Spectral Sensitivity of the Goldfish ERG and Optic Tectum Before and After Optic Nerve Damage

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SPECTRAL SENSITIVITY OF THE GOLDFISH ERG AND OPTIC TECTUM
BEFORE AND AFTER OPTIC NERVE DAMAGE

A Thesis
Presented to the Faculty of the Department of Psychology
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
of the Requirements for the
Degree of Master of Arts

by
Jenel Cassidy Pile
August 2001
SPECTRAL SENSITIVITY OF THE GOLDFISH ERG AND OPTIC TECTUM
BEFORE AND AFTER OPTIC NERVE DAMAGE

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It is well known that visual neurons in lower vertebrates regenerate after damage. Thus, the visual system has served as a useful model for neural regeneration studies. However, most of the research on neural regeneration has focused on the recovery of anatomical connections; little has been done assessing the return of visual function. The purpose of this study was to determine the return of function of the goldfish optic nerve at four time intervals after damage (crush). An increment threshold procedure was used to obtain electroretinogram (ERG) and tectal responses to light onset and termination to various wavelengths of light. Spectral sensitivity functions were derived from both the ON- and OFF-components at both visual levels. By examining the spectral sensitivity functions of these responses, it was possible to determine if there were differences in shape and absolute sensitivity among the ON- and OFF-responses at each wavelength within each level, as well as between the visual levels. The results of this study found that there are differences in the cone contributions between the ON-and OFF-components at both the ERG and tectal level of normal adult goldfish. Tectal responses from optic nerve crush subjects returned at approximately 41- days postcrush (dpc). In conclusion, the results of the present study show that the optic nerve re-establishes functional properties within the tectum after approximately 60-dpc.
Chapter 1
Introduction and Literature Review

Neural regeneration is perhaps one of the most important areas in neuroscience under investigation for both basic science and clinical reasons. Neural regeneration refers to the ability of a neuron to repair damage inflicted upon it. Research on higher vertebrates has emphasized the recovery from damaged neurons following peripheral nervous system injury, while lower vertebrate research has examined recovery from damage of CNS neurons. The reason for this distinction is because neurons in the Central Nervous System (CNS) of higher vertebrates are not able to regenerate leaving permanent and devastating damage to the brain and/or spinal cord. On the other hand, lower vertebrate CNS neurons, including sensory neurons, are able to regenerate. Roger Sperry (1943) showed that the optic nerve of the newt was capable of regeneration after lesioning. He removed the newt’s eye, rotated it 180 degrees, and reinserted it back into the eye socket. The newts demonstrated visual functioning, although somewhat distorted, by shooting their tongues downwards at objects (e.g., flies) located above them. Sperry (1943) concluded that the nerve axons “found” their way back to the original connections in the brain.

Since then, the visual system has been a valuable model for studying neural
regeneration. Most of the work in the area has focused on anatomical recovery and restoration of neural connections. Little research has been done assessing the return of function of these connections. It cannot be assumed that normal functioning has returned just because connections have been re-established without investigating post-damage performance. In addition, because neurons make numerous connections with other cells in many different directions and locations, it is virtually impossible to determine whether the system has completely returned to normal by examining anatomy.

The main purpose of this study was to examine the return of function to the optic nerve of the goldfish after damage. Neural responses were obtained from two levels of visual processing: the retinal level (responses at a location prior to optic nerve damage) and the tectal level (responses at a location after optic nerve damage). The responses of control subjects, sham subjects, and optic nerve crush subjects at various intervals up to 60 days, were recorded and compared.

The following literature review will briefly describe what is currently known about the anatomy and physiology of the retinal and optic tectum areas of the visual system. This review will focus primarily on lower vertebrates including goldfish when possible. Following the review will be a discussion of the usefulness of the spectral sensitivity function in assessing visual processing, including color vision. Assessing visual processing includes determining the number of cone contributions, the type of contributions, as well as the presence of opponent and nonopponent processes. Thirdly, the review will describe the processes of early neural development and the formation and survival of neural synapses. In addition, current literature and knowledge of retinotectal
regeneration will be described. Finally, the purpose of this study and specific objectives will be discussed.

**Anatomy and Physiology of the Retina**

Visual processing begins at the retina. The retina, which is part of the CNS, is the neural layer in the back of the eye. It is here that incoming sensory information from the environment is initially processed, coded, and transmitted to the appropriate areas of the brain. The vertebrate retina contains at least five major classes of neurons; each linked in an intricate design of synaptic connections. These five classes include the photoreceptors, horizontal cells, bipolar cells, amacrine cells, and ganglion cells (Dowling, 1987).

In most vertebrates, there are two types of photoreceptors: rods and cones. Both types have an outer segment that contains photopigments which undergo a chemical transformation following the absorption of light, and initiate the flow of events that changes the membrane potential of the photoreceptor, and thus, the amount of neurotransmitter released to the contacting cells. Rod photoreceptors are extremely sensitive to light and, therefore, typically mediate night vision. Studies conducted on primates indicate that the rods are dispersed throughout the retina although they lie primarily on the peripheral outskirts of the retinal field and are nonexistent in the center (fovea) of the retina. Cone photoreceptors are responsible for daytime and color vision. They have higher visual acuity than rods and better spatial and temporal resolution (Kandel, Schwartz, & Jessel, 1995). Currently, there are at least three known cone types, each with a single photopigment that has different spectra with maximal sensitivity in
the long-wavelengths, referred to as L-cones, middle-wavelengths, referred to as M-cones, and short-wavelengths, referred to as S-cones (Liebman & Entine, 1964). Cones are dispersed throughout the primate retina but are primarily located in the fovea. Goldfish also possess three types of cone photopigments; their maximum sensitivities are 450 nm (S-cones), 533 nm (M-cones), and 625 nm (L-cones; Marks, 1965).

Cones and rods make synaptic connections with the horizontal cells and bipolar cells. Horizontal cells not only supply a supportive layer beneath the photoreceptors but also provide lateral communication across the retina. When light first reaches the photoreceptors, it is absorbed by the photopigments and creates a change in membrane potential sending information to bipolar cells. This membrane potential is a graded potential that produces a change in ionic fluxes across the membrane. A graded potential represents small voltage potential changes on the order of 5-20 mV. As a result of bipolar cell activation, the bipolar layer then sends information to the ganglion cells. There are two types of bipolar cells based on their response to light: ON- and OFF-bipolar cells. ON-bipolar cells are excited, or depolarized, to the onset of light and inhibited, or hyperpolarized, to the offset of light. Respectively, OFF-cells are excited at the termination of light and are inhibited by the onset of light. Bipolar cells also synapse with amacrine cells. The amacrine cells, like horizontal cells, form a lateral supportive layer but are located beneath the bipolar cells. Finally, bipolar cells synapse with either ON- or OFF-ganglion cells. The ON-ganglion cells directly synapse with ON-bipolar cells and are excited by the onset of light; the OFF-ganglion cells directly synapse with
OFF-bipolar cells and are excited by the termination of light (Famiglietti, Kaneko, & Tachibana, 1977). The way in which any retinal neuron responds to visual stimuli is dependent upon the type of connections it receives from other neurons. These connections are referred to as its receptive field. For example, the response properties of a bipolar cell are a function of the number and type of connections it makes with photoreceptors. Consequently, the response properties of a ganglion cell are a function of the number and type of connections it makes with bipolar cells. The receptive field organization of a cell is often used to describe the cell's response properties.

Ganglion cell receptive fields have two significant features. First, the receptive fields of ganglion cells are circular and they are smaller where visual acuity is finest (this feature is a result of a small number of photoreceptors "connected" to this cell). Where acuity is low, the receptive fields are much larger. Second, the receptive field of ganglion cells is separated into two parts: a small center circular zone referred to as the receptive field center and the remaining area known as the surround. The center and surround respond differently to the light stimulus (this response is the result of the type of synaptic connection, i.e., excitatory or inhibitory). For instance, ON-center ganglion cells are excited by light presented to the center field. Light presented to the surround produces an inhibitory response. OFF-center ganglion cells are inhibited by presenting light to the center field, but are excited when light is directed at the surround. As the final neural layer of retinal cells, ganglion cells possess long axons that converge and create the optic nerve, which extends to the visual processing areas of the brain. In order for the electrical signal to travel along the axon, the ganglion cell response is in the form
of action potentials. When an action potential is generated, it is an all-or-none signal that relays neural information to connecting cells. Once an action potential is generated by the ganglion cells, the signal is conducted down the optic nerve in order to communicate with the visual processing areas of the brain.

**Anatomy and Physiology of the Optic Tectum**

The main area of termination of retinal ganglion cells in teleost fish, such as goldfish, is the optic tectum. It is one of the largest structures in the brain, including the cerebellum or telencephalon. The tectum is a bilateral structure that receives information from the optic nerve of the contralateral eye. The two lobes of the tectum are joined at the commisura posterior. With few exceptions, the anatomical structure of the tectum shows little variation between teleost species (Schwassmann & Kruger, 1965).

Studies from a variety of teleost fish suggest that the tectum has five main layers. The innermost layer, the Stratum Griseum Periventriculare (SGP), contains many small nuclei that extend their processes through all layers to form the external membrane. Above this layer lies the Stratum Fibrosum Profundum (SFP) which is a fleshier layer where bundles of fiber tracts penetrate the periventricular gray. The Stratum Griseum Centrale (SGC) is located above the SFP and is the thickest area in the fish tectum. It consists of numerous kinds of neurons and is primarily a synaptic neuropil. A neuropil refers to a dense tangle of axon dendrites, terminals, synapses, and glial cells. The fourth layer, the Stratum Plexiforme et Fibrosum Externum (SPFE), holds optic nerve fibers and probably some of their terminations. Large clusters of unmyelinated axons are interspersed in this layer. Finally, the Stratum Fibrosum Marginale (SFM) is the top layer
and forms the external tectal membrane. It too contains many optic nerve fibers. The two most superficial layers of the tectum possess large numbers of connections and neural activity (Schwassmann & Kruger, 1965).

Physiological studies have revealed that the receptive fields of tectal neurons are elliptical or circular in shape and vary in size (Schellart & Spekreijse, 1976). The shape of tectal receptive fields differs from the circular, concentric receptive fields of retinal ganglion cells. Furthermore, Schellart and Spekreijse revealed that the tectal receptive fields could be divided into four categories: circular, elongated, irregular, and multi-center, all of which differ in diameter. The circular fields, similar to the center/surround organization of the ganglion cells, are often much smaller than the other fields.

The discussion over receptive field shape and size is important to note because it is argued whether these recordings have a true tectal origin (Schellart, Riemslag & Spekreijse, 1978). Since recordings are typically taken from the superficial layers of the tectum, it has been argued that they may be retinal ganglion cell recordings. However, Grafstein (1967) obtained tectal spike responses from deeper layers of the tectum where no optic nerve fibers have been found. She discovered that these responses differed from ganglion cell responses by the latency of the spike elicited; tectal cell responses had a longer latency. In addition, Schellart et al., (1978), showed that tectal cell spike duration (half peak width) is larger than that of the optic nerve. Further electrophysiological studies of tectal visuotopic receptive fields reveal that, beyond the superficial layers, responses of true tectal origin can be obtained (Northmore, 1989a; 1989b).
Neural activity has been recorded in the tectum of goldfish providing researchers with evidence that the tectum plays a role in visual processing. This evidence is provided by evoked (gross) potentials as well as single spike responses (individual action potentials). Riemslag and Schellart (1978) investigated goldfish evoked and spike responses to opposite moving stimuli. They found that the tectal evoked responses contain directionally selective components as well as luminance components, indicating that the tectum is not only activated by light intensity, but also by light movement. Direction selectivity also has been found in goldfish ganglion cells (Bilotta & Abramov, 1989).

Tectal electrophysiological studies also have been done in order to investigate the tectum’s ability to detect color. Goldfish have been used extensively in color vision research, and goldfish tectal evoked responses to different wavelengths of light have been measured in order to assess the tectum’s sensitivity to certain wavelengths of the spectra. Regan, Schellart, Spekreijse and Van Den Berg (1974) conducted a study in which they flickered different wavelengths of light at the goldfish while recording tectal evoked responses. They found that the tectum did respond to various wavelengths along the visual spectrum, although they did not exhibit any color inhibition or opponency because the use of flicker stimuli may have “fused” the ON- and OFF-responses together. Color opponency or inhibition occurs when an excitatory response from one cone input (e.g., L-cones) is suppressed by an inhibitory response from another cone input (e.g., M-cones). Our laboratory has shown that spectral sensitivity of the goldfish tectum based on tectal ON-responses are most sensitive to long- and short- wavelength stimuli, while tectal
OFF-responses are most sensitive to long-wavelength stimuli. Tectal ON-responses appear to receive both L- and S-cone contributions and weak, inhibitory S- and M-cone contributions (Cassidy & Bilotta, 2000). S-cones make excitatory synapse with some neurons and inhibitory synapses with other neurons.

Similar findings have been reported from extracellular, single unit recordings from the rainbow trout (*Oncorhynchus mykiss*) optic tectum (McDonald & Hawshryn, 1999). The rainbow trout exhibited an opposing response pattern for middle to long (M-L) and middle to short (M-S) wavelengths of light.

Thus, there is evidence to suggest that the teleost optic tectum contains both excitatory and inhibitory cone contributions, and that the number and type of cone contributions differ between ON- and OFF-response components.

**Spectral Sensitivity**

Many vertebrate species share the same basic neural processing as well as retinal physiology and anatomy. However, species differ in their responses to various wavelengths of light. Sensitivity to light is a result of several factors. First, visual system sensitivity depends on the number of different cone photoreceptor types in the retina, Zrenner, Abramov, Akita, Cowey, Livingstone, and Valberg’s study (as cited in Hughes, 1996). These cone types determine the spectral range that the visual system can respond. The sensitivity of an individual cone photopigment to light is indicated by its cone absorption spectra. The spectra shows how much light energy is absorbed by the photopigment across a restricted region of the spectrum. The wavelength at which a photopigment absorbs the maximum amount of light is called it’s lambda-max. Relative
to the photopigment's lambda-max, less light energy is absorbed by the photopigment as the wavelength is increased or decreased. If a wavelength does not fall within a particular photopigment spectral range, it is not absorbed, and that cone type is not responsive to that wavelength.

There are two basic requirements that a system demands for color vision. There must be at least two cone types possessing different photopigments (with different lambda-max's), and these cone types' spectral sensitivities must overlap (Cornsweet, 1977). As mentioned above, each cone type contains a photopigment that is most sensitive to light from either the long (L-cones), middle (M-cones), or short (S-cones) wavelength region of the spectrum (Sperling & Harwerth, 1971; Zrenner et al., 1990). Species differ in the number and types of cone photopigments they possess. For instance, the tree shrew (Petry, 1993) possess cones that are sensitive only to long- and middle-wavelengths of light. Furthermore, some vertebrates possess cone types that are sensitive to ultraviolet wavelengths of light in addition to containing cone types that respond to long-, middle- and short-wavelengths (Goldsmith, 1994). Ultraviolet sensitivity has been demonstrated in rodents (Jacobs & Deegan, 1994), a number of birds (Goldsmith, 1994), as well as some fish including rainbow trout (Hawryshyn & Harosi, 1994), carp (Hawryshyn & Harosi, 1991), zebrafish (Hughes, Saszik, Bilotta, DeMarco, & Patterson, 1998; Robinson, Schmitt, Harosi, Reece, & Dowling, 1993), and the goldfish (Hawryshyn & Harosi, 1991).

One way to assess the number, type, and type of contribution (opponent or nonopponent) to the visual response is to examine the spectral sensitivity function. A
common method for deriving spectral sensitivity is the increment threshold method. The increment threshold procedure superimposes monochromatic light onto a white background and varies the monochromatic stimulus intensity until a response criterion is reached. Sensitivity is defined as the reciprocal of the log intensity necessary to reach criterion response at a particular stimulus wavelength. By plotting log sensitivity as a function of stimulus wavelength, a spectral sensitivity function can be created. The increment threshold method has been used to determine spectral sensitivity functions using psychophysical methods in primates (Sperling & Harwerth, 1971), fish (Neumeyer, 1984), tree shrews (Petry, 1993), rodents (Jacobs & Deegan, 1994), cats (Loop, Millican & Thomas, 1987) and humans (King-Smith & Carden, 1976). Furthermore, this increment threshold method has been used to determine spectral sensitivity from electrophysiological responses in a variety of species including primates (Mills & Sperling, 1990) and goldfish (Cassidy & Bilotta, 2000).

The spectral sensitivity function is a useful tool because it provides researchers with a way to ascertain the different cone type contributions to visual processing. To determine the cone contributions from the spectral sensitivity functions, two methods have been commonly used. One method involves comparing the absorption spectra of each individual cone type to the spectral sensitivity. To conduct this procedure, cone absorption spectra templates are superimposed over the spectral sensitivity function (see Mackintosh, Bilotta & Abramov, 1987). Although this method enables one to qualitatively determine the spectral sensitivity cone contributions, it does not enable one to determine the exact contribution of each single cone type. Consequently, to better
ascertain cone contributions from spectral sensitivity, computational modeling has been used (DeMarco & Powers, 1991; Hughes et al., 1998). Computational modeling allows the use of an equation to determine the weight each cone type contributes to the overall spectral sensitivity. As opposed to the template method, this procedure also enables a quantitative analysis of cone contributions as well as whether there are both excitatory and inhibitory contributions.

Overall, the spectral sensitivity function allows one to determine which individual cone types contribute to the visual response. Furthermore, functions across numerous conditions have been used that suggest the presence of separate visual channels. Each channel serving a unique role in overall visual functioning. For example, there is evidence that many species possess both luminance and chromatic channels. A luminance channel typically receives only excitatory contributions from the cone types, whereas a chromatic channel receives both excitatory and inhibitory contributions. Therefore, an analysis of the spectral sensitivity function can be used to satisfy two main purposes: as a way of validating that there are diversified functional channels in the visual system and to decipher what contribution, if any, do the different cone types make to a visual channel.

**Neural Development**

The mature human brain contains approximately 100 billion neurons in addition to a larger number of glial cells. Until recently, it was thought that the entire neural network was completed before birth. Recent research with adult primates (Macaca fascicularis) has demonstrated that new neurons are being born continuously throughout
the lifespan (Gould, Reeves, Graziano, & Gross, 1999). Furthermore, the synaptic connections between neurons continue to be modified throughout adulthood (Kemperman, Kuhn & Gage, 1997).

During early CNS development, a neural tube is formed from a single group of cells. These tubular cells divide and progressively form a layer of cells called the ventricular zone. It is from this zone that cells divide and eventually produce all neurons and glial cells. The creation of nerve cells is referred to as neurogenesis. Following neurogenesis, nerve cells migrate to establish distinct nerve cell populations (e.g., a layer of the cortex), and then continue to differentiate to form different types of neurons. Once these neuron types are formed, it is pertinent that they grow and make the appropriate synaptic connections with other neurons, a process called synaptogenesis (Purves, Augustine, Fitzpatrick, Katz, LaMantia, & McNamara, 1997).

One of the most fascinating traits of the nervous system is the ability of neurons to extend their axons and guide themselves through elaborate cellular territory to find synapses with cells that may be as far as centimeters away (Purves et al., 1997). The beginning of this growth process arises from an area at the tip of the axon called the growth cone. Fine outgrowths, called filopodia, pull the growth cone in a particular direction. As the axon extends through the maze of cells, it is important to note that every step in synaptogenesis appears to be a premeditated choice; it appears that there are no haphazard, random movements. For example studies done by Rakic (1985) show that neurons are guided with the help of radial glial cells, which act as "spokes" for cells to glide along.
Along with the guidance of radial glial cells, additional guidance comes from the target cells that each neuron is seeking. Target cells secrete specific molecules and chemicals that provide directional guidance to the extending axon, or, in simpler terms, they "attract" the axon. Such agents are referred to as chemotropic molecules. These chemotropic molecules are extremely selective and attract only the appropriate type of neuron in order to increase the chances of the correct synaptic connection. The first molecular family to meet the criteria for chemotropic attractants was the netrins (Purves et al., 1997). These proteins produce attractive behaviors, and injecting netrins into non-neurons results in the secretion of additional netrins from those cells. Following the discovery of the netrin family was the semaphorin family (Marx, 1995). Two members, semaphorin I and semaphorin II, are secreted from target cells to lure axons, as well as regulate axon steering and branching. Finally, target cell receptors form a family that enhances attraction of growing axons called receptor protein tyrosine kinases (Barinaga, 1995). These are surface molecules that appear to play a role in chemoattraction, although little more is known about their specific purpose.

In addition to axon guidance, the support, growth, survival of neurons and their processes are maintained by factors called trophic factors. These trophic factors, such as nerve growth factor (NGF) and brain derived neurotrophic factor (BNDF), also are secreted by target cells and provide nourishment to the neurons (Purves et al., 1997). This nourishment is important because in order to continue growing and establish healthy, long-lasting connections, the neuron must not only grow and be guided but must also survive.
Over 30 years ago, a substance was found that affected the growth and survival of neurons in the sympathetic nervous system called nerve growth factor (NGF) (Levi-Montalcini & Cohen, 1956). It appears that the amount of NGF that is produced from a target cell is correlated with the amount of innervation the target cell receives. Support for the role of NGF in neural survival emerged from various demonstrations using mice, newborn rodents and birds (Purves et al., 1997). Such support includes the observation that the absence of NGF increases the rate of cell death, the survival of an increased amount of neurons in the presence of higher levels of NGF, the production and occupancy of NGF in target cells, and the existence of NGF receptors in innervated target cells (Purves et al., 1997).

In addition to NGF, there are currently three other members of the neurotrophin family: brain-derived neurotrophic factor, neurotrophic-3, and NT-4/5 (Purves et al., 1997). The precise purpose of these factors is under investigation. Furthermore, the activity of neurotrophic factors arises from receptor proteins such as Trk receptors. These receptors enable the particular connecting neuron to respond to the corresponding neurotrophin (Purves et al., 1997).

While target cells are secreting molecules to promote guidance and growth of axons, they must also tell axons where not to grow. In fact, cells do this simultaneously as they attract the appropriate axons towards them and repel inappropriate axons away from them. Such inhibitors are just as important for networking as growth-promoting factors (Purves et al., 1997).
There are currently two main classes of chemorepellents. One class binds to cell surfaces and prevents the outgrowth of surrounding axons. An example is protein IN-1 (inhibitor-1) which is embodied into myelin sheaths encircling axons. A second class of diffusible molecules comes from the semorphin family, more specifically, collapsin. During an experiment by Raper (as cited in Messersmith, Leonardo, Shatz, Tessier-Lavigne, Goodman, & Kologkin, 1995), collapsin was found in chick brain tissue and appeared to discourage axonal extension in vitro. Further studies have shown that the secretion of some semorphins repel particular groups of axons and not others. In addition to the chick research, a study by Bonhoeffer’s group found a repelling substance named repulsive axon guidance signal in the developing chick optic tectum (Walter, Henke-Fahle, & Bonhoeffer, 1987). This signal was distributed across the optic tectum and prevented nasal and temporal ganglion cell axons from forming synapses with tectal neurons.

After neurons finally reach their target region, they must make an important “decision” to finalize synaptogenesis: that is, which specific target to innervate and form a synapse with. Neurons will not innervate near glial or connective tissue cells; however, when synapses are created, they appear to be specific, absolute, and secure. This means that once synapses are established, there becomes a strong bond between neurons, and synaptic modification is only likely when external factors demand it (Purves et al., 1997). There are four types of synapses that neurons can form: axosomatic (axon-cell soma), axodendritic (axon-dendrite), axoaxonal (axon-axon), and dendrodendritic (dendrite-dendrite). Axodendritic are the most abundant in the CNS. Once a synaptic connection
is established, neurons become dependent on their targets for constant nourishment and survival. The long-term dependency between neurons is called trophic interaction, which is based on the constant signaling of neurotrophic factors into the synapse.

If a neuron is deprived of neurotrophic factors, it is unable to survive and will die through a process called apoptosis (Oppenheim, 1991). The proportion of cells that decease varies according to region, and ranges from 20-80% (Rosenzweig, Leiman & Breedlove, 1999). Neuronal cell death is an important part of neural development because, during the embryonic stages, surplus numbers of neurons are produced. Therefore, neurons must compete with one another in order to establish strong trophic interactions with appropriate synapses. In competition, the strongest and healthiest neurons will innervate and sustain the connection demonstrating a concept termed "Neural Darwinism" - survival of the fittest neuron (Purves, 1994).

These processes of neural development can be generalized to visual development in the CNS. Neural processing in the form of electrical signals starts at the retina and proceeds to visual processing areas of the brain. The axons of the ganglion cells form the optic nerve and extend to seek connections on the lateral geniculate nucleus, suprachiasmatic nucleus, assorted pretectal nuclei, and optic tectum (Finlay & Sengelaub, 1989). The optic nerve’s journey is unidirectional and travels a long distance to reach the visual processing centers of the brain. Since there are not webs of connections to cipher through and plenty of space (and distance) between the back of the eye and the tectum, it is easier to examine, record and manipulate than neurons located in
the brain. It is these axons that will terminate and form synapses where trophic interactions are maintained and visual processing in the brain can be achieved. In goldfish, retinal axons synapse with a high degree of specificity in the tectum; that is, there is a one-to-one correspondence of a retinal area to the tectal area it connects with. For example, temporal ganglion cell axons synapse with posterior tectal neurons and nasal projections synapse with anterior tectal neurons (Swassmann & Kroger, 1965). This highly specific retinotectal organization makes the retinotectal pathway an excellent model to study neural development.

**Neural Regeneration**

CNS regeneration has been studied extensively in lower vertebrate species primarily because they possess the ability to regenerate CNS neurons while higher vertebrates cannot. The retina, although structurally separate from the brain, is part of the CNS. Therefore, retinal neurons possess identical properties as any other CNS neuron. For the same reasons investigators use the optic nerve to study neural development, many investigators have used the optic nerve to study neural regeneration. The advantage of studying retinal neurons is that their primary connection to the brain consists of a long cable - the optic nerve (which is comprised of ganglion cell axons). For the most part, input about the visual world travels from the retina to the visual processing areas of the brain. However, researchers have found evidence for the existence of efferent myelinated fibers running parallel to the optic nerve and terminating in the goldfish retina (Witkovsky, 1971). Thus, the retino-tectal connection is capable of supplying scientists with a sufficient amount of visual information. Also, since the optic
nerve is a cable-like extension and is one of the largest nerves in the visual system, it is easy for researchers to access and damage without damaging other neural areas.

One of the first experiments with neural regeneration was done by Sperry (1943). He removed the eye of the newt, severed the optic nerve, and reininserted the eye, literally turning their world upside down by rotating the eye 180 degrees. He tested them behaviorally by presenting an object above them (e.g., a fly). The newts responded by shooting their tongues towards the ground as if their visual field were upside down. Through this experiment, Sperry demonstrated that the ganglion cell axons reconnected themselves to the brain and found their way back to their original connections. In addition, Sperry suggested that tectal cells possess a unique “tag” and that the growing terminal of ganglion cells possess complementary “tags” such that they seek out certain tectal locations, articulating what is referred to as the chemoaffinity hypothesis (as cited in Purves et al., 1997).

More recent studies on neural regeneration were conducted by Freidrich Bonhoeffer's group (Walter et al., 1987) using the chick. Under normal conditions, axons from the temporal region of the chick retina synapse with the anterior pole of the optic tectum. When the retinal axons were lesioned and presented with a choice of destination, they grew towards the anterior pole and avoided growing to the “wrong” areas of the tectum. These studies, and others, clearly demonstrate that the optic nerve is capable of regenerating and appears to be able to “find” its way back to the appropriate tectal area.
Unfortunately, most research has concentrated on the anatomical reconnections of axons after lesioning. Little has been done assessing the return of function after damaged axons have re-established connections. Several studies examining the recovery of visual functioning have been done using a variety of fish species. For example, behavioral and electrophysiological assessment was done in the bluegill sunfish (*Lepomis macrochirus*) (Northmore & Masino, 1984) and the goldfish (*Carassius auratus*) (Northmore, 1989a,b; Northmore & Celenza, 1992) after lesioning the optic nerve. Northmore & Masino (1984) were attempting to ascertain if tectal functioning returned all at once following optic nerve crush. Brief flashes of red light-emitting diodes (LED) were presented to the fish. They found that primitive behavioral responses, such as overall sensitivity to dimming stimuli, first appeared at 20 days postcrush (dpc). Orientation accuracy and high spatial frequency detection (a more sophisticated visual task) were obtained by 55-dpc. In another study by Northmore (1987), electrophysiological tectal evoked responses were obtained between 32- and 56-dpc. In addition, the retinotectal projection was mapped in order to characterize the size of multiunit receptive fields (MURFs). MURFs can be described as the massed electrical potential of ganglion cell arbors distributed upon the superficial surface of the tectum (Schmidt & Edwards, 1983).

Northmore (1989a,b) found two distinct phases in the restoration of neural responses in the tectum during regeneration after neural injury: the first phase being the recovery of only OFF-responses, the second phase being the return of both ON- and OFF-responses. An evoked OFF-response was recorded at approximately 20-dpc, and appeared to be initially weak, but progressively strengthened as the receptive field size
shrunk (Northmore & Masino, 1984). Reasoning seems to indicate that when these “new” axons first arrive at the tectum, they find tectal cells that are already occupied with synapses from the previous generation of axons and a competitive battle brews in which the “newer,” recovered axons displace the older ones and establish connections (Marcus, Delaney, & Easter, 1999). At around 40-dpc, ON-responses appear in the tectum. Furthermore, mapping indicated that the ON- and OFF-responses of MURFs re-establish normal sensitivity, dimensions and shape by approximately 40-dpc (Northmore & Celenza, 1992). Studies conducted by Schmidt and Edwards (1983) utilized intraocular injections of tetrodotoxin (TTX) in order to determine whether electrical activity plays a role in regenerating neurons. TTX blocks sodium channels along axon membranes and, therefore, disables the production of action potentials. Regenerated fibers following TTX injections usually reach the tectum around 15-dpc, and electrophysiological recordings are attainable by approximately 35-dpc. However, although TTX injected fish re-established the same organizational map as those of optic nerve crush fish who did not receive TTX, their MURF size was reduced, suggesting that the TTX treatment decreased the size of receptive field centers, as well as increased the amount of time it took for synaptic activity to recover. These results suggest that TTX slowed down the regeneration process and that the growth and regenerative abilities of a neuron are dependent upon their firing activity (Schmidt & Edwards, 1983).

In summary, the discovery that neuronal functioning after regeneration does not recover all at once has been a valuable one for understanding neural regenerative properties. Moreover, it has led researchers to postulate on the order and timing of the
return of various visual functions. However, this research still lies towards the introductory boundary of visual regeneration research. Other areas of visual functioning (e.g., color vision) remain open to investigation.

Summary and Purpose of Study

CNS regeneration studies have relied primarily on lower vertebrate species such as frog (newt) and goldfish. Researchers have focused most of their attention on the anatomical recovery of regenerated neurons. However, functional recovery experimentation is increasing. Physiological studies investigating the return of function from regenerated neurons have utilized the retinotectal projection, or the optic nerve, as a representative of overall CNS functioning. Such studies have investigated whether the return of functioning arises all at once, as well as the timing it takes for lesioned (crushed) optic nerves to recover. However, no research has been done assessing the return of the properties of color vision.

The main purpose of this study was to examine the return of function to the optic nerve of the goldfish after damage. Neural responses were obtained from two levels of visual processing: the retinal level (responses at a location prior to the optic nerve) and tectal level (responses at a location after optic nerve damage). The responses of 3 groups—control subjects, sham subjects, and optic nerve crush subjects at various time intervals up to 60 days—were recorded and compared. To that end, there are two main objectives to this study. The first is to investigate the spectral sensitivity functions of the ON- and OFF- components of both ERG and tectal responses under normal conditions. This objective was investigated by obtaining spectral sensitivity functions at both visual
processing levels. Comparisons between the two levels, as well as comparisons between
the ON- and OFF-responses, were made. This information by itself provides new and
important information of the differences between ON- and OFF-responses at two
different levels of the visual system. In addition, these data were used to compare visual
processing between normal subjects and those who received optic nerve damage.

The second main objective was to investigate the order in which neural
functioning returned following optic nerve damage. It was investigated by recording
tectal responses at various intervals postcrush until 60 days (see Methods). Spectral
sensitivity functions were obtained from both ON- and OFF-responses in order to
determine the sequence and timing of the recovery of tectal responses. A quantitative
analysis of the spectral sensitivity function determined the return of the cone
contributions to tectal response functioning.
Chapter 2

Method

Participants

Adult goldfish (*Carassius auratus*) measuring 3 to 6 cm in length were used. They were obtained from local pet stores. Fish were kept on a 14-hr on/10-hr off cycle under normal illumination levels and housed for two weeks before use. Fish were fed daily with Tetramin basic flakes. Standard procedures for animal care were used. All procedures were approved by the Institutional Animal Care and Use Committee of Western Kentucky University on January 3rd, 2000.

Apparatus

The optical system used to display the visual stimuli was the two-channel system currently in the laboratory (see Hughes et al., 1998). The light source for one channel was a 250-W tungsten-halogen bulb (Oriel, Stratford, CT, Model 6334) and was used to present a broadband background. The other channel presented monochromatic light to the subject. The light source for this channel was a 150-W xenon arc lamp (Spectral Energy, Westwood, NJ, Model LH 150). Interference (Oriel, Stratford, CT, Model 54161) and neutral density filters (Reynard, Calle Sombra, CA, Model 390) controlled stimulus wavelength and irradiance, respectively. The light from both channels was focused via a lens in front of a 5 mm diameter liquid light guide (Oriel, Strafford, CT,
Model 22556); the light completely filled the guide. Surgical procedures were done using Vannas dissecting scissors (WPI, Sarasota, FL, Model 14003), a Tyrell hook (WPI, Sarasota, FL, Model 14136), and Dumont #5 tweezers with a 0.05 mm tip (WPI, Sarasota, FL, Model 14400). ERG recordings were obtained using a 36-gauge chlorided silver electrode. Tectal recordings were obtained using a 36-gauge chlorided silver wire inserted into a saline filled glass pipette with a tip diameter of approximately 15 microns. Also, a 36-gauge chlorided silver wire was used as a reference electrode.

**Optic Nerve Crush Procedures**

Fish were anesthetized by being placed in a 0.04% tricaine methanesulfonate (MS-222) solution until respiration ceased and then placed under an illuminated stereomicroscope. The Tyrell hook was used to cut around the right eye muscles in order to pull the eye away from the eye socket. Once the eye was pulled away and the optic nerve in view, the nerve was compressed for 10-15 seconds with the surgical tweezers. This 10-15 second compression ensured the tearing of the axons, but kept the main artery, blood vessels, and ganglion cell sheath intact enabling the axons to regenerate. Animals then were placed back into their home tank and antibiotic medicine (Maracyn, Glendale Heights, IL, 500 mg per 10 gallons) was administered to the water daily for 5 days in order to prevent infection.

"Sham" subjects also were anesthetized and placed under the illuminated stereoscope. The muscles of the right eye were cut using the Tyrell hook and the eye pulled away from the socket. When the optic nerve was in view, the tweezers were placed around the nerve, but no compression of the nerve was done. The eye was then
positioned back into the socket, and the fish were placed into their homes and treated with antibiotics.

**Testing Procedures**

Optic nerve crush fish were divided into 6 groups and tested at various time intervals. The first group underwent ERG and tectal recording between 3 to 7-dpc; since ERG responses appeared normal at this stage following the optic nerve crush, no ERG responses were obtained at later stages. The second group was tested for tectal responses at about 14-dpc. The third group was tested for tectal responses at about 21-dpc. The fourth group was tested for tectal responses between 32 to 35-dpc. The fifth group was tested between 40 to 45-dpc, and the sixth group was tested between 55 to 60-dpc.

“Sham” subject testing was conducted between 3 to 7 days post-surgery. Fish were anesthetized by being placed in a 0.04% MS-222 solution. Animals then were paralyzed with an intramuscular injection of 20 μg gallamine triethiodide and placed under illumination for surgery. The surgery was conducted using the dissecting scissors by making two major cuts from the left eye towards the back of the skull. Another cut was done from the left eye to the right eye to ease skull removal. The skull then was lifted with the tweezers and extracted with the scissors. The fish was positioned in a holding chamber which stabilized the animal during recordings. This chamber consisted of a plastic tube holder placed in a cut portion of a small sponge. The sponge was placed in a rectangular plexiglass chamber inside the light-tight Faraday cage. Fish were artificially respirated and anesthetized by passing a 0.01% MS-222 aerated solution through the gills throughout the testing session. The glass pipette electrode was placed in the left
superficial tectum and the reference electrode was placed in the nostril. Tectal evoked potentials were differentially amplified with a band-pass of 0.1 to 300 Hz (Grass, West Warwick, RI, P55). Signals then were sent to an oscilloscope (Tektronix, Beaverton, OR, Model TDS340) and simultaneously recorded by a computer at a 1ms data acquisition rate. The liquid light guide from the optical system was placed in front of the right eye. A broadband background of 5µW/cm² was then turned on, and the fish adapted to this illumination for at least 5 minutes.

Following adaptation, the test session began. Sessions consisted of presenting monochromatic light at numerous irradiances. Wavelength presentation was from 320 nm to 700 nm and staggered in 40 nm steps. This procedure avoided adaptation to any one cone type as well as ensured that each fish had a sufficient number of wavelength presentations across the spectra. There were five stimulus presentations, each 500 ms in duration. The computer then averaged these waveforms to obtain one waveform for analysis. The beginning wavelength was chosen from either the long or short spectral end and starting order was varied across test sessions. At the completion of the series, wavelengths were presented 20 nm apart. Data were collected for 50 ms prior, 500 ms during, and 500 ms after the stimulus presentation. When the full series of wavelength presentations was finished, the fish was removed from the holding chamber and disposed of using approved methods.
Chapter 3

Results

Overview of the Analyses

Spectral sensitivity functions. Spectral sensitivity functions were obtained from the tectal and ERG waveforms. Log stimulus irradiance-response functions were produced by plotting the log stimulus irradiance (quanta/s/cm²) against the peak amplitude (μV) of the response component. Sensitivity at each wavelength was determined by taking the reciprocal of the log stimulus irradiance that produced a response criterion of +50 μV (Hughes et al., 1998). This process was done separately for the ON- and OFF-components of the ERG and tectal waveforms. Analyses comprised of comparing the spectral sensitivity functions obtained from the tectum and ERG responses from normal and optic nerve crush subjects at various intervals for both the ON- and OFF-components. As a control, tectal responses from shams and ERG responses between 3 to 7-dpc were examined. Spectral sensitivity differences were examined by performing within subjects factorial analyses of variance (ANOVAs). Missing values for individual subjects were controlled by substituting the mean log sensitivity for each wavelength. Missing values were those in which unreliable responses were obtained due to procedural errors, i.e., electrical malfunctions, equipment malfunctions or poor health of the subject.
Error bars in all figures represent +/- 1 standard error of the mean (SEM).

**Cone contributions.** It was predicted that the spectral sensitivity functions of the different visual levels would be composed of different combinations of cone types. It also was anticipated that the spectral sensitivity functions at the various time intervals following optic nerve damage would re-establish cone contributions that were similar to those of normal subjects. To determine the cone contributions to the spectral sensitivity function, a multiple mechanism model was applied to the spectral sensitivity data (Hughes et al., 1998). This multiple mechanism model takes the form

\[ S_\lambda = k_1(A_{\lambda_{\text{max}}(1)}) + k_2(A_{\lambda_{\text{max}}(2)}), \]

where \( S_\lambda \) depicts the sensitivity at each wavelength, \( A_{\lambda_{\text{max}}(x)} \) depicts the sensitivity of a cone type with \( \lambda_{\text{max}} \) of \( x \), and \( k_1 \) and \( k_2 \) are the weights of the cone inputs. Cone weights can be negative or positive indicating inhibitory or excitatory contributions to the response. The model is designed so that any given mechanism describes only a portion of the spectrum. For example, the M-cones may provide excitatory contributions to one mechanism (e.g., M - L) and inhibitory contributions to another mechanism (e.g., L - M). To determine the cone contributions, data values were converted to proportions and normalized with reference to their maximum value. A nonlinear regression algorithm was utilized to determine the cone weights that minimized the least-squares fit to the data.

Previous work supports the use of this multiple mechanism model, as opposed to the linear-additive model (Hughes et al., 1998), as the best-fit model for determining cone contributions to the spectral sensitivity function. Sperling and Harwerth (1971) utilized a
similar multiple mechanism model while describing behavioral increment-threshold data in primates. In addition, Hughes et al. (1998) used this model in order to explain the differences between the cone spectra and the spectral sensitivity data while determining cone contributions to the spectral sensitivity of zebrafish (Danio rerio). The success of this model across different species enables it to be utilized with the goldfish data in the present study.

Normal Subjects

Figure 1 shows sample ERG and tectal responses. The stimulus wavelength for the ERG response was 540 nm and the stimulus irradiance was 14.2 log quanta/s/cm²; the stimulus wavelength for the tectal response was 660 nm and the stimulus irradiance was 14.1 log quanta/s/cm². The horizontal line depicts stimulus onset and termination. Stimulus presentation was for 500 ms. Note that both the ERG and tectum produce responses to both light onset (ON-response) and termination (OFF-response).

Comparisons of the spectral sensitivity functions from the tectal and ERG responses of normal goldfish were made. In addition, comparisons of the ON- and OFF-components of both tectal and ERG responses were made. Differences between the spectral sensitivity functions were examined by conducting a 2 (response type: ON- and OFF-components) x 2 (visual level: ERG and tectum) x 20 (wavelength) ANOVA. Response type and wavelength were within subjects measures and visual level was a between subjects measure. The ANOVA found a significant three-way interaction among response type x visual level x wavelength, F(19, 285) = 4.93, p < .001. The three-way interaction was examined first by comparing the response type (ON- and OFF-
components) at each visual level (ERG and tectum). This step was followed by comparing the visual level (ERG and tectum) at each response type (ON- and OFF-components). The Bonferroni correction method was used to establish alpha levels for these comparisons. With the use of this correction, one can establish a “protection level” against the chance of an inflated Type I error due to making several comparisons (Hayes, 1994). The possibility of making one or more Type I errors in a set of tests is termed familywise error rate and can be calculated as

$$\alpha_{FW} = 1 - (1 - \alpha_{PC})^k$$

where \(\alpha_{FW}\) is the familywise error rate, \(\alpha_{PC}\) is the error rate per comparison, and \(k\) is the number of comparisons. An alpha level of .0025 was established by plugging the twenty-eight comparisons, \((k)\), in the above equation to get the probability of error at \(\alpha_{PC}\), which was \(p < .05\) before Bonferroni’s. This procedure was done to minimize the chance of Type I errors. In addition, there was a significant interaction between response type \(\times\) wavelength, \(F (19, 285) = 7.08, p < .001\) and visual level \(\times\) wavelength, \(F (19, 285) = 2.99, p < .001\). There was a nonsignificant interaction between visual level \(\times\) response type, \(F (19, 285) = .054, p < .819\).

Comparison of the ON- and OFF-components. Figure 2 shows the absolute spectral sensitivity functions from eight normal subjects obtained from the ERG ON-response (b-wave, open circles) and OFF-response (d-wave, filled circles). Paired sample t-tests were conducted to test the significance of the differences between the two ERG response types at each wavelength in order to assess which individual wavelengths elicited responses that were significantly different. Significant differences (all tested at p
<.0025, Bonferroni’s correction) were not found for any wavelengths. Thus, the b-wave and d-wave components are not significantly different from one another.

Figure 3 shows the absolute spectral sensitivity functions from nine normal subjects obtained from the tectal ON-response (open squares) and OFF-response (filled squares). Paired sample t-tests were conducted to test the significance of the differences between the tectal response types at each wavelength in order to assess which individual wavelengths elicited responses that were significantly different. Significant differences (all tested at p < .0025, Bonferroni’s correction) were found at 560 nm. The tectal ON-response is significantly less sensitive at the middle wavelength of 560 compared to the tectal OFF-response.

Comparison of the ERG and tectal responses. A comparison of the absolute spectral sensitivity of the ERG b-wave and the tectal ON-response illustrates the difference in sensitivity between the two visual system levels. Figure 4 compares the absolute spectral sensitivity of the ERG b-wave response (open circles) and the tectal ON-response (open squares). Independent sample t-tests were conducted to test for significance between the two visual levels at each wavelength in order to assess which individual wavelengths elicited responses that were significantly different. Significant differences (all tested at p < .0025, Bonferroni’s correction) were found for the following wavelengths: 420, 500, 660, and 700 nm.

The results from the cone model analysis showed that the ERG b-wave response appears to receive only L-cone contributions. However, the tectal ON-response appears to receive contribution from the S-, M- and L-cone types, including an L-cone inhibited
by M-cone (L - M). The letters in Figure 4 indicate the type of cone contribution to the response.

Figure 5 shows the absolute spectral sensitivity of the ERG d-wave response (filled circles) and the tectal OFF-response (filled squares). Independent sample t-tests were conducted to test for significance between the response type at each wavelength. Significant differences (all tested at $p < .0025$, Bonferroni’s correction) were found for the following wavelengths: 460, 480, 500, 520, 540, 560, and 580 nm. The ERG d-wave was significantly less sensitive at the middle wavelengths compared to the tectal OFF-response.

The results from the cone model analysis showed that both the ERG d-wave response and the tectal OFF-response appear to receive M- and L-cone contributions; however, the tectal OFF-response can be best-fit with a model that ‘sums’ the contributions of the M- and L-cones. This additive contribution produces higher sensitivity to wavelengths of 580 – 620 nm than would be predicted by the M- and L-cones separately. The letters in Figure 5 indicate the type of cone contribution to the response. Table 1 reveals the cone model weights for S-, M-, and L-cone types at each visual level for both the ON- and OFF response types. The positive values represent excitatory contributions and the negative value represents inhibitory contributions.

**Optic Nerve Crush Subjects**

Optic nerve damage was assessed in several ways. First, the tectum was divided into 5 sections in order to identify the tectal regions where recordings were obtained. The 5 sections consisted of 4 outer quadrants and a center area. For each optic nerve crush
subject that was tested, at least 3 of the 5 sections were examined for responses to 3 stimulus wavelengths (400, 500, and 600 nm) at the maximum stimulus irradiance. If there were no substantial responses in a given tectal area then a 'no response' decision was made and another section was examined. This procedure was repeated in at least three subjects per postcrush group. If there were any substantial responses at any wavelengths, then spectral sensitivity functions were derived.

3 to 7-dpc. Between 3 to 7-dpc, the retina produced a response but the tectum did not. Figure 6 shows a sample ERG (Fig. 6a) and tectal response (Fig. 6b) at 6 days after optic nerve damage. The stimulus wavelength for the ERG was 640 nm and the stimulus irradiance was 13.7 log quanta/s/cm$^2$; the stimulus wavelength for the tectal recording was 600 nm and the stimulus irradiance was 15.1 log quanta/s/cm$^2$. The horizontal line depicts stimulus onset and termination. Stimulus presentation was for 500 ms. Note that there is a response to both light onset and termination at the ERG level, but there is no response at the tectal level. Since ERG responses appeared normal at this stage following the optic nerve crush, no ERG responses were obtained at later stages.

14-dpc. Tectal recordings were taken from three 14-dpc fish. Figure 7 shows a representative tectal response at 14-dpc. The stimulus wavelength for the tectal recording was 600 nm, and the stimulus irradiance was 15.1 log quanta/s/cm$^2$. The horizontal line depicts stimulus onset and termination. Stimulus presentation was for 500 ms. Note that there is no response at the tectal level.

21-dpc. Tectal recordings from three 21-dpc fish were obtained. Figure 8 shows a sample tectal response at 21-dpc. The stimulus wavelength was 600 nm, and the
stimulus irradiance was 15.1 log quanta/s/cm². The horizontal line depicts stimulus onset
and termination. Stimulus presentation was for 500 ms. Each of the three fish elicited a
slight response to light onset and termination at a wavelength presentation of 600 nm. At
400 and 500 nm wavelength presentations, responses were inconsistent. An extremely
slight response to light onset was obtained from one fish at 500 nm. A slight OFF-
response was obtained from one fish at 700 nm.

40 to 45-dpc. Responses to both light onset and termination at the tectal level
were obtained from three fish between 40 to 45-dpc; however, the responses at each
wavelength were abnormal. Figure 9 shows a sample tectal response at 41-dpc. The
stimulus wavelength was 580 nm, and the stimulus irradiance was 14.3 log quanta/s/cm².
The horizontal line depicts stimulus onset and termination. Stimulus presentation was for
500 ms. Note that there is a very slight response to light onset and a stronger OFF-
response.

55 to 60-dpc. Tectal responses were obtained from five fish between 55 to 60-
dpc. Figure 10 shows a sample tectal response at 60-dpc. The stimulus wavelength was
660 nm, and the stimulus irradiance was 14.1 log quanta/s/cm². The horizontal line
depicts stimulus onset and termination. Stimulus presentation was for 500 ms. Note that
there is both a substantial tectal ON- and OFF-response.

Comparisons of the spectral sensitivity functions obtained from the tectal
responses from normal and 60-dpc subjects were made. Differences between the spectral
sensitivity functions were examined by conducting a 2 (response type: ON- and OFF-
components) x 2 (treatment: normal and 60-dpc) x 20 (wavelength) ANOVA. Response
type and wavelength were within subjects measures, and treatment was a between subjects measure.

**Comparisons of the normal and 60-dpc responses.** A comparison of the absolute spectral sensitivity of the normal tectal response and the 60-dpc tectal response illustrates the similarities in sensitivity between these groups. Figure 11 compares the absolute spectral sensitivity of the normal ON-response (open squares) and the 60-dpc ON-response (open triangles). Figure 12 compares the absolute spectral sensitivity of the normal OFF-response (filled squares) and the 60-dpc OFF-response (filled triangles). The ANOVA found a nonsignificant interaction among response type x treatment x wavelength, $F(19, 247) = 1.61, p > .05$. There also was a nonsignificant interaction between response type x treatment, $F(19, 247) = .987, p < .339$ and treatment x wavelength, $F(19, 247) = 1.21, p < .257$, while there was a significant interaction between response type x wavelength, $F(19, 247) = 4.80, p < .001$. However, a main effect of treatment indicated that the 60-dpc ON- and OFF-components were significantly less sensitive than the normal ON- and OFF-components, $F(1,13) = 18.40, p < .001$. Therefore, there is a significant difference among the absolute sensitivities of the two groups; normal subjects were significantly more sensitive overall than those subjects tested at 60-dpc.

**Sham Subjects**

Comparisons of the absolute spectral sensitivity functions from tectal ON- and OFF-responses of normal and 3 sham subjects were made. Differences between the spectral sensitivity functions were examined by conducting a 2 (response type: ON- and
OFF-components) x 2 (treatment: normals and shams) x 20 (wavelength) ANOVA. Response type and wavelength were within subjects measures, and treatment was a between subjects measure.

Figure 13 shows the absolute spectral sensitivity functions obtained from the normal tectal ON-response (open squares) and the sham ON-response (open diamonds). The ANOVA found a significant interaction among response type x treatment x wavelength, $F(19, 190) = 1.717, p < .05$. There also was a significant interaction between response type x wavelength, $F(19, 190) = 6.94, p < .001$ and treatment x wavelength, $F(19, 190) = 1.93, p < .014$. There was a nonsignificant interaction between response type x treatment, $F(19, 190) = .537, p < .48$. Independent sample t-tests were conducted to test for significance between the ON-responses of the two treatment levels at each wavelength. Significant differences (all tested at $p < .0025$, Bonferroni’s correction) were not found between any wavelengths; the sham ON-response was not significantly different at these two wavelengths compared to the normal ON-response. The overall shape of the spectral sensitivity function is similar to that of the normal ON-response.

Figure 14 shows the absolute spectral sensitivity functions obtained from the normal tectal OFF-response (filled squares) and the sham OFF-response (filled diamonds). Independent sample t-tests were conducted to test for significance between the OFF-responses of the two treatment levels at each wavelength. Significant differences (all tested at $p < .0025$, Bonferroni’s correction) were not found between any
wavelengths; the sham OFF-response was not significantly different compared to the normal OFF-response.
Chapter 4

Discussion

The first objective of this project was to compare the neural responses of normal goldfish at two different levels of the visual system. To that end, comparisons were made between the ON- and OFF-responses at both the ERG and tectal levels. The ERG response is primarily the responses of the retinal neurons with the exception of the ganglion cells. Thus, the ERG is representative of neural processing before the ganglion cells, while the tectal responses reflect the responses of ganglion cell axon terminals on the tectum (Northmore, 1989a). Thus, comparing ERG and tectal responses provides information about the different levels of the visual system and how these levels process information. It was expected that there would be differences between the ON- and OFF-components, as well as between the two visual levels.

The second objective was to assess the return of function at the tectal level at various time intervals following damage to the optic nerve. It was anticipated that, over time, the spectral sensitivity functions obtained from the nerve crush subjects would be similar to the functions obtained from the control group. However, several possible outcomes leading to the above finding were anticipated. It was possible that functional recovery would be in the form of an overall increase in sensitivity; the shape of the spectral
sensitivity function of recovering subjects would be similar to that of normal subjects but less sensitive overall. It also was possible that specific processes or cone contributions would recover first. As a result, the shape of the spectral sensitivity function of the regenerating nerve would differ from the function obtained from normals. This difference would suggest that certain mechanisms regenerate before others, thereby providing valuable information about the role that cone contributions play in the return of visual function.

**Normal Subjects**

This study found that the spectral sensitivity functions of the normal goldfish ERG ON-component (b-wave) and OFF-component (d-wave) were not significantly different. The b-wave appeared to be relatively more sensitive at the long wavelengths and less sensitive at the short wavelengths compared to the d-wave, however no significant differences were found. Therefore, the retinal bipolar cells that produce a response to either light onset (ON-cells) or light termination (OFF-cells) do not significantly differ in the specific cone contributions that they receive. However, there appears to be some difference in the type of cone contributions the ERG b- and d-wave receives based on the results of the cone modeling analysis. The ERG b-wave appears to receive only L-cone contributions (which supports the results of Regan et al., 1974) while the ERG d-wave appears to receive L + M cone contributions. The cone modeling analysis also found that the spectral sensitivity function of the normal goldfish tectum showed a slight difference between the ON- and OFF-components. The tectal ON-response appeared to receive S-, M-, and L – M cone contributions while the tectal OFF-
response appeared to receive L- and M-cone contributions. Thus, the tectal cells that are responsible for responses to light onset or termination possess different synaptic connections with the retinal ganglion cells axons (optic nerve). Also, since it is unknown whether tectal responses are purely tectal in origin (meaning that responses obtained from the tectum may possess retinal ganglion cell activity), these differences in spectral sensitivity confirm the work of DeMarco and Powers (1991) who found that there were spectral sensitivity differences between the ON- and OFF-components of goldfish optic nerve response. They found that the ON-response received strong input from the S-cones and antagonism from the M- and L-cones. The OFF-response appeared to receive input from S- M-, and L-cones with a dominant input from the L-cones (DeMarco & Powers, 1991).

Furthermore, this study conducted a comparison of the absolute spectral sensitivity functions of the ERG b-wave and the tectal ON-response and, based on the cone modeling analysis, found differences between the two visual system levels. The results of the present study contradict the results from Regan et al. (1974) which found that both the ERG and tectal responses to the flickering stimuli appeared to be dominated by L-cone contributions. In the present study, the cone model analysis showed differences among cone contribution between the two levels. The ERG b-wave appeared to receive only L-cone contributions, similar to the Regan et al. (1974) findings, while the tectal ON-response appeared to receive contributions from the S-, M-, and L- cone types, including an L – M cone contribution.
A comparison of the absolute spectral sensitivity functions of the ERG d-wave and the tectal OFF-response was done as well. This comparison also revealed differences between the two levels indicating that the retinal OFF-bipolar cells and the tectal OFF-cells do not possess the same cone contributions that elicit light responses. Interestingly, the OFF-components of the retinal and tectal levels appeared to receive cone contributions from both the L- and M-cones. However the tectal OFF-response was best fit with a model that ‘summed’ the L- and M-cone contributions, which produced higher sensitivity values than would have been predicted by the L- and M-cones separately.

**Optic Nerve Recovery**

The second objective was to compare spectral sensitivity functions of tectal and ERG responses between normal subjects and optic nerve crush subjects at various time intervals following damage. No differences in the ERG functions between the controls and optic nerve crush subjects between 3 to 7-dpc were found. This outcomes seems logical since the nerve damage occurred after this level of processing. However, large differences in the functions of the tectal responses between these two groups were observed. A tectal response in the optic nerve crush subjects at 6-dpc was nonexistent.

The time intervals that were chosen in order to assess the return of tectal function after optic nerve damage were based on the findings of past experiments such as Northmore and Masino (1984), Northmore and Celenza (1992), and Northmore (1987; 1989a,b). This researcher found no evidence of optic nerve recovery until 21-dpc. However, even at 21-dpc, the responses were small and insubstantial. The first substantial responses were obtained at 41-dpc, where an initial OFF-response was seen.
This result contradicts the results of Northmore’s (1989a,b) studies which found that the recovery of tectal OFF-response could be seen at approximately 20-dpc. Northmore and Celenza (1992) found that at around 40-dpc, ON-responses also appeared in the tectum. The present study was able to obtain weak and insubstantial ON-responses from one fish at 41-dpc, but, more noteworthy, this study obtained the first substantial OFF-responses at 41-dpc. This study confirms Northmore’s (1989a,b) conclusion that the OFF-response does, in fact, return before the ON-response, since the present study obtained OFF-responses at 41-dpc and did not obtain ON-responses until about 60-dpc. It was not until 60-dpc that substantial tectal ON- and OFF-responses were obtained and spectral sensitivity functions were derived.

This researcher did not find significant differences in the shapes of the spectral sensitivity functions of the normals and the 60-dpc subjects. However, it did find a significant difference between the absolute sensitivity of the two groups. The 60-dpc ON-response exhibited an overall spectral sensitivity function that was significantly less sensitive compared to the normals. The 60-dpc OFF-response also exhibited an overall spectral sensitivity function that was significantly less sensitive than the normals.

This variation in absolute sensitivity is most likely due to the fact that the optic nerve has not completely re-established its connections with the appropriate tectal cells. The correct synapses between the cells have been established, but they are not yet as strong as the normal synapses. This finding may be the result of a decreased amount of trophic factors due to the cell damage. Purves et al. (1997) suggested that it is not only important for neurons to be guided and repelled from the appropriate cells but also once
synaptic connections are established there must be a sufficient amount of trophic factor(s) present for the cells to maintain healthy, long-lasting connections. If these healthier connections have not yet been established, then the responses of these cells will not be as sensitive to stimuli as the responses of normal cells.

Overall, the results of this study suggest that the optic nerve re-establishes synaptic connections and that these connections are functionally similar to those of normal subjects between 55-60 days after nerve damage. It can be expected that recovered tectal responses (after damage) would have eventually elicited nonsignificant differences in absolute spectral sensitivity functions if given more time to recover.

Sham Subjects

As a control measure, spectral sensitivity functions of sham subjects and those in the control condition were compared. No differences in either the tectal or ERG responses between these two conditions were anticipated.

It was expected that the sham subjects would derive a similar absolute spectral sensitivity function compared to the normals. Nonsignificant differences in spectral sensitivity between the shams and the normals were found. There was a significant interaction suggesting differences in the shape of the spectral sensitivity functions. On the other hand, although the sham responses appeared to be different from those of normals, the sham procedure did not produce the deficits that the optic nerve crush procedure did. The shams exhibited a clear response to both light onset and termination between 3-7 days post-surgery.
Perhaps having a larger sample size of sham fish would help decrease or diminish the difference in shape seen in the study. On the other hand, since the shams underwent the surgical procedures of tearing the muscles around the eye and pulling the eye out of its socket, it is possible that there may have been some trauma inflicted upon the optic nerve. The pulling of the eye may have strained some of the axons causing them to ‘break’ and eventually die. Also, the possible stretching of the optic nerve and insertion of the surgical tweezers may have decreased the blood supply to the axons causing damage to, or the death of, some neurons. It would be beneficial for future researchers to record from more sham subjects and at a later time interval, such as 14-days post-surgery, in order to better assess the degree of the damage inflicted upon the optic nerve.

In addition, it would be beneficial to future researchers if they were able to record the responses from the tectum at each time interval from each individual fish. However, a different procedure would be needed, such as inserting an electrode through the skull as opposed to removing the skull. It would also be advantageous for researchers to test optic nerve crush subjects after 60-dpc in hopes of obtaining responses that are nonsignificant from normals in absolute sensitivity. Lastly, researchers may be able to acquire tectal recordings at an increased number of time intervals in addition to the four utilized in the present study. Such an increase would enable scientists to better assess the specific timing of the return of function (of the ON- and OFF-responses) as well as the order of the return of cone contributions to the responses.
References


Table 1
Cone Model Weights

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<th>Cone Type</th>
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<th>ERG Off</th>
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<th>TECTAL Off</th>
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</table>
Figure Captions

**Figure 1.** Figure 1a shows an example of a goldfish electroretinogram (ERG) obtained with a 540 nm stimulus at a stimulus irradiance of 14.2 log q/s/cm$^2$. Both the ON-and OFF-responses are present. Figure 1b shows a goldfish tectal response obtained with a 660 nm stimulus at a stimulus irradiance of 15.1 log quanta/s/cm$^2$. Like the ERG, the tectal response contains a response component to both light onset and termination. In both figures, the ordinate represents the response in microvolts, and the abscissa represents time (ms). The horizontal line depicts light onset and termination.

**Figure 2.** Figure 2 shows the absolute spectral sensitivity of the goldfish ERG b-wave (open circles) and d-wave (filled circles). The ordinate represents the log absolute sensitivity (q/s/cm$^2$), and the abscissa represents the stimulus wavelength (nm).

**Figure 3.** Figure 3 shows the absolute spectral sensitivity of the tectal ON- (open squares) and OFF-response (filled squares). The ordinate represents the log absolute sensitivity (q/s/cm$^2$), and the abscissa represents the stimulus wavelength (nm).

**Figure 4.** Figure 4 illustrates the spectral sensitivity of the ERG b-wave (open circles) and tectal ON-response (open squares). The ordinate represents the log absolute sensitivity (q/s/cm$^2$), and the abscissa represents the stimulus wavelength (nm). The letters indicate the type of cone contribution to the response based on the cone modeling analysis. The ERG b-wave appears to receive cone input from the L-cones while the tectal ON-response appears to receive cone input from S-, M-, and L - M cones.

**Figure 5.** Figure 5 illustrates the spectral sensitivity of the ERG d-wave (filled circles) and tectal OFF-response (filled squares). The ordinate represents the log absolute sensitivity (q/s/cm$^2$), and the abscissa represents the stimulus wavelength (nm).
sensitivity \((q/s/cm^2)\), and the abscissa represents the stimulus wavelength (nm). The letters indicate the type of cone contribution to the response based on the cone modeling analysis.

**Figure 6.** Figure 6a shows an ERG response of a 6-dpc subject obtained with a 640 nm stimulus at a stimulus irradiance of 13.7 log \(q/s/cm^2\). Notice there is a response to both light onset and termination. Figure 6b shows a tectal response of a 6-dpc subject obtained with a 600 nm stimulus at a stimulus irradiance of 15.1 log \(q/s/cm^2\). Notice there is no response at the tectal level. In both figures, the ordinate represents the response in microvolts, and the abscissa represents time (ms). The horizontal line depicts light onset and termination.

**Figure 7.** Figure 7 illustrates a tectal response at 14-dpc. The stimulus wavelength was 600 nm and had a stimulus irradiance of 15.1 log \(q/s/cm^2\). The ordinate represents the response in microvolts, and the abscissa represents time (ms). The horizontal line depicts light onset and termination. There is no response at the tectal level 14-days after optic nerve crush.

**Figure 8.** Figure 8 illustrates a tectal response of a 21-dpc subject obtained from a 600 nm stimulus at a stimulus irradiance of 15.1 log \(q/s/cm^2\). The ordinate represents the response in microvolts, and the abscissa represents time (ms). The horizontal line depicts light onset and termination. Notice there are very weak responses to both light onset and termination.

**Figure 9.** Figure 9 shows a tectal response of a 41-dpc subject obtained from a 580 nm stimulus at a stimulus irradiance of 14.3 log \(q/s/cm^2\). The ordinate represents the
response in microvolts, and the abscissa represents time (ms). The horizontal line depicts light onset and termination. Notice there is a weak response to light termination but little if any response to light onset.

**Figure 10.** Figure 10 illustrates a tectal response of a 60-dpc subject obtained from a 660 nm stimulus at a stimulus irradiance of 14.1 log q/s/cm$^2$. The ordinate represents the response in microvolts, and the abscissa represents time (ms). The horizontal line depicts light onset and termination. Notice there is a substantial response to both light onset and termination.

**Figure 11.** Figure 11 shows the absolute spectral sensitivity of the normal tectal ON-response (open squares) and the 60-dpc ON-response (open triangles). The ordinate represents the log absolute sensitivity (q/s/cm$^2$), and the abscissa represents the stimulus wavelength (nm). Notice that the two functions are similar in shape, but the 60-dpc response is less sensitive overall compared to the normal ON-response.

**Figure 12.** Figure 12 shows the absolute spectral sensitivity of the normal tectal OFF-response (filled squares) and the 60-dpc OFF-response (filled triangles). The ordinate represents the log absolute sensitivity (q/s/cm$^2$), and the abscissa represents the stimulus wavelength (nm). Notice that the two functions appear to be similar in shape, but the 60-dpc response is less sensitive overall compared to the normal OFF-response.

**Figure 13.** Figure 13 shows the absolute spectral sensitivity of the normal tectal ON-response (open squares) and the sham ON-response (open diamonds). The ordinate represents the log absolute sensitivity (q/s/cm$^2$), and the abscissa represents the stimulus wavelength (nm).
Figure 14. Figure 14 shows the absolute spectral sensitivity of the normal tectal OFF-response (filled squares) and the sham OFF-response (filled diamonds). The ordinate represents the log absolute sensitivity (q/s/cm²), and the abscissa represents the stimulus wavelength (nm).
ERG Response

Stimulus wavelength: 540 nm

Tectal Response

Stimulus wavelength: 660 nm
Spectral Sensitivity of the ERG Components

Log Absolute Sensitivity (q/s/cm²)

- ERG ON
- ERG OFF

Wavelength (nm)
Spectral Sensitivity of Tectal Components

- Tectal ON
- Tectal OFF

Log Absolute Sensitivity (q/s/cm²)

Wavelength (nm)
Spectral Sensitivity of ON-Responses

Log Absolute Sensitivity (q/s/cm²)

- ERG ON
- Tectal ON

Wavelength (nm)

300 400 500 600 700

S, M, L - M, L
Spectral Sensitivity of OFF-Responses

Log Absolute Sensitivity (q/s/cm²)

Wavelength (nm)

ERG OFF

Tectal OFF

M

L

L + M
ERG Response (6-dpc)

Stimulus wavelength: 640 nm

Tectal Response (6-dpc)

Stimulus wavelength: 600 nm
Tectal Response (14-dpc)

Stimulus wavelength: 600 nm
Tectal Response (21-dpc)

Stimulus wavelength: 600 nm
Tectal Response (41-dpc)

Stimulus wavelength: 580 nm
Tectal Response (60-dpc)

Stimulus wavelength: 660 nm
Normal vs. 60-dpc (OFF-response)

Log Absolute Sensitivity (q/s/cm²)

- 60-dpc OFF
- Normal OFF

Wavelength (nm)