

Cell-Cell Coupling as Possible Final Mechanism for Neural Light Adaptation?

Light adaptation *per se*, and DA and NO (both of which are released preferentially in the light), are known to reduce coupling between retinal neurons (Bloomfield and Völgyi, 2009; Piccolino et al., 1984; Hampson et al. 1992; Pottek et al., 1997; Xin and Bloomfield, 2000). Therefore, we tested the hypothesis that modulation of cell-cell coupling in the retina could underlie the effects of light adaptation, DA and NO on optokinetic CS, by using gap junction blockers (as we had used DA agonists) to mimic light adaptation. Surprisingly, meclofenamic acid (MFA, 20 mM), a highly soluble gap junction blocker that has been widely used and validated in retinal research (Pan et al., 2007; Veruki and Hartveit, 2009), did not increase CS in the dark as predicted (Figure 3.5). Mefloquine (MFQ) – a blocker reported to be relatively selective for connexin (Cx)36- and Cx50-mediated gap junctions, between N2A cells transfected with rat Cx36 or mouse Cx50 (Cruikshank et al., 2004) – is poorly soluble, and we could not test it at concentrations higher than 2 mM; and even at this rather high concentration, MFQ also did not alter CS in the dark.

It has been suggested that lateral inhibition operates mainly via HCs in the outer retina under strongly light-adapted (photopic) conditions, whereas it operates mainly via ACs in the inner retina (mainly through GABAergic and glycinergic mechanisms) under more weakly adapted (mesopic) conditions (Cook and McReynolds, 1998; Taylor, 1999; McMahon et al., 2004). Since cell-cell coupling alters the strength of modulatory inputs to the retinal through-pathway (and

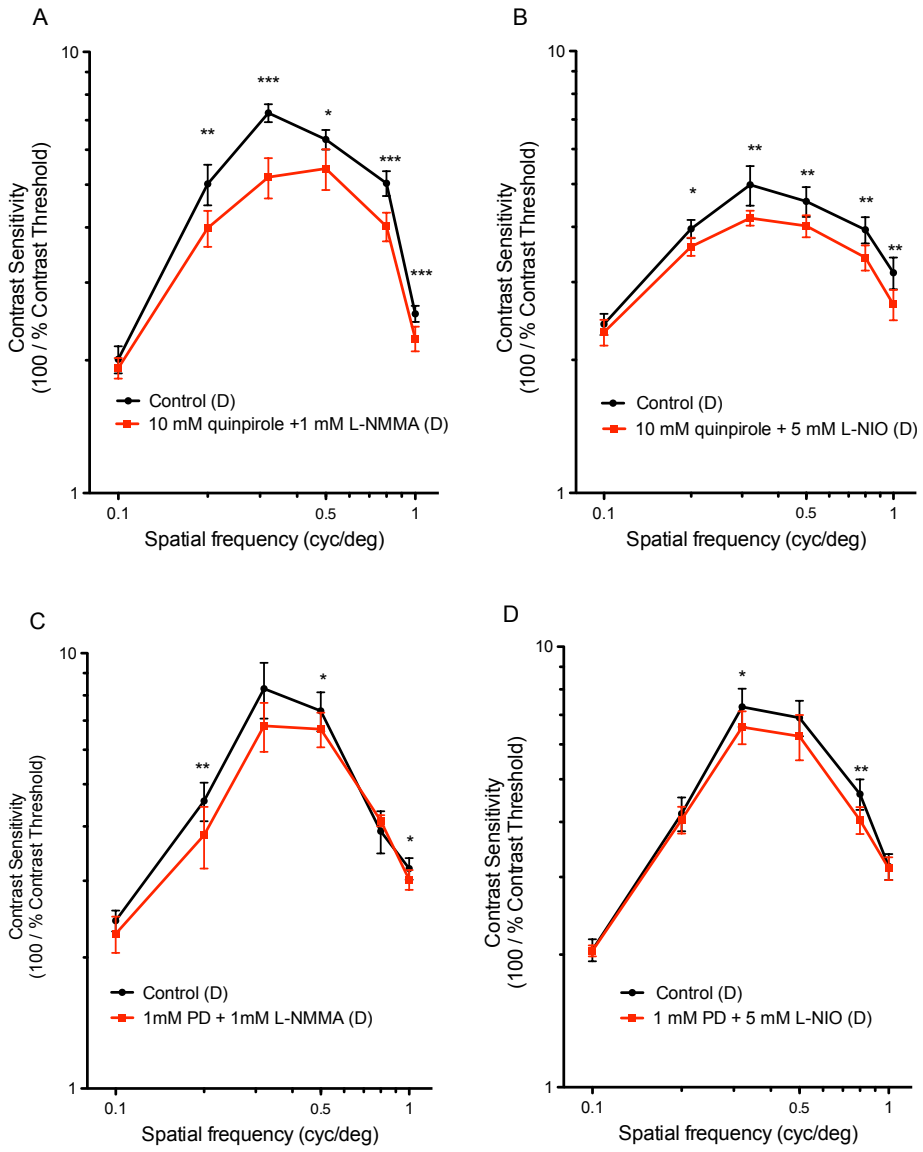

Figure 3.4 The effects of NOS inhibitors on DA agonist-treated CS. (A,B): the increase in CS seen in the D2R agonist quinpirole (10 mM) alone was abolished when NOS inhibitor L-NMMA (1 mM) or L-NIO (5 mM) was present. Both NOS inhibitors also decreased CS to different degrees (L-NMMA: **p<0.01 at SF=0.2 cyc/deg; ***p<0.001 at SF=0.32, 0.8, and 1.0 cyc/deg; *p<0.05 at SF=0.5 cyc/deg, N=9, paired t-test; L-NIO: *p<0.05 at SF=0.2 cyc/deg; **p<0.01 at SF=0.32, 0.5, 0.8, and 1.0 cyc/deg, N=9, paired t-test). (C,D): similarly, the increase in CS caused by the D4R agonist PD168077 (1 mM) alone was also unobservable in the presence of NOS inhibitors; both inhibitors decreased CS at some SFs (L-NMMA: **p<0.01 at SF=0.2 cyc/deg, *p<0.05 at SF=0.5 and 1.0 cyc/deg, N=8-9, paired t-test; L-NIO: *p<0.05 at SF=0.32 cyc/deg, **p<0.01 at SF=0.8 cyc/deg, N=9, paired t-test). Data are presented as mean ± SD.

thereby, spatiotemporal tuning in retinal circuits) (Veruki et al., 2010), and since manipulation of coupling between retinal neurons failed to modulate CS under weak illumination, we tested whether MFA could influence CS under stronger illumination. Meclofenamic acid did indeed increase CS in light-adapted chicks, at all but the two lowest SFs tested (Figure 3.5: SF=0.32 cyc/deg, $p<0.0001$, N=9; SF=0.5 cyc/deg, $p=0.0156$, N=8; SF=0.8 cyc/deg, $p=0.002$, N=8; SF=1.0 cyc/deg, $p=0.0156$, N=8; paired t-test). This suggests that changes in cell-cell coupling (possibly between HCs) may mediate light-adaptational changes in CS, in a luminance-dependent manner.

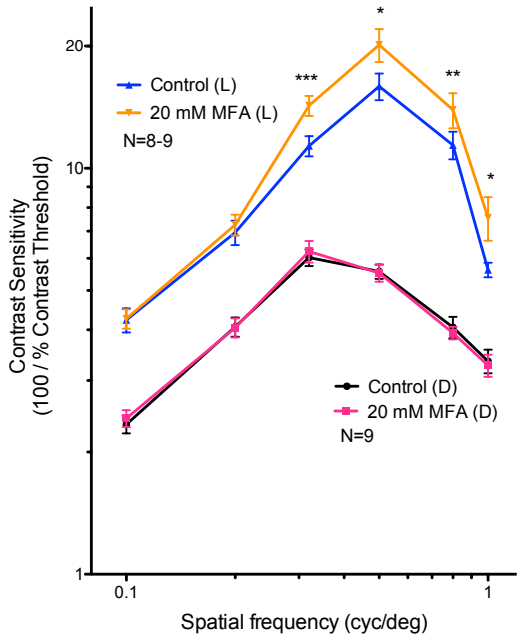

Figure 3.5 The effect of a gap junction blocker, meclofenamic acid (MFA), on dark- and light-adapted CS. MFA (20 mM) did not cause any changes in dark-adapted CS (below: black and pink traces). It did *increase* CS of light adapted chicks (above: blue and orange traces) at all but the lowest two SFs tested (***$p<0.0001$ at SF=0.32 cyc/deg, *$p=0.0156$ at SF=0.5 cyc/deg, **$p=0.002$ at SF=0.8 cyc/deg, p=*0.0156 at SF=1.0 cyc/deg, N=8-9; paired t-test). Data are presented as mean ± SD.

DISCUSSION

In the present study we have further validated the chick optokinetic response (OKR) for understanding the effects of light adaptation and two important messenger systems on spatiotemporal processing in the retina. We have replicated the effects of manipulating adaptation state and dopamine (DA) signaling, previously described in mice, and presented new findings that implicate nitric oxide (NO) signaling and modulation of cell-cell coupling. The chick, with its large eyes, retinas specialized for photopic vision, and ease of conditionally altering visual processing in the retina in situ, is a promising model for understanding fundamental visual mechanisms that are shared across species.

Dopamine as Light-Adaptive Neuromodulator

Dopamine acts as a neuromodulator for a variety of light-adaptive retinal properties: regulating eye growth in chick (Feldkaemper and Schaeffel, 2013; Stone et al., 2013; Cohen et al., 2012), Purkinje shift in quail (Manglapus et al., 1999), horizontal cell spinule formation in fish (Yazulla et al., 1996) and cell-cell coupling (Lasater and Dowling, 1985; DeVries and Schwartz, 1989; Tornqvist et al., 1988; Hampson et al., 1992) in various species. DA synthesis is increased upon light adaptation and decreased upon dark adaptation (Godley and Wurtman, 1988; Boatright et al., 1989; Kirsch and Wagner, 1989). In mice carrying a conditional knockout of tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, optokinetic CS at middle to high SFs was lower than in wild type (Jackson et al., 2012). Now, using a rapid, reversible, time- and eye-specific method, we have shown that DA plays a similar role in chicks.

In dark-adapted retinas, a D2R- or D4R-specific surrogate for dopamine mimicked the effect of light adaptation (Figure 3.2), whereas a D1R agonist was ineffective. Both D2R and D4R are expressed in chick retinal neurons: D2R-immunoreactive proteins in photoreceptor inner segments, outer and inner plexiform layers, and the ganglion cell layer, and D4R mRNA mainly in inner retinal cells (Rohrer and Stell, 1995). We suggest that the D2-like agonists acted via inner-retinal cells expressing D4R, because the effect of the D4R agonist was significantly greater than that of the D2R agonist (Figure 3.2B), and because certain direction-selective ganglion cells (DSGCs) of the mouse retina express D4R (Huberman et al., 2009). While the D2R- and D4R-induced increases in CS were not comparable in magnitude to the light-adapted CS (Figure 3.1), D2Rs also serve as release-suppressing autoreceptors on dopaminergic cells (Veruki, 1997), which could truncate any postsynaptic effects of D2-like agonists. Similarly, a D4R agonist but not D1R agonist increased CS in TH knock-out mice (Jackson et al., 2012).

It was reported that DA increased glutamate- or KA-induced current in perch HCs (Knapp et al., 1990). In another study on perch HCs, DA suppressed desensitization of iGluRs, switching transient glutamate-gated currents into sustained currents (Schmidt et al., 1994). Since the DAergic interplexiform cells extend processes to the OPL (Dowling and Ehinger, 1975), it is not surprising to see such DA-induced modulation of HCs. Further exploration using more specific DA- and protein kinase A agents showed that DA enhanced glutamate-gated currents through a D1R-cAMP-PKA pathway in salamander OFF BCs (Maguire and Werblin, 1994). We did not see D1R agent-evoked modulation of CS function, and the OKR is driven by RGCs postsynaptic to the *ON* BCs; however, we cannot exclude the possible influence of DA on iGluRs, since the

displaced ON-center RGCs certainly express iGluRs. The GluR6 subunit of GluR contains phosphorylation site for protein kinase modification, whereas other subunits (GluR1-4) lack it (Raymond et al., 1993). If ON-center displaced RGCs in the chick express GluR6 and are a target for DA, DA's inhibition of desensitization of iGluRs, i. e., switching a transient current to a sustained one, will likely alter CS to high temporal-frequency stimulus and thereby alter the *temporal* coding properties of the retina.

Nitric Oxide as Neuromodulator for Light Adaptation

The synthesis of NO, like that of DA, is increased upon light adaptation (Neal et al., 1998). However, unlike DA – which is released by cells of a single type and may act over large distances – NO is synthesized by NO synthase (NOS), in many types of cells in the chick retina (Fischer and Stell, 1999; Haverkamp et al., 1999; Wilson et al., 2011; Tekmen-Clark and Gleason, 2013); NO has a short half-life and can act only locally. The many sources, and consequently many local targets, make the retinal NO system more complex than that of DA. Furthermore, NO as neurotransmitter/neuromodulator is thought to act mainly by a single target, activating soluble guanylyl cyclase to produce cGMP, increasing the probability that any agonist treatment activates many mechanisms, confounding the detection of site-specific actions. Nevertheless, NO shares several light-adaptational properties with DA, such as myopia-prevention in chick (Nickla et al., 2006), cell-cell coupling in bass HCs (Lu and McMahon, 1997), and spinule formation in carp HC dendrites (Pottek et al., 1997).

As reported here, NOS-inhibitors L-NIO and L-NMMA strongly reduced CS in light-adapted chicks (Figure 3.3), thus simulating dark adaptation, whereas the inactive enantiomer, D-NMMA, had no effect. This clearly indicates that NO, derived from NOS activity inside the eye, exerts a light-adaptational influence on retinal circuitry in the chick. However, the NO donors – SNAP and SNP – did not affect CS in dark-adapted chicks, except at high, probably toxic doses. We suggest that NO-dependent light adaptation in chicks is due to modulation of OKR-related signaling in only one of numerous local circuits, and that the widespread and diverse actions of exogenous NO (donor) obscure many local effects. In our experiments, NO donor SNAP conserved band-pass CS functions and attenuated CS only at higher SFs – perhaps affecting microcircuits having a fine spatial scale, such as BCs or narrow-field ACs, rather than the OKR-generating displaced RGCs themselves, which cover ~3-4 degrees of visual angle (Reiner et al., 1979; Prada et al., 1989). Sodium nitroprusside (15.6 mM) caused a permanent, nonvisual head movement in the opposite direction of the expected OKR. The long-lasting effect of this short-lived agent is likely due to toxicity in the retina, which created an imbalance between the excitatory and inhibitory inputs to the higher centers of the OKR circuit (accessory optic system).

Possible Interaction Between DA and NO Systems in the Retina

We tested for possible interactions between DA and NO in modulating CS of the chick, because it has been reported that DA controls NO synthesis in carp retina (Sekaran et al., 2005), and acts through NO to regulate eye growth in chicks (Moinul et al., 2012; Nickla et al., 2013) and horizontal-cell spinule formation in fish (Haamedi and Djamgoz, 2002). Our data suggest

similarly that DA attenuates CS by activating NOS – i.e., that in the dark-adapted retina some NO-driven activity contributed to DA-dependent changes in the OKR. Other interactions have not been excluded, for example, the effect of NOS inhibitor in this experiment might simply be too strong that it masked the parallel increase in CS caused by DA agonist. These questions could be addressed in subsequent studies.

Cell-cell Coupling in Retinal Adaptation

Using a Cx57 knock-out mouse line in which HC coupling was abolished, Dedek et al. (2008) found no difference in adaptation-induced shift in spatial tuning of either RGC responses or OKR compared to wild type. Although it could be that compensatory changes happened downstream in retinal circuitry during development, this finding together with our own led us to question the role of HC-HC coupling in the OKR (as we initially hypothesized), and to rethink the interpretation of our MFA results. Synchronization of firing, through electrical coupling, has been shown in RGCs (Mastronarde, 1983; Meister et al., 1995). Did MFA in our experiments act directly on the ON-center, displaced RGCs that drive the OKR in the chick? In the mouse, ON-OFF DSGCs that code upward movement show coupling (Trenholm et al., 2013), and although coupling-mediated inputs contribute little to spiking events (due to strong attenuation caused by junctional resistance) in the neighboring cells, they prime them for anticipatory responses. However, we note that (1) at least in mammals, the ON-OFF and ON DSGCs are functionally distinct and project to different visual centers (Kay et al., 2011), and (2) the OKR reflects *supra*threshold processes, i. e., optokinetic signals have to have been sent to the accessory optic system before an OKR is evoked, regardless of adaptation state. In contrast, rabbit ON DSGCs,

coupled indirectly through a common wide-field AC, encode null-direction movement by GABA-mediated desynchronization (Ackert et al., 2006). Our observation that MFA increased CS at *higher* SFs suggests that uncoupling of some retinal cells (not yet identified) shifts their spatial tuning to a finer scale. Moreover, although we do not yet know the coupling characteristics of displaced RGCs in the chick, coupling of rabbit a-RGCs (with their neighbors and ACs) *increases* in the light (Hu et al., 2010); thus, in this case uncoupling should mimic *dark* adaptation. Uncoupling of RGCs, e. g., using MFA, theoretically should desynchronize them and broaden their temporal responding range, thereby improving their temporal resolution. This would be an interesting area for future exploration.

Supplemental Material

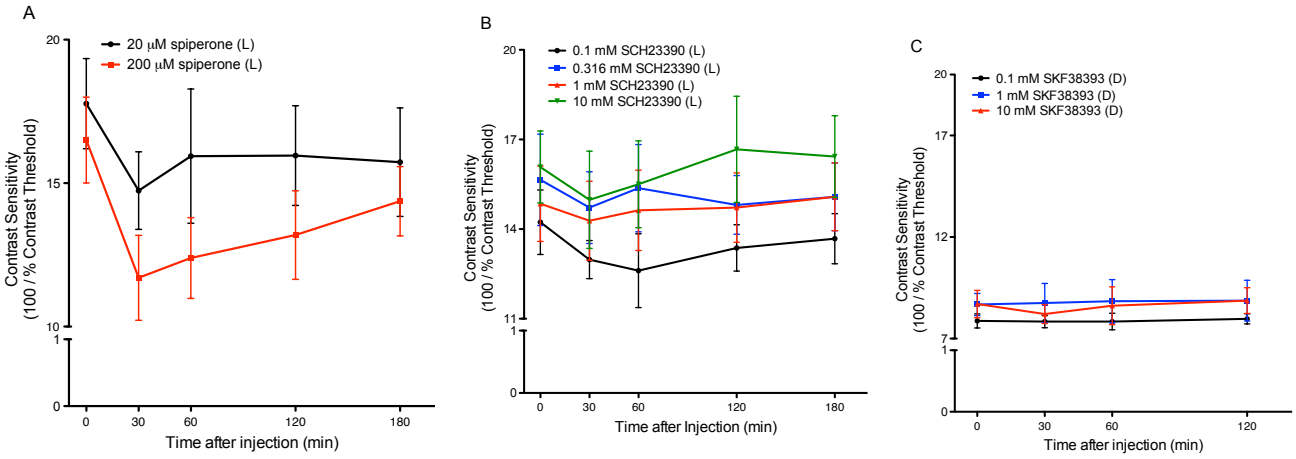

Figure S1 Effects of dopamine ligands on optokinetic contrast sensitivity (CS; linear scale) at SF=0.5 cyc/deg, at different concentrations and times after intravitreal injection in the light (L). (A): Spiperone, a D2R antagonist, at 20 μM, decreased CS significantly only 30 minutes after injection ($p=0.0121$; repeated-measures ANOVA, N=9), whereas at 200 μM, its depressive effect on CS persisted for ≥3 hours after injection ($p<0.001$ at 30 min, 1 hr, and 2 hrs, and $p<0.05$ at 3 hrs; repeated-measures ANOVA, N=9). (B): SCH23390, a D1R antagonist, had an almost significant influence on CS in the light (L), at 0.1 mM ($p=0.0533$, N=9), but no statistically significant effect at other concentrations ($p=0.38$-0.63; repeated-measures ANOVA, N=9). (C): SKF38393, a D1R agonist, exerted no significant effects on CS in the dark (D), at any concentration ($p=0.20$-0.93; repeated-measures ANOVA, N=9).

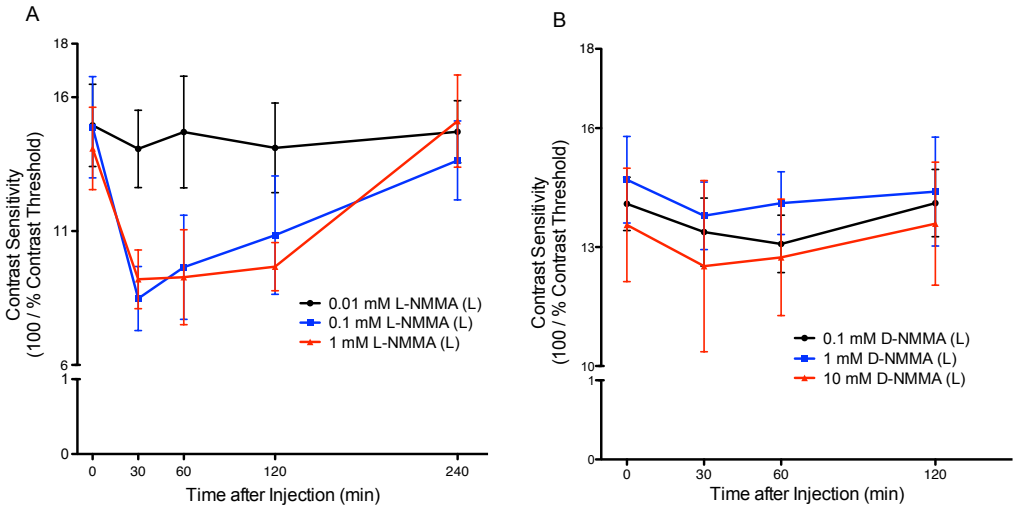

Figure S2 Effects of active and inactive inhibitors of nitric oxide synthase (NOS) on optokinetic contrast sensitivity (CS; linear scale) at SF=0.5 cyc/deg, at different concentrations and times after intravitreal injection in the light (L). (A): L-NMMA, an active NOS-inhibitor, had no significant effect on CS at the lowest dose tested (0.01 mM; $p=0.62$; repeated-measures ANOVA, N=6), whereas at 0.1 mM and 1.0 mM it decreased CS significantly (0.1 mM: $p=0.024$ at 30 min and 1 hr; 1.0 mM: $p<0.001$ at 30 min, 1 hr, and 2 hrs; repeated-measures ANOVA, all N=5). (B): D-NMMA, a NOS-inactive enantiomer of L-NMMA, marginally decreased CS at 0.1 mM ($p=0.043$) but had no significant effect on CS at 1 mM ($p=0.22$) or 10 mM ($p=0.27$); all repeated-measures ANOVA, N=9.

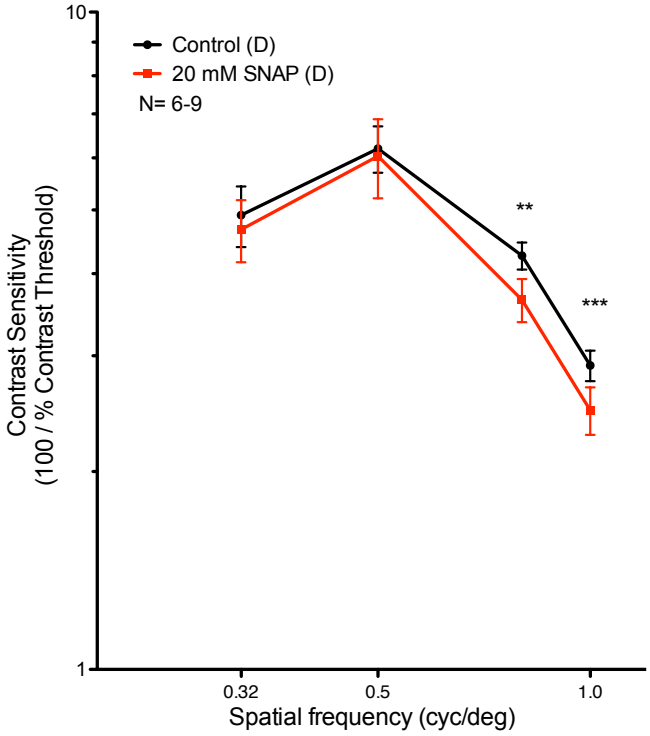

Figure S3 Effect of SNAP (20mM), a nitric oxide donor, on optokinetic contrast sensitivity (CS; $\log_{10}$ scale), after intravitreal injection in the dark (D). SNAP decreased CS at higher SFs (SF=0.8 cyc/deg: p=0.0018, paired t-test, N=6; SF=1.0 cyc/deg: p=0.0006, paired *t*-test, N=9).

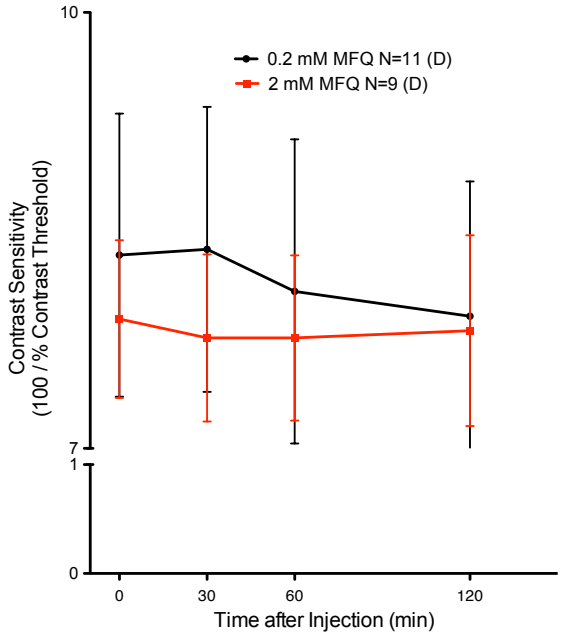

Figure S4 Effects of a gap junction blocker, mefloquine (MFQ), on optokinetic contrast sensitivity (CS; $\log_{10}$ scale) at SF=0.5 cyc/deg, at different concentrations and times after intravitreal injection in the dark (D). At either dose tested (0.2 mM, p=0.5253, N=11; 2 mM, p=0.9435, N=9; repeated-measures ANOVA), MFQ did not cause a difference in CS compared to that before injection (0 min).

Chapter 4: Global Discussion

In the present work, I used a fast, noninvasive, and specific method- the optokinetic response (OKR)- to examine retinal spatiotemporal processing under different adaptational conditions. The chick retina, like that of the mouse, adapts by using a ‘‘day/night’’ or ‘‘cone/rod’’ switch in tuning preference during changes in lighting conditions. Additionally, I explored the roles of dopamine (DA), nitric oxide (NO), and cell-cell coupling in adaptation, and found that DA and NO are adaptation-linked modulators of spatiotemporal processing in the retina, whereas gap junctions are certainly important, but only conditionally.

Limitations of the “All-perfect” OKR

Although, as mentioned in the preceding chapters, the OKR is fast, noninvasive, easy-to-test (compared to learned behavioral tests), and specific to a single type of RGC (the displaced, ON type DSGCs in the chick), it has its own limitations. One is that the OKR cannot reveal events that happen under a temporal range of milliseconds. In optokinetic CS measurement, a critical factor one needs consider is “contrast adaptation”, the slow phase of which in both mammals and nonmammals can take tens of seconds to complete (Smirnakis et al., 1997; Baccus and Meister, 2002). To avoid obtaining CS results at different stages of contrast adaptation, CS in this study was tested repeatedly (4-out-of-5 criterion), which means that we cannot study behaviors that happen within a very short time window, for example, desensitization/ recovery of the AMPA receptor (Mosbacher et al., 1994; Sun et al., 2002). Another limitation of the OKR is indeed its “strength”- the specificity to the ON DSGCs (in the chick, the ON displaced RGCs); hence the OKR can only display the neural signaling, and modulation of signaling within this specific circuit. For example, a study on rabbit showed that ON DSGCs that project to the accessory optic

system (AOS) are indirectly coupled with their neighbors through a common wide-field AC (Ackert et al., 2006). Since I applied the gap junction blocker, meclofenamic acid (MFA), "globally" via intravitreal injection, the effect of MFA might be a result of uncoupling of these cells from the ACs plus uncoupling of any gap-junctioned cells in the retina. As a result of OKR's specificity, this interpretation of our MFA results cannot be extrapolated to other DSGC subtypes; e. g., ON-OFF DSGCs that do not project to the AOS (Oyster and Barlow, 1967; Kay et al., 2011; Huberman et al., 2009), and certainly not to other types of RGC. Therefore, the mechanisms that underlie adaptation of the optokinetic pathway revealed here do not necessarily apply to other circuits in the retina, which can hardly be investigated by my method. Moreover, the OKR (as the final output of the optokinetic circuit) cannot easily probe individual excitatory and inhibitory input, even in studies that measured the gain (Bonaventure et al., 1992a; Bonaventure et al., 1992b), which in the OKR is the eye- or head moving velocity divided by the stimulus velocity. For example, an increase in gain or in threshold of contrast, can result from an increase in excitatory input or a decrease in inhibitory input, or both. Finally, the AOS has considerable interactions with other visual centers and brain structures (for review, see Giolli et al., 2006), which makes the OKR less desirable a method to investigate purely retinal properties.

Dopamine's "Side Effect": Modulation of Glutamate Receptor Physiology?

The research presented in Chapter 2 follows a logic of "light→DA release→uncoupling of gap junctions→light adapted CS". In isolated, coupled HC pairs, junctional resistance was greatly increased upon delivery of brief DA pulse (Lasater and Dowling, 1985). Injection of cAMP into one of the paired HCs caused a similar effect, so did injection of the catalytic subunit of cAMP-dependent kinase (Lasater, 1987). It has been appreciated that DA modulates connexin proteins

by phosphorylation or dephosphorylation (Kothmann et al., 2009), mainly of serine amino acids on the carboxyl tail or intracellular loops of Cx proteins (Lampe and Lau, 2000, 2004). What about other roles that DA plays in the retina?

In a group of studies, DA showed modulatory effects on non-NMDA ionotropic glutamate receptors (iGluRs). In a study on isolated HCs from the perch retina, application of DA increased glutamate- or KA-induced current by increasing the frequency of channel openings (versus altering the number of channels or single-channel conductance) (Knapp et al., 1990). Since, in this study, DA was applied in the bathing solution- presumably only acted on plasma membrane outside the patched area- and hence had no access to GluRs being studied, this effect was assumed to be mediated by intracellular second messengers such as cAMP. In another study, also on perch HCs, DA strongly suppressed desensitization of iGluRs, switching transient GluR-gated currents into sustained currents (Schmidt et al., 1994). Since the DAergic interplexiform cells have processes extending to the outer plexiform layer (Dowling and Ehinger, 1975; Boycott et al., 1975; Kleinschmidt and Yazulla, 1984; Dacey, 1990), it is not surprising to see such DA-induced modulation in the outer retina. Further exploration on this topic using more specific DA agents (for D1R and D2R) and protein kinase A agents showed that DA enhanced glutamate-gated currents through a D1R-cAMP-PKA pathway in OFF BCs of the salamander retina (Maguire and Werblin, 1994). I have not seen D1R agent-evoked modulation of CS function (Chapter 2), and the OKR is driven by the ON displaced RGCs postsynaptic to the ON BCs; however, I cannot exclude the possible influence of DA on iGluRs, since the displaced ON RGCs certainly express iGluRs. It will be interesting to know where in the chick retina DA might exert such an effect on glutamate-gated currents. One way to address this is to screen for

GluR6 subunit - a subunit that has phosphorylation site for protein kinase modification, whereas other subunits (GluR1-4) lack it (Raymond et al., 1993) - in the chick retina, using *in situ* hybridization or immunocytochemistry; and to co-localize the GluR6-containing cells with D1R. In agreement with the above physiological findings, GluR6/7 immunoreactivity has been reported present in the cat HC and OFF BCs (Morigiwa and Vardi, 1999). In rats and goldfish, GluR6/7 was seen more widespread, including HCs, ACs, RGCs, and cone outer segments (goldfish), in addition to the two plexiform layers (Peng et al., 1995). What can be done using the optokinetic CS, though, is to examine temporal coding properties of the retina; that is, if displaced RGCs in the chick retina do contain GluR6 and are a potential target for DA, DA's inhibition of desensitization of iGluRs, i. e., switching a transient current to a sustained one, will likely alter CS to high temporal-frequency stimulus.

The Mystery: SNP-induced Spontaneous Head Movement

Upon injection of sodium nitrosprusside (SNP), I observed a non-stop, non-visual head movement that is in the opposite direction of the expected OKR, i. e., the nasal-temporal direction of the injected eye. In a study that examined horizontal optokinetic nystagmus (OKN) of the chicken, intravitreal injection of 2-amino-4-phosphonobutyric acid (APB), a glutamate receptor agonist that blocks the ON pathway of the retina, also caused spontaneous eye- and head-movement, in addition to strongly reducing the OKN, as seen in other species (frog: Yücel et al., 1989; rabbit: Knapp and Schiller, 1984; turtle: Knapp et al., 1988). More intriguingly, in their study, the spontaneous movement became more robust when the chickens were in total darkness, and unobservable when the optic nerve of the injected eye was sectioned. It remains unknown whether their finding was due to toxic effect since there is no information of long-term

observation. The fact that darkness and optic nerve sectioning could modulate the movement made it more likely a result of processing of the retina. Additionally, intravitreal injections of picrotoxin, a GABA antagonist, produced spontaneous, temporal-nasal nystagmus in a few species (Ariel et al., 1988; Ariel, 1989). In another study of the chick, ocular application of both GABA agonist and antagonists caused spontaneous head- and eye movement (Bonaventure et al., 1992). Therefore, if SNP in my experiment influenced the vertical pathway (in which glutamate serves as a signaling messenger) or the GABA input provided by HCs and/or ACs, then we might have mimicked the effects of the above studies. On the other hand, these "mainstream" neurotransmitters in the above studies could have affected NO-producing cells and provoked spontaneous movements *through* NO. In fact, in the chick retina, two of the four main types of neuronal NOS-expressing ACs have dendrites ramifying in the ON sublamina of the IPL (Fischer and Stell, 1999; Wilson et al., 2011; Tekmen-Clark and Gleason, 2013); so do the "displaced" nNOS-containing ACs of the mammalian retina (Kim et al., 1999; Lee et al., 2003; Pang et al., 2010). These indicate that these NOS cells are downstream in the ON pathway, and hence liable to modulation by APB.

Could this movement arise from central stages of the optokinetic pathway? In the cat, lesioning of the lateral and dorsal terminal nuclei of the AOS produced spontaneous, horizontal nystagmus (Clement and Magnin, 1984). In the turtle, the nBOR - the nonmammalian homologous of mammalian nucleus of AOS - receives "push-pull" excitatory (glutamatergic) input directly from the retina and inhibitory (GABAergic) input from nucleus of optic tract (NOT) (Ariel and Kogo, 2001), suggesting interactions between AOS and NOT. Therefore, SNP could have caused an imbalance between glutamatergic and GABAergic tone.

Caveats

One problem is that not all cells exhibit a "light→uncoupling, darkness→coupling" behavior. Simply injecting a gap junction blocker cannot fully mimic the effect of light for all cells in the retina. A concrete example is that coupling of rabbit αRGCs (with their neighbors and ACs) *increases* in the light (Hu et al., 2010). Consequently, gap junction blockers can certainly mimic "light adaptation" in cells such as photoreceptors, HCs, and ACs - those that show uncoupling upon light - but probably also mimics "dark adaptation" in coupling between certain RGCs.

Could horizontal cells (HCs) play a major role in modulating CS function according to ambient light level? Contrast detection is a functional result of the center-surround organization of the receptive field of most retinal neurons. Horizontal cells are the first-order interneurons that provide lateral inhibition to the direct flow of information (Thoreson and Mangel, 2012). The most direct evidence is that injection of hyperpolarizing current into HCs caused hyperpolarization in simultaneously recorded ON-center BCs and depolarization in OFF-center BCs of the carp retina (Toyoda and Kujiraoka, 1982), that is, mimicking light-induced HC responses by artificial current injection resulted in surround responses in BCs. Current injections into HCs also affected surround responses of nearby RGCs (Naka and Nye, 1971; Naka and Witkovsky, 1972). In a similar study, both depolarizing and hyperpolarizing currents into HCs increased firing rate of ON-OFF DSGCs of the rabbit retina (Mangel, 1991).

In photopic conditions, RGCs show strong surround inhibition, whereas dark adaptation strongly decreases, if not abolishes, their receptive field surround (Barlow et al., 1957; Donner, 1981; Muller and Dacheux, 1997). Moreover, coupling between HCs is constantly modulated

according to ambient illumination, and hence the strength in their inhibitory input to the vertical pathway is constantly modified; for example, as light intensity is increased, the decrease in HC coupling (but see Xin and Bloomfield, 1999) goes in parallel with the decrease in the size of RGC's surround receptive field (Barlow et al., 1957; Troy et al., 1993; 1999). Finally, HC-HC coupling is modulated by DA and NO agents (Teranishi et al., 1983; Pottek et al., 1997). Therefore, I originally sought to examine whether HC-HC coupling was the final mechanism of the adaptation-induced modulation of the CS function. Surprisingly, the gap junction blockers I used did not increase CS in the dark-adapted chicks as anticipated. Since gap junctions are present in every major type of retinal neurons (Bloomfield and Völgyi, 2009), and since both blockers used in my study are nonspecific, it is possible that universal uncoupling of all gap junctions in the retina may produce opposite effects on CS, which results in no net effect.

However, HC-HC coupling is not the only target for gap junction blockers in HCs. There are a few theories regarding the mechanisms of HC-mediated feedback to photoreceptors, among which a unique one is the ephaptic mechanism. According to the ephaptic model, *hemichannels* at the tips of HCs mediate the HC-driven feedback (Kamermans et al., 2001), and blocking these hemichannels with a broad-spectrum gap junction blocker, carbenoxolone, diminished feedback, which often is measured as a shift in voltage-gated Ca^{2+} currents of photoreceptors to more negative potential (Verweij et al., 1996). In support of this is the presence of connexin 26 (Cx26) hemichannels on HCs of carp (Kamermans et al., 2001; Janssen-Bienhold et al., 2001). Since MFA (or MFQ) did not increase CS as expected, could it be that MFA blocked hemichannels on HCs and hence abolished HC-driven feedback to cones? When it comes to interpreting hemichannel mechanism, one needs to note that carbenoxolone, the gap junction blocker used by

Kamermans et al. (2001), has been shown to reduce Ca^{2+} current directly in *isolated* cones of salamander retina (Vessey et al., 2004). Moreover, had MFA or MFQ diminished this feedback, I should have seen a decrease in CS instead of no difference. Additionally, our further observation that MFA *increased* CS under unattenuated light intensity also disproved this hypothesis. A fancy yet simple solution to this is to target specific Cx gap junctions with mimetic peptide, a relatively novel way of blocking Cx-mediated (both gap junctions and hemichannels) conductance (for review, see Evans et al. 2012). These short-sequence peptides correlate with part of the Cx protein, mainly extracellular loops; and upon binding with Cx, blocks gap-junction or hemichannel conduction. Peptides designated to Cx26 and Cx56 - the chicken version of Cx57 (Rup et al., 1993) that mediates HC-HC coupling - can be applied to answer this question. Since the sequence of these peptides can be very specific to a certain Cx, this way will really help me pinpoint the role of certain gap junctions in adaptation, if there is any.

In a parallel myopia study of our lab, MFA has shown to halt experimental myopia in young chicks (Teves et al., 2014, *Invest Ophthal Vis Sci (ARVO Abstracts)*, 331:3036). More recently, we have tested a mimetic peptide specific to part of the 2nd extracellular loop of chicken Cx36 and observed similar effect on eye growth as did MFA (unpublished data). This suggests a possible role of Cx36-comprised gap junctions in myopia development. Since form-deprivation myopia follows a light-deprivation→decreased retinal DA and/or NO→ (uncoupling of retinal neurons→) excessive eye growth pathway (which is very similar to my hypothesis of optokinetic adaptation), it will be interesting to see if these peptides have any light-adaptation influence on CS in dark-adapted chicks in the future. However, we do not yet have conclusive evidence that the OKR and eye-growth signaling share a common pathway.

Consistent with my finding, in a study of the mouse where Cx57 was knocked out (Dedek et al., 2008), neither optokinetic CS nor RGC spiking behavior showed adaptation-induced modulation, compared to wild-type results. This finding makes us to question whether HC-HC coupling alone is important in modulating spatial CS function according to ambient illumination.

When it comes to the optokinetic pathway, one needs to be aware that the OKR-driven ON DSGCs are quite "atypical", as do other DSGCs. Although these cells inevitably receive HC input indirectly from their presynaptic ON BCs, their selective responsiveness to moving stimuli in a certain direction (i. e., the preferred direction) adds an additional layer of "receptive field" to them. When studying the receptive field of DSGCs, a special type of AC, the starburst ACs (SACs) that plays a major role in shaping DSGCs' responses by inhibiting them asymmetrically (Vaney and Young, 1988; Masland et al., 1984), must be taken into account. Such cells have been anatomically characterized in the chick retina (Spira et al., 1987). It has recently been reported that in the mouse retina certain types of DSGCs are morphologically asymmetrical; their dendiritic arborization is not concentric about their soma but oriented toward the preferred direction measured physiologically (Kim et al., 2008). Therefore, since I do not know on what cells the drugs have acted, further investigations are required to understand adaptational mechanisms of this pathway (see previous section on "limitations of the OKR").

Future Directions and Conclusion

In the first part of my study (Chapter 2), I have found that the chick retina exhibits a "night→day" or "rod→cone" switch in spatiotemporal tuning preference. Could this shift be

mediated by DA and/or NO as well? In an ERG study on Japanese quail, Purkinje shift – a shift of peak spectral sensitivity from longer (560-600 nm) to shorter wavelength (510-520 nm) light from day to night – could be mimicked with D2R agents injected (Manglapus et al., 1999). Agonists or antagonists of DA and NO can be used to mimic "daytime" or "nighttime" vision- an interesting future experiment. Also, to find out the specific gap junction (Cx) that is involved in light-adapted CS function (Chapter 3), mimetic peptides selective to other Cx, for example, Cx56, can be designed and tested. Furthermore, if such peptides can be conjugated with a fluorophore or neurobiotin (which requires further binding with fluorophore-tagged avidin for visualization), we can even visualize to which cells these peptides bind, study their binding kinetics, and eventually have a better understanding of the role of gap junctions in optokinetic adaptation.

In summary, using the OKR, I comprehensively explored light- and dark-adaptional changes in CS function for the first time in the cone-dominated retina of an avian, diurnal species. Resembling the mouse retina, the chick retina adapts by switching between rod/cone in tuning preferences from dark to light. Additionally, I used pharmacology to prove that DA, NO, and cell-cell coupling are critical mechanisms in daytime, cone-dominated, adaptation. The chick's large eyes, easiness to experiment on, and similarities in neural processing to the mammalian retina make them a great model for future retinal research.

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