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Exploring Animal Photoreceptors and Eyes

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In this exercise, students examine an array of invertebrates and vertebrates to explore structure-function relationships among animals, using photoreception and vision as a context. This exercise was developed to accompany a physics-based lab (Barney et al. 2009), in which students model the physical relationships of different structural "eye" types. Building on the biodiversity component, students examine simple eyespots (*Dugesia*); a variety of eyes that use "pinholes" to channel light rays; and the eyes of scallops (*Pecten*) which include reflective "mirror" surfaces. Students also study microscope slides to examine structure-function relationships in insect compound eyes and to compare the retinas of various vertebrates. Additionally, they dissect a representative mammalian eye (*Bos*). In an optional investigation, students collect data from humans to test a working hypothesis about the relationship between age and accommodation.

Keywords: eyes, photoreceptors, vision, dissection, retina, zoology, structure-function relationships, animal anatomy and physiology

Introduction

In this exercise, students examine a diverse array of invertebrates and vertebrates to explore structure-function relationships in the Animal Kingdom, using photoreception and vision as a context for their comparisons. This lab exercise was developed to provide a "wet lab" experience to accompany a physics-based "bio-optics" lab (Barney et al. 2009) in which students model the physical relationships achieved by different structural types of "eyes", including pinhole, mirror and lens mechanisms for focusing light on photoreceptors. In addition to reinforcing the physical aspects of vision previously investigated, this exercise is intended to build in a biodiversity component, such that students will better appreciate some of the range of vision types exhibited in the animal kingdom, as well as gaining exposure to a wider array of animals.

During the exercise, students examine simple eyespots (*Dugesia*); a variety of eyes that use "pinholes" to channel light rays; and the eyes of scallops (*Pecten*), which include reflective "mirror" surfaces. Students also view microscopic slide preparations to examine structure-function relationships in insect compound eyes and to compare the retinas of various vertebrates. Lastly, they dissect a representative mammalian eye (*Bos*). In an optional extension, students collect data from individuals on campus to test a working hypothesis about the relationship between age and accommodation. Students plot and analyze data from individuals with and without corrective lenses. The results of this comparison reinforce what they have learned about physical structure-function relationships important in vision.

Most comprehensive introductory biology textbooks include a comparative treatment of photoreception (usually incorporated into chapters on sensory systems), e.g. Brooker et al. 2011 (see pp. 902-910) or Campbell and Reece 2002 (see pp. 1063-1069). For more comprehensive detail, we also relied heavily on Land and Nilsson (2002), and we use a photographic atlas (Rust 1983) that includes detail of microscope slides as a supplement in lab. Our students have previously completed the Bio-optics lab (Barney et al. 2009), in the same freshman/sophomore level course; therefore our treatment is fairly advanced and could be used in a post-introductory zoology, comparative anatomy/physiology, or neuroscience course. The components we have included are modular, however, and therefore can easily be packaged by instructors to be more basic, more comprehensive, or with additional, self-selected examples. Self-selection of components will also allow one to construct an exercise within a variety of time-frames; the description below is a very busy three-hour lab at our institution. The accommodation and age investigation included as an extension will likely require institutional Human Subjects and Review Board approval; please refer to your institutional guidelines.

Student Outline

Objectives

1. To understand diversity of structure/function relationships in the Animal Kingdom, using vision as a context for comparison.
2. To build biological examples of the structural mechanisms by which various animals (invertebrate and vertebrate) achieve vision according to the physical mechanisms of pinholes, mirrors, and lenses.
3. To further understand physical principles of optics and vision by in-depth examination of animal examples.
4. To become fluent with the major structures of a typical vertebrate eye, through dissection, and to understand the functions of those structures in achieving various aspects of vision (e.g. focusing, protection, accommodation, nervous transmission, etc.)
5. To become fluent with the microscopic anatomy of several examples of animal eyes by recognizing the patterns in construction, as well as understanding how the differences in construction achieve different "fine-tuned" aspects of vision.
6. To be able to construct a scatterplot graph from raw data, and to make inferences about the biological and physical relationships portrayed from this graphical data set.
7. To be able to carry out simple accommodation measurements (optional).

Introduction

In a previous laboratory exercise investigating physical optics of animal eyes, we focused separately on three general physical arrangements that model structural characteristics found in eyes among different kinds of animals: pinholes, mirrors, and lenses. During that exercise, you were able to demonstrate both the advantages and disadvantages of each kind of "model" and you explored, in particular, the relationship that is evident when a lens (or mirror) changes in shape/thickness and the distance at which an object is brought into focus. You should review those relationships before commencing today's lab (Brooker et al. 2011, Fox 2008, Land 1978, Land 2005, Land and Nilsson 2002). Several other kinds of models for sensing light and/or forming images exist in the animal kingdom. In addition, most animals, including humans, are characterized by a combination of structural features that exemplify more than one of these models. For example, the pupil of a human eye functions like a pinhole and we have two structures that function as "lenses" (the cornea achieves very coarse focus, and the fine focus of your eye is achieved primarily by your lens; Tortora 2002). As you investigate different animal eyes in today's lab, keep in mind that there is not a one-to-one correspondence of models and animal species. Actual organisms exemplify a rich array of multiple modifications of multiple models. This is why it is both important and exciting to study biological diversity! Refer to Figure 1.9, p. 13 in Land and Nilsson (2002) for a schematic showing the major optical types of eyes found in multicellular animals.

Sensations and the perceptions they evoke in the brain (for example, vision) begin with sensory reception, the detection of the energy of a stimulus by sensory cells. Most sensory receptors are specialized neurons or epithelial cells that exist singly or in groups with other cell types within sensory organs, such as eyes and ears. All stimuli represent forms of energy, and the general function of receptor cells is to convert the energy of stimuli into changes in membrane potentials and then transmit signals to the nervous system (Campbell and Reece 2002, Booker et al. 2011). A great variety of light detectors has evolved in the animal kingdom, from simple clusters of cells that detect only the direction and intensity of light to complex organs that form images. Despite their diversity, all photoreceptors contain similar pigment molecules suggesting they are *homologous.* Animals as diverse as flatworms, annelids, arthropods, and vertebrates have some of the same, ancient genes associated with the development of photoreceptors in embryos. Thus, the genetic underpinnings of all photoreceptors may have evolved in the earliest bilateral animals (Campbell and Reece 2002).

The functional unit of all vision-forming eyes is the receptor cell, which in vertebrates typically contains retinal and opsin proteins (light-sensitive) and responds to light by initiating a nerve impulse (details of the sequence of cellular and biochemical events involved in this response are described in Brooker et al. 2011, p. 907-908). Light sensitive opsins are borne on a hairy layer, to maximize the surface area. The nature of these "hairs" differs; in the protostomes, they are microvilli – extensions of the cell wall. But in the deuterostomes, they are derived from cilia. The opsin proteins surround the chromophore, a pigment that distinguishes colors. Groups of such cells are called "eyespots". Eyespots permit animals to gain a basic sense of the direction and intensity of light, but not enough to discriminate an object from its surroundings (Campbell and Reece 2002; Brooker et al. 2011; simplified design shown in Fig. 1.3 p. 4; Land and Nilsson 2002).

Investigating Photoreceptors in Animals - Invertebrate Examples

A. Eyespots and Dugesia

Most invertebrates have photoreceptors, which range from simple clusters of photoreceptor cells to complex image-forming eyes. One of the simplest is the **eye cup** of planarians, which provides information about light intensity and direction without actually forming an image. Photoreceptor cells are located within a cup formed by a layer of cells containing a screening pigment that blocks light. Light can enter the cup and stimulate the photoreceptors only through an opening on one side where there is no screening pigment (Figure 43.13, p. 902, Brooker et al. 2011). The opening of one eye cup faces left and slightly forward, and the opening of the other cup faces right-forward. Thus, light shining from one side of the planarian can enter only the eye cup on that side. The brain compares the rate of nervous impulses coming from the two eye cups, and the animal turns until the sensations from the two cups are equal and minimal. The result is that the animal moves directly away from the light source and reaches a shaded location beneath a rock or some other object, a behavioral adaptation that helps hide the planarian from predators (p. 1063 Campbell and Reece 2002; Burr et al. 2000, Inoue et al. 2004).

1. Observe live *Dugesia*, representatives of the Phylum Platyhelminthes, the flatworms. Note their response to the direction of light and record notes on this response in your notebook.
2. Examine prepared, stained whole mounts of *Dugesia* (p. 57 in Rust 1983) and draw the animal, including its eye cups. As noted previously, the "eyespots" of *Dugesia* do not form images. Why would it be important for this animal to be able to discriminate light vs. dark, even without forming images? List at least 3 other possible benefits to the animal that would depend upon it being able to sense information about the direction of light vs. dark, and/or the timing of light/dark?
3. Other demonstration material may be available highlighting other animals that are characterized by light-sensitive cells or specialized photoreceptive structures that do not form images. Figures of *Amphioxus*, the lancelet (Phylum Chordata) appear in Rust (1983: p. 76). Note the location of the photoreceptors along the dorsal axis of the body in this marine animal.

B. Mirrors and other Reflective Surfaces

As with lenses, the value of mirrors as optical components depends on their ability to alter the direction of light rays. The law of reflection states that the angle an incident ray makes with a normal (right angle) to the surface is the same as the angle made by the reflected ray and the normal. This property makes it possible to use curved reflecting surfaces in the formation of images. In general, the more curved the mirror, the greater the ability to focus on nearby objects (Barney et al. 2009). A number of bivalve mollusks (Phylum Mollusca) have evolved optical structures that enable them to detect the approach of predators. Ark shells (*Arca, Pectunculus*) have basic but effective compound eyes in the mantle surrounding the opening of the shells, that evolved independently of the more familiar compound eyes of insects and crustaceans (see part C. below). Scallops of the genus *Pecten* and some close relatives have evolved unique concave reflector eyes for the same purpose (Fig. 6.2, p. 106 in Land and Nilsson 2002, Land 1978). Scallops have 60-100 small (1 mm) eyes peeping out between the tentacles of the mantle that protects the gap between the two shells (Fig. 33.20, p. 657 in Campbell and Reece 2002; **http://dels-old.nas.edu/USNC-IBRO-USCRC/resources_methods_scallop.shtml#scallop**). The back of the scallop's eye is accurately spherical and lined with a green-reflecting mirror, the *argentea*, which appears silvery if you remove the outer connective tissue of the eye.

Your instructor will demonstrate safety techniques to be observed while you open a preserved scallop. Locate the many eyes and draw their position on the animal in your lab notebook. If you are able to peer into a dissected scallop eye through a dissecting scope you will see an image: an inverted image of yourself! Write a few sentences speculating on the possible adaptive benefits to the animal of having eyes located in this spatial arrangement; relate these observations to the habitat and lifestyle of the animal.

Visit (**http://dx.doi.org** and type in the doi box: 10.1016/j.cub.2008.11.061) to view images of "mirror" eyes in a deep-sea fish (*Dolichopteryx longipes*) that also has a pair of "typical" eyes with lenses (Wagner et al. 2008). What is the function of the dorsally-oriented eyes in this fish? What is the function of the ventrally-oriented mirror eyes? This link also contains a short animation demonstrating how mirror eyes function optically.

Many arthropod and vertebrate eyes have mirrors behind the retina, but unlike the scallop mirror their function is not to form an image. Rather, their function is to reflect the light already focused by the lens, and return it anteriorly through the retina, giving the retina a "second chance" of capturing photons missed on the first pass. This structure is called the *tapetum* *lucidum* and is best developed in animals that live in deep water or are active at night. Light reflected by the tapetum lucidum is returned through the lens as a narrow beam, "eyeshine", and will be visible only from the direction of the original lamination. You have undoubtedly seen this returned beam in animals suddenly caught by your flashlight or headlamps at night (cats, deer, wolf spiders, raccoons, etc.). The tapeta of vertebrates are comprised of a wide variety of materials, all having in common a high refractive index (crystals of guanine in the tapeta of many fish, riboflavin in the tapeta of bush-babies, and rods containing the zinc salt of cysteine in the tapeta of cats) (Land and Nilsson 2002). Be sure to identify the tapetum lucidum on the cow or sheep eye that you dissect today.

C. Compound Eyes and Lenses

Image-forming eyes of two major types have evolved in invertebrates: the compound eye and the single-lens eye. Compound eyes are found in insects and crustaceans (Phylum Arthropoda) and very primitive compound eyes are found in some polychaete worms (Phylum Annelida). Compound eyes function in two distinct ways – and are referred to as apposition compound eyes or superposition compound eyes. Superposition eyes resemble apposition eyes in having the same general design of multiple facet-like structures, but they produce a single deep-lying erect image in the vicinity of the retina. They are common on moths, other night-flying insects, and among some deep-sea crustaceans (Figure 7.4, p. 129 Land and Nilsson 2002).

For this exercise, we will focus on apposition compound eyes, which characterize most other insects active during the day. This type of compound eye consists of up to several thousand light detectors called *ommatidia*. These resemble "facets" of the eye, and each has its own light-focusing *lens* (Fig. 43.14, Brooker et al. 2011). The lens (cornea and crystalline cone) focuses light onto a long structure called a *rhabdom*, which is comprised of a column of photosensitive microvilli. The microvilli project from the membrane of the photoreceptor cells which are called *retinulae*. Each ommatidium registers light from a tiny portion of the visual field. Differences in the intensity of light entering the many ommatidia result in a mosaic image. The animal's brain may sharpen the image when it integrates the visual information. The compound eye is extremely acute at detecting movement, an important adaptation for flying insects and small animals constantly threatened with predation. For comparison, consider that the human eye can distinguish light flashes up to about 50 flashes per second. For this reason, the individual images of a movie, which flash at a faster rate, fuse together to create the perception of smooth motion. The compound eyes of some insects, however, recover from excitation rapidly enough to detect the flickering of a light flashing 330 times per second. Such an insect viewing a movie could easily resolve each frame of the film as a separate still image (Land and Nilsson 2002). Insects also have excellent color vision, and some (including bees) can see into the ultraviolet range of the spectrum, which is invisible to us. In studying animal behavior, we must be careful not to extrapolate our sensory world to other species; different animals have different sensitivities and different brain organizations (Campbell and Reece 2002). For an interesting example of how artificially constructed insect eyes are used in scientific research, visit: **http://news.bbc.co.uk/go/em/fr/-/2/hi/science/nature/4946452.stm** .

1. Use a dissecting microscope and the dried insect specimens available, examine external views of insect eyes and draw at least one diagram of the arrangement of the ommatidia; write the name of the insect with your diagram. Indicate whether your insect specimen is more likely to use superposition compound eyes, apposition compound eyes (or perhaps a combination of both), and why.
2. Examine prepared slides of *Drosophila* internal eye structure, in cross section, using Fig. 43.14, p. 903 (Brooker et al. 2011) as a schematic guide. You should be able to identify the chain-like ommatidia (in cross section), and the individual components of each ommatidium: cornea, crystalline cone, rhabdom, and the "hairy" looking membranes lining the photoreceptor cells, the retinulae. Begin at low power (e.g. 40X), noting the fly's brain in close proximity to the right and the left eyes. Center one of the eyes, and switch to higher power (100X) to identify and draw individual structures in your lab notebook.
3. Watch the "movie" entitled "Insect Vision" from **www.physioviva.com,** which demonstrates how insect ommatidia function. Animations in this video will model the type of image the insect "sees" after multiple images are formed. Summarize your findings from this animation in your lab notebook in a paragraph.

D. Single Lens Eye and Pinholes

Among the invertebrates, single-lens eyes are found in some jellies, polychaetes, spiders, and many mollusks. A single-lens eye works on a camera-like principle. The eye of an octopus or a squid, for example, has a small opening, the pupil, through which light enters. Analogous to a camera's adjustable aperture (F-stop), the iris of a single-lens eye changes the diameter of the pupil; behind the pupil, a single lens focuses light onto the retina, which consists of light-transducing receptor cells. Also similar to a camera's action, muscles move the lens forward or backward to focus images on the retina (Fig. 33.22, p. 658 in Campbell and Reece 2002). Examine a preserved *Nautilus* (Phylum Mollusca) or other cephalopods on demonstration, if available. On-line images may be substituted if preserved specimens are not available. The *Nautilus* eye is unusual in that it operates with a pinhole, but *no lens* (Fig. 4.1 p. 57, Land and Nilsson 2002). The eye of *Nautilus* is relatively large, however, measuring about 1 cm in diameter, and this pinhole pupil can vary its diameter (between about 0.4 and 2.8 cm) as light intensity changes.

In order to obtain any significant resolution of an image does the *Nautilus* need to increase or decrease the diameter of its pupil?

What is the immediate trade-off (disadvantage) of doing so, given the habitat the Nautilus lives in?

In contrast, terrestrial vertebrates use a pupil "pinhole" in combination with a lens, and as a group, exhibit several different adaptations for adjusting the aperture of this pinhole (Fig. 5.11, p. 89, Land and Nilsson 2002).

Investigating Photoreceptors in Animals - Vertebrate Examples

A. Dissection of Mammalian Eye

1. Your instructor will give you instructions on wearing protective gloves and safety goggles, safe use of sharp instruments such as scalpel and scissors, and proper cleaning and disposals of your dissection pan and utensils. Note that in addition to the liquid preservative, the eyes will be filled with fluid of their own; you should take care when you cut into the eye that you do not splash liquid in your eyes or on someone else. If you do, make sure you are aware of the location of the eyewash station, etc.
2. Obtain a preserved cow or sheep eye and place it in your dissection pan. Use the eye models which will be on demonstration to help you locate structures, as well as diagrams in the pictorial biology lab guides available in lab (e.g. Rust 1983, p. 99).
3. First examine external structures (Fig. 2 Ward's Natural Science Establishment 2001), then cut the eye carefully with a scalpel to separate anterior and posterior portions (Fig. 3, Ward's Natural Science Establishment 2001). Ideally, you should try to retain the fluid in the posterior chamber of the eye and set this half in your pan like a small bowl holding the fluid. This will help keep the specimen's retina in place and intact, fanned out against the posterior wall of the eye.
4. Create Table 1 in your lab notebook and complete by writing the functions of each structure, using information in the assigned readings in Brooker et al. (2011). Use Ward's Vertebrate Eye copymaster 1 (Ward's 1984) to label external and internal eye structures.

It is unlikely that you will observe the fovea centralis directly, since it is quite small. Refer to Brooker et al. (2011, p. 904) to locate the structure and learn about its function.

What structures are responsible for changing the shape of the lens in this eye?

What is the significance of being able to change the shape of the lens in terms of focusing on objects at various differences?

When you examine prepared microscope slides of a mammalian retina (Part II.B.) carefully note the position of these structures next to the lens, in microscopic view. Draw their position, relative to the lens, in your lab notebook.

Does this mammal have a tapetum lucidum? If so, where is it located (indicate on your lab notebook diagram).

B. Micro-Structure of the Retina – A Comparison of Several Animals

1. At your table, you will find prepared slides showing the micro-structure of the eyes of a small mammal, bird, zebrafish, frog, and *Loligo*, a large species of squid. Each person at your table should select one of the vertebrate slides and make a careful, labeled diagram in your lab notebook (one for each lab notebook) showing the location of each of the following structures from anterior to posterior: *pupil, lens, ganglion neurons, bipolar neurons, rods and cones (the photoreceptor cells), pigmented epithelium of the retina, choroid, and sclera*

   Draw an arrow showing the direction of the path of light as it enters the eye and strikes the retina– the point of the arrow should be pointing posteriorly relative to the animal eye. Helpful diagrams are located in Rust (1983, p. 99 -100) and Brooker et al. (2011, p. 908, Fig. 43.22).

   As mentioned previously, also note the location, on each vertebrate, of the ciliary muscles and suspensory ligament – how are these arranged relative to the lens?

   Recall from your background reading assignment (pp. 905-909 in Brooker et al. 2011) that light strikes the photoreceptor cells (rods and cones). Next, this signal is carried *anteriorly* to the bipolar and then to the ganglion cells. Where do the axons of the ganglion cells now travel, and are they passing anteriorly, or posteriorly to the retina, relative to the direction that light is coming in? What is the obvious problem with this arrangement?

Table 1. Structure-function relationships of a mammalian eye.

Main Region	Structure	Function
	ciliary body/ciliary muscle	
	suspensory ligament	
	aqueous humor	
	conjunctiva	
	pupil	
	lens	
	cornea	
	iris	
posterior cavity	vitreous humor	
	retina	
	choroid	
	sclera	
	optic disc	
	optic nerve	
	fovea centralis	
extrinsic eye muscles		

2. Now examine the slide for *Loligo*, the squid (octopuses have eyes structured very similarly to the squid eye). In your lab notebook, write the sequential order of the same anatomical parts that you observed in the vertebrate eye, from anterior to posterior, relative to the same arrow in your vertebrate diagram that shows the direction traveled by light entering the eye and striking the retina. There will not be a one-to-one correspondence of the nerve cells, but the important differences will be in relative positions of the photoreceptor cells as compared with the other neurons used for transmission of the signals generated by the photoreceptors.

What structures are arranged in a different order (or are different structures altogether)? Which arrangement (vertebrate or squid?) results in a blind spot and which arrangement avoids a blind spot? Why? What is the function of the pigmented epithelium at the posterior of all of these eyes?

As you compare the relative costs and benefits of different form and function arrangements in animal eyes, keep in mind that the evolution of eyes is not a linear, progressively "better" trajectory, culminating with the "best" eyes landing with *Homo sapiens*. "Better" and "worse" are highly relative terms, and relative terms should always be considered within the context of the environment and lifestyle of the organism in question. Our eyes are far from perfect and you have only to glance around the room at the proportion of us wearing corrective lenses (even at a very young age in our total lifespan) to get a good sense of that! For another testimony to variation in "good, better, and best", refer to Table 2 showing the extreme range of sensitivity exhibited by various animal eyes. The sensitivity of animal eyes can derive from several different eye structures, including the aperture of the pinhole, the density and arrangement of rods and cones, and most fundamentally, from the size of the eye itself.

In what environments do we generally find animals with the most sensitive eyes?

Table 2. The sensitivity (S) of a selection of animal eyes. From Table 3.2, p. 52. Land and Nilsson (2002); original references for the S values given in Land and Nilsson (2002).

Name	Sensitivity	Light habitat
Cirolana (marine isopod)	4200	deep sea
Oplophorus (decapod shrimp)	3300	deep sea
Dinopis (ogre-faced spider)	101	nocturnal
Limulus (horseshoe crab)	83-317	coastal mainly nocturnal
Ephestia (moth)	38	nocturnal/crepuscular
Onitis aygulus (dung beetle)	31	nocturnal/crepuscular
Phronima (hyperiid amphipod)	38-120	mid-water
Homo (peripheral rod pool)	18	crepuscular
Pecten (scallop)	4.0	coastal sea-floor
Bufo (toad)	4.0	mainly diurnal
Leptograpsus (shore crab)	0.5	diurnal
Onitis ion (dung beetle)	0.35	diurnal
Apis (worker bee)	0.32	diurnal
Phidippus (jumping spider)	0.04	diurnal
Homo (fovea in daylight)	0.01	diurnal

C. Accomodation in the Human Eye

Speaking of "imperfect", most humans in the more developed countries of the world (like us) can expect to lose some near vision as we age, due to the gradual decline of lens elasticity known as *presbyopia.* As we age, our ability to focus on near objects becomes progressively diminished; the condition may involve changes in both the crystalline lens and the ciliary muscles that bend and straighten the lens, changing its curvature. The change in lens curvature to achieve focus of objects being brought closer to the eye is known as *accommodation* of the lens. As an object is brought closer to the eye, a point is reached where accommodation of the lens becomes maximum (Brooker et al. 2011, p. 904, Fig. 43.15). This is known as the *near point of accommodation*. Objects brought closer to the eye than the distance of the near point cannot be properly focused on the retina and appear blurred. With advancing age, the lens of the eye (and perhaps the ciliary muscles and suspensory ligaments) gradually loses its elasticity and capacity for accommodation. As a result, the near point of accommodation should increase with age. This prediction will constitute our working hypothesis for a short investigation on vision in on our own species. Because we are using human subjects, this investigation will require that your instructor has obtained Human Subjects Review Board approval at your own institution. Your instructor will explain this process and provide you with instructions for "informed consent" as you enlist people's help in making measurements.

1. Obtain a meter stick and a card that has a small letter a (10 pt. Font) printed on one side. Place the end of the meter stick at chin level and hold the meter stick at a right angle to your face. Hold the card at the end of the meter stick (enlist the help of a lab partner to do this). Focus on the letter with one eye closed, and ask your partner to slowly slide the card forward (toward your eye). When you can no longer focus on the letter, ask your partner to stop sliding the card. Then, measure the shortest distance from the cornea of the eye where the letter can be sharply seen. Make a data table in your lab notebook with the following columns, and enter your own data in the first row: Age of human, eye measured (right or left), human subject wearing contacts or not? (Yes or No), and near point of accommodation in centimeters.
2. You and your group members should now embark on a short field excursion to collect the same information from 6 other human beings, attempting to obtain as broad a range of ages as is possible (so…other students in your labs are not fair game). You will be armed with your meter stick, the test card, your lab notebook, and some colored stickers. No individual should be measured more than once, so provide each subject with a sticker so that individuals will not be re-sampled. It is your responsibility to inform your potential subjects of what you are doing, and why, and that their name will not be recorded nor associated with the data collected. You should inform them they may decline to participate. If they do elect to participate, they will need to give their age (truthfully!) and will be asked to remove corrective glasses, but not contact lenses. If they are wearing contacts, they will be asked to inform you. Try to sample at least some subjects younger than 20 and/or older than 45 (the older, the better!) and return to class. This should take no more than 20 minutes.
3. Statistical Analysis and Preparation of Graph. Your instructors will compile the data that you collect with the rest of the class. You will be instructed in lab as to what type of statistical analysis you will perform on the compiled data set to investigate the hypothesized effect of age on the near point of accommodation. You may also want to investigate the effect of other variables on the near point of accommodation, such as right vs. left eye and use of corrective lenses. Your statistical results of this analysis should be included in your lab notebook. Construct a graph that includes your hypothesized dependent variable on the y axis and the independent variable on the x axis. Construct 2 lines on the same graph – one line for persons sampled who were using contacts, and the second line for those without contacts. Write a paragraph in your lab notebook summarizing the results of this experiment.

Materials

There are many potential extensions and additional examples that can be included with this lab (see Notes for the Instructor); alternatively, portions of the previous Student Outline can be deleted to produce a package that is appropriate to instructional level and time available. This list is intended to cover the components that we have included in the Student Outline text for a lab section of 24 students working in pairs (for the eye dissection) and in table groups of 4 (for the microscope slides); additional suggestions for extensions are included in Notes for the Instructor.

Equipment per entire class:

- 12 compound microscopes
- 4 dissecting microscopes (for use with dried insects, scallop eyes) with lamps; alternatively,
- 10X or 16X hand magnifiers can be substituted for the dissecting microscopes.
- 1 additional compound microscope (or dissecting microscope) at each display stations for additional demonstration examples (e.g. *Amphioxus* whole mount slide)
- Giant eye model (e.g. Ward's 81-3336, approx. $85).
- "Insect Vision" animated movie – Item #13; **www.physioviva.com**
    - ◦ If "Insect Vision" video is used, a computer and projection unit to play the video is needed
    - ◦ If live *Dugesia* are observed as a demo, a culture demonstration with desk lamp is needed

Supplies per class of 24:

- Nitrile or vinyl protective gloves
- Dissection aprons (optional), roll of 100, frog (Ward's 15-1006)
- Squid – optional demonstration (preserved, double injected; Ward's 68-7391)
- *Amphioxus* whole mount slide – optional demonstration (Ward's 92-3013)
- *Amphioxus* whole mount plastimount – optional demonstration (Ward's 55-8100)
- Lamprey, preserved –optional demonstration of pineal eye (e.g. Ward's 69-1022)
- Planaria (*Dugesia*) , living – optional demonstration of phototaxis (Ward's 87-2500)
- Cow eye dissection kit (includes copy masters for diagrams and sheets for students to label mentioned in the student outline; Ward's 62-2070).
- Cow eyes, preserved, pail of 50 (Ward's 69-7183); also available from biologyproducts.com
- Scallops (preserved; jar of 10; Ward's 68-7122)
- 12 meter sticks (accommodation experiment)
- 12 printed "a" cards (accommodation experiment)
- One sheet of 12 colored stickers (accommodation experiment)
- 12 small dissection pans or trays (primarily for cow eye dissection and opening scallops)
- 12 sets of dissection utensils, each including: 1 scalpel, 1 blunt probe, 1 forceps, 1 pair sharp scissors

Per table:

- One sharps disposal for scalpel blades
- Lens paper; lens cleaner, 4" finger bowls (2)
- One pair of safety glasses per student
- One box of prepared slides including: whole mount *Dugesia* (planaria; Ward's 92-0822); Comparative eye slide set (one set per table group: Ward's 95-0834), Insect Compound Eye (sect) mas (radial section; Ward's 92 W-2416).
- One copy of: Rust, T.G. 1983. *A Guide to Biology Lab, 3rd Edition.* Southwest Education Enterprises, Boerne, TX. 112 pp. (ISBN: 0-937029-01-7). This photographic atlas is out of print but available from amazon.com. An alternative atlas is Photographic Atlas for the Biology Laboratory (Carolina # DH-451710).
- One box per table: 3-4 dried, pinned insect specimens if available from your department's entomology collection. Samples might include dragonflies, grasshoppers, honeybees, beetles, butterflies, and/or large flies

Other optional supplies that could be used for extensions:

- Sclerotic rings (bird; alligator skeletons)
- Preserved flounder (this fish shows interesting developmental "migration" of eye position)
- Pineal eyes (on live reptiles, such as *Anolis carolinensis*)
- Live *Euglena* (stigma near flagellar end contains a red pigment which shades a collection of light sensitive crystals – this "eyespot allows the organism to move toward light).
- Aquatic vs. terrestrial preserved eyes (fish, frogs, mice); the comparative eye slide set from Ward's includes both terrestrial and aquatic vertebrate examples.
- Preserved *Nautilus* (see **http://www.utmb.edu/nrcc/LiveAnimRes.htm)** for possibilities of live or preserved cephalopods.

Notes for the Instructor

Laboratory Safety

The scallop and cow eye dissections involve the use of scalpels and scissors and handling of items in preservative. There is always the possibility that a student may splash preservative or eye fluid into his/her own eye while cutting, so prepare your students in advance for minimizing this possibility by pointing out precautions deliberately, and in advance. In particular, review the location of the eyewash station and eyewash procedure. The instructor should demonstrate proper use of scalpel and scissors and have Sharps disposal containers handy at each table. Encourage students to use scalpels and scissors only minimally – they are only needed to separate anterior from posterior halves of the cow eye (and to initially open the scallop). They are *not* needed after that, and we prefer to have the students put these items away completely at this point to avoid sharp items being left in inappropriate places on tables and around specimens during this very busy lab. In general, there is a lot going on simultaneously in this lab (preserved items, microscope and lamp cords, glass slides, movement of students to and from demonstration stations), so be alert for on-going hazards that get created by students along the way. If live animals are used (e.g. using live *Anolis* to show pineal eyes), students will also need to be instructed in safe handling of live animals so as not to injure animals or leave residues from preservative on sensitive animal skin.

Human Subjects Review Board

Conducting the accommodation experiment (Part II. C. in the Student Outline) will most likely require approval of your own institutional Human Subjects Review Board, since students are asked to collect data from other humans. Because the procedure itself is neither invasive nor traumatic, and because students are asked to inform potential participants that they may "opt out" (participation is by consent only), approval should be relatively straightforward. It is important for students to know how the HSRB process works at your institution and why it exists. They may not be aware that procedures that seem simple and harmless may still involve approval by an oversight committee. This is probably something very valuable for them to learn about and including this portion of the lab may provide you with a welcome opportunity to discuss such issues with your class (for example, you might want to compare the process for human subjects vs. other vertebrate animal subjects by comparing HSRB vs. IACUC policies and guidelines).

Picking and Choosing Vision Elements

This exercise lends itself to great flexibility in the "modules" chosen for inclusion; an instructor can easily adapt additional modules or leave out items to fit various goals for time available, level of students, or particular animal groups that are desirable to introduce. We deliberately chose most of the examples (modules) included in this student outline to encompass three "physical" themes (pinholes, mirrors, and lenses) investigated with an optical light apparatus in a previous ABLE lab (Barney et al. 2009: *BioOptics Corporation: An investigative interdisciplinary case study on the eye*). Other organisms provide equally striking examples of other "form and function" adaptations and might be more readily available, or serve other program-specific curricular goals at your own institution.

Other themes that we think would make outstanding modules for this lab, but that we have not yet developed, include: a) color vision (for example, see recent science news articles on mantis shrimps and squids for amazing color vision adaptations: **(http://arthropoda.wordpress.com/2010/03/10/mantis-shrimp-vision-preview/; http://www.americanscientist.org/science/content1/7725),** b) aquatic vs. terrestrial vision, including how lens shape and position demonstrate physical aspects of the difference in refractive indices for air vs. water, c) sensing of polarized light and circular polarized light (mantis shrimps are the only known animals that can see circular polarized light), and d) the importance of studying vision in other animals in order to gain important insights for human health (e.g. see **http://www.scientificamerican.com/podcast/episode.cfm?id=gene-therapy-cures-color-blind-monke-09-09-16).**

Answers to Imbedded Questions in the Student Outline

Invertebrate Examples

A. Eyespots and *Dugesia*

Why would it be important for this animal to be able to discriminate light vs. dark, even without forming images?

Orientation; photoperiod gauge.

List at least three other possible benefits to the animal that would depend upon it being able to sense information about the direction of light vs. dark, and/or the timing of light/dark.

Moving toward food, moving away from danger, dispersal to new habitats, sensory input on when to reproduce, when to disperse, sensing up vs. down in the water column, or location of substrate (e.g. shelter).

B. Mirrors and other reflective surfaces

Write a few sentences speculating on the possible adaptive benefits to the animal of having eyes located in this spatial arrangement; relate these observations to the habitat and lifestyle of the animal.

Scallops have eyes almost completely ringing the outer edge of the part of the body that is exposed when the shell is open. Thus, there are eyes looking in almost any direction for potential danger and/or potential food. As an adult, this animal is fairly stationary while feeding and anchors itself on a substrate such as a rock.

What is the function of the dorsally-oriented eyes in this fish?

Scanning for danger/other fish; these eyes are oriented toward the brighter surface and can collect some sensory information from light coming from this direction.

What is the function of the ventrally-oriented mirror eyes?

The brown snookfish scans for bioluminescent prey in darker water in deep ocean; the ventrally oriented eyes may reflect bioluminescent light from potential prey located below the swimming snookfish, allowing it to detect prey in a dark habitat.

D. Single lens eye and pinholes

In order to obtain any significant resolution of an image does the *Nautilus* need to increase or decrease the diameter of its pupil?

Decrease the diameter.

What is the immediate trade-off (disadvantage) of doing so, given the habitat the *Nautilus* lives in?

The Nautilus lives in deeper oceanic waters, where light reaching those depths may be limited; decreasing the diameter of the pupil, while increasing resolution, will cut down on the amount of light entering the eye, and therefore decrease sensitivity and the brightness of the image.

Vertebrate Examples

A. Dissection of Mammalian Eye

Does this mammal have a tapetum lucidum? If so, where is it located?

Cow eyes have tapeta, the bluish, reflective, almost rainbow-like surface on the anterior portion of part of the black, pigmented surface of the choroid. Typically, the tapetum covers only a portion of the choroid. The tapetum "aims" anteriorly, toward the front (pupil) of the eye.

B. Micro-structure of the retina – a comparison of several animals

What is the location of each of these structures from anterior to posterior (in a typical vertebrate):

ganglion cells (neurons), bipolar cells (neurons), rods and cones (photoreceptive neurons), pigmented epithelium, choroid, sclera.

How are these arranged relative to the lens? (ciliary muscles and suspensory ligaments of typical vertebrates, including humans.)

On the retina slides, students will note that the suspensory ligaments and ciliary muscles can be seen as tiny "bridging" structures on the each lateral side of the lens, near the anterior (front) of the lens. Surprisingly small in cross section, it is the action of these muscles and ligaments that variously allow the lens to flatten (far sight accommodation) or cause the lens to bulge (near sight accommodation).

Where do the axons of the ganglion cells now travel, and are they passing anteriorly or posteriorly to the retina, relative to the direction that light is coming in? What is the obvious problem with this arrangement?

Table 1. Structure-function relationships of a mammalian eye.

Main Region	Structure	Function
anterior cavity	ciliary body ciliary muscle	change shape of lens
	suspensory ligament	change shape of lens
	aqueous humor	nourish lens
	conjunctiva	support/protection
	pupil	functions as pinhole; regulates amount of light entering eye
	lens	focus light on retina; accommodation
	cornea	protection; secondary lens (coarse focus)
	iris	muscles that dilate or contract pupil
posterior cavity	vitreous humor	helps maintains shape of eye & retina
	retina	photoreception
	choroid	protection; may include a reflective surface (tapetum lucidum); pigmented epithelium absorbs potentially scattered light that did not strike rods & cones
	sclera	protective connective tissue
	optic disc	where the optic nerve leaves the eye carrying nerve impulses to the brain
	optic nerve	transmits sensory information from the retina to the brain
	fovea centralis	in humans and some other vertebrates – an area of high acuity due to a dense concentration of cones
extrinsic eye muscles		moves the eyeball to point different directions

The axons of the ganglion cells are grouped together, clustering into the large optic nerve as they pass anterior to the other neurons, including the photoreceptor cells of the retina itself! This arrangement potentially creates a significant blockage of light that is coming from anterior to posterior to reach the retina, and where the optic nerve itself "turns" to leave the eye posteriorly (after having passed over the retinal cells), there is typically a "blind spot" at the optic disc, as occurs in humans.

What structures (in *Loligo*) are arranged in a different order (or are different structures altogether)? Which arrangement (vertebrate or squid) results in a blind spot and which arrangement avoids a blind spot? Why?

The Loligo retina contains pigmented epithelium, and a stacked series of neurons in the retina, similar to the vertebrate retina, but there appears to be one layer "fewer" neuronal cells. Importantly, the rods and cones are located anterior to the accessory neurons, such that the axons of the neurons that constitute the optic nerve do not obstruct the light hitting the rods and cones. Thus, the arrangement in Loligo avoids this particular blind spot cause, the optic nerve leaving the eye posteriorly behind the photoreceptor cells.

What is the function of the pigmented epithelium at the posterior of all of these eyes?

A heavily pigmented layer seems to be almost universal behind the photoreceptors, and it probably functions to absorb photons of light that have managed to miss photoreceptor cells while passing through the retina. To avoid scattering this light, which could potentially reflect back onto the retina from "behind", resulting in loss of image acuity, heavily pigmented cells absorb the light at the back of the eye. Note the trade-off here between the benefit of eliminating potentially scattered light from reaching the retina (under high light conditions where visual acuity can be sharp) and the benefit of "deliberately" reflecting scattered light back onto the retina via the tapetum lucidum when light availability is minimal (e.g. at night). Note the parallels here with the two forms of insect compound eye vision: apposition and superposition.

In what environments to we generally find animals with the most sensitive eyes?

These animals generally tend to be deep sea dwellers or nocturnal/crepuscular, conditions when light availability is poor. (Crepuscular refers to animals that are most active at sundown and shortly thereafter.)

Sample Results – Accommodation Experiment

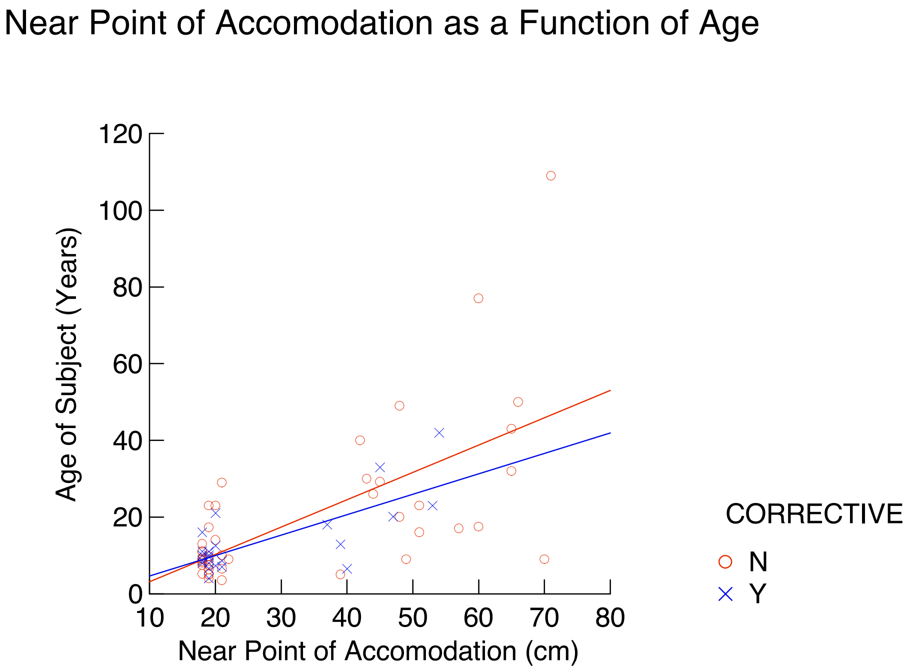

Figure 1. Compiled class results for an investigation of near point of accommodation as a function of age (2009; Hope College). Pearson correlation coefficient for right eyes only for individuals using corrective lenses r = 0.741, df = 21, P < 0.01; for right eyes only without corrective lenses, r = 0.646; df = 42, P < 0.01). Although the correlation coefficients suggest a significant relationship between the variables, please note that this relationship may not be a linear one.

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