ABSTRACT

Four inquiry-based laboratories are described that introduce students to the properties of human brain cells. These experiments require no technical equipment, are inexpensive and safe, and introduce students to genuine research using neuropsychological investigations as a means of studying the properties of brain cells. Students design and conduct an experiment using optical illusions to explore the activity and response of specific nerve cells of the visual system. Some of the successes and pitfalls of such an activity are discussed.

Key Words: Inquiry-based pedagogy; neuroscience laboratory; vision; brain; optical illusion.

The human brain is a topic of interest to many students yet is seldom explored through laboratory investigation. Here, I describe a means of introducing students to brain research. The four activities outlined below engage students in a true research experience. Students are required to design experiments guided by their hypotheses. Neuropsychological methods are used to study the properties of specific types of brain cells. This approach uses simple equipment and self-reports from human subjects. It is safe, inexpensive, and easy to set up. Optical illusions are used to investigate the property of the cells of the visual system. Depending on the optical illusion selected, students can study retinal photoreceptor cells, thalamic cells of the lateral geniculate nucleus, simple cells, or complex cells of the visual cortex.

Vision Research Projects

Below is a brief account of how visual information is conveyed and processed from eye to brain. Readers are referred to any introductory neuroscience textbook (e.g., Pinel, 2006) for a complete explanation of this process. Only information useful for understanding the experiments is offered. Following a description of important cells involved in visual processing, optical illusions whose effects rely on the cells described in the section are introduced, as well as possible ways to modify the basic illusion to investigate the properties of these cells.

A Summary of Visual Processing

Light that enters the eye stimulates light-sensitive receptors at the back of the eye (Figure 1). The visual data at this point are like a pixilated image. They must be processed by the brain to extract meaning—for example, to identify where objects begin and end and to recognize movement. The visual data collected by the eye are processed in stages by specialized cells in precise areas of the brain. At each step, specific information is obtained, and the processed information is relayed to the next processing center.

Research Project 1: The Distribution of Rods & Cones in the Retina

Light that enters the eye is detected by receptors called rods and cones. Our ability to perceive colors is conferred by the cones. Cones come in three varieties, each maximally sensitive to a different color: orange, green, or violet (Stein & Stoodley, 2006: p. 119). Rods are more sensitive to light than cones, and they allow us to see in dim light. Since there is only one variety of rods, they do not confer any color information to our nervous system. Light coming from the center of gaze is imaged on the back of the eye in an area called the fovea. The fovea is densely packed with cone receptors. The rest of the eye, which receives light from our peripheral vision, consists mostly of rods. The net consequence of this arrangement is that humans have color vision only in the center of their gaze, and black-and-white vision in the surrounding regions. The precise distributions of rods and cones in the eye...
have been mapped. The number of cones peaks in the fovea and decreases sharply within 10° of the center of gaze (Lindsay & Norman, 1977). Rods are absent from the fovea, rise sharply at 20° from the center of gaze, and slowly decrease in density farther away from the fovea (Lindsay & Norman, 1977). There are more rods in the side of the eye near the nose than in the side near the temple.

It is possible to investigate the distribution of rods and cones in one eye using a simple customized diagram and self-reports from subjects. The diagram consists of a clearly indicated point for the center of gaze. Subjects should direct their attention to this point. They should view this diagram from a prescribed distance and with one eye closed. Viewed in this manner, the point of gaze will be imaged on the fovea. Objects drawn on the diagram progressively farther away from the point of gaze are imaged at a greater distance from the fovea. The precise location in the eye can be calculated using basic trigonometry and information about the distance of the object from the point of gaze and the distance of the subject from the diagram (Figure 2). The angle between the point of gaze and the object is identical to the angle from the fovea to the image of the object on the retina. While subjects maintain their focus on the point of gaze, objects are uncovered and subjects are asked about the color of the uncovered object (note: hide the objects until the test). If the image of the object falls in an area of the eye where there are no cones, the subject will not be able to identify the color of the object (they will report a gray object). By creating a diagram with objects of predetermined colors drawn at specific distances and in precise orientations from the center of gaze, it is thus possible to map the locations of the cones sensitive to each color and of the rods.

The diagram must be specifically designed for each experiment (depending on the question investigated), and appropriate controls must be devised to ensure that subjects are keeping their gaze on the center spot. Some innovative students, who had devised a unique approach, were able to use the same technique to investigate cones at the periphery, to ensure that subjects were truthful in their responses. Appropriate controls will depend on the question investigated, and the students must identify these and include them in their study.

Using this set-up, and customizing their diagram, students can investigate a variety of questions about the distribution of rods and cones.

**Figure 1.** Summary of some of the brain’s visual processing centers. This diagram represents a human brain, viewed from the top. Light that enters the eye stimulates receptors at the back of the eye. The location and color of objects in the visual field can be obtained from these data. This information is relayed to cells in an area of the brain called the thalamus. There, some processing of the information takes place that allows the perception of contours and allows the detection of shapes and edges. This processed information is sent to the simple cells of the visual cortex in the occipital lobe (back of the head). At this location, lines of particular orientation and color are identified from the visual input. This information is conveyed to complex cells of the visual cortex, where movement of these lines is first recognized. This input then goes to other areas of the brain for increasingly complex analysis of the visual image, resulting in our visual perception of the world.
in the eye (Figure 3). The retina can be mapped in all directions around the fovea. The rod-and-cone distributions in the two eyes can be compared. The effects of experience on rod-and-cone distributions can be explored (e.g., comparing control, color-blind subjects, athletes who use peripheral vision, subjects with a “lazy eye”). Different groups (sex, age) can also be compared. Student-generated hypotheses should guide the design of each experiment.

**Research Project 2: Edge Detection by On-Center-Off-Surround Cells**

The next step in processing visual information involves the detection of shapes, or where objects begin and end. The brain identifies the edge of objects by identifying sharp contrasts between light and dark areas, or the juxtaposition of two areas of different colors. This type of processing takes place in brain cells called on-center-off-surround cells, which are found in several places in the brain, most notably in an area called the thalamus.

Each on-center-off-surround cell collects data from several photoreceptors from only one eye. Each of these cells compares the level (or color) of light in one central circular spot in the visual field with the level of light (or color) in a surrounding disk. If there is a difference between the two areas, the cell signals that it has perceived an edge or boundary (an abrupt change in the intensity or color of light in a short distance) and sends that data forward for further processing.

The Hermann grid is an optical illusion that relies on the properties of on-center-off-surround cells (Figure 4; Spillmann, 1994). In this illusion, subjects view a grid and report seeing illusory gray dots at intersections of the grid that are away from the center of gaze. On-center-off-surround cells that “look for contrast” between the lines (see Figure 4, area A) receive white light in their middle spot and dark bands on their surrounding disk. They therefore sense an edge and fire strongly. The effect is the perception of a strong contrast between the white lines and dark squares in that area. By comparison, the on-center-off-surround cells that look for contrast at the intersections (see Figure 4, area B) receive a lot of white light in their center spot, but they also receive a lot of light in their surrounding disk. As a consequence, the cell is not as strongly stimulated. The effect is the perception of less contrast between the light and dark areas of the diagram, which manifests itself as the perception of a shadow or gray circle at the intersections.

This effect is seen only in the periphery of vision because the acuity of visual perception is much more refined at the center of gaze. Hence, the area of the visual field to which an on-center-off-surround cell is sensitive is much smaller in the center of gaze than in the periphery. As illustrated by Figure 4, area C, at the center of gaze there is no difference in the level of activation of on-center-off-surround cells looking for contrast, amid cells attentive to contrast between lines and those attentive to the intersections. For more detailed explanations, see Schiffman (2000: p. 145).

The Hermann grid can be used to investigate many properties of the on-center-off-surround cells. By varying the color of the grid and lines, students can investigate which colors are contrasted by the cells to detect edges. Students may also wish to investigate whether off-center-on-surround cells exist by creating Hermann grids of reverse intensity (with white squares and black lines) and evaluating whether subjects report seeing white spots at intersections. By varying the size of the grid, it may be possible to determine the diameter of the area in which on-center-off-surround cells look for contrast at the center of gaze and in the periphery of vision. Students may investigate whether these cells receive input from one or two eyes by testing for the effect with one eye closed. Students may also like to hypothesize whether this effect can be detected when the grid is rotated, based on the properties of the on-center-off-surround cells.

**Research Project 3: Simple Cells of the Primary Visual Cortex**

Once the brain has detected edges, the data are forwarded to the primary visual cortex in the occipital lobe (at the back of the brain). There, simple
cells analyze the data and detect the presence of lines of specific color oriented in a particular direction. Each simple cell responds only in the presence of its preferred stimulus. Some respond best to green lines oriented at 30° from the horizontal axis, others to purple lines at 50°, and so on. These cells receive input from only one eye.

In 1965, Celeste McCollough devised an illusion based on the properties of simple cells (McCollough, 1965). In the McCollough effect, subjects move their eyes over a horizontal grid of alternating black and green bars, as well as a vertical grid of alternating purple and black bars. Following 10 minutes of adaptation to this stimulus, subjects are presented with a black-and-white stimulus composed of horizontal and vertical bars (note: the bar width is identical to that of the original stimulus; Figure 5). In the horizontal white bars, subjects report perceiving a faint purple color; in the vertical white bars, they report a faint green color. This image aftereffect is thought to be a result of neural fatigue (overstimulation) of simple cells of the visual cortex that respond specifically to green horizontal lines and purple vertical lines. When the stimulus is removed, the neurons exhausted by this stimulus recover and their activity is inhibited. The brain compares this activity to the activity of simple cells with preference for lines of similar orientation but complementary color and, sensing a stronger activation of the cells responsive to the complementary color, interprets the signal to imply the presence of lines of complementary color. For more information, see Stein and Stoodley (2006: p. 122).

Modified McCollough stimuli can be used to ascertain the properties of simple cells of the visual cortex (Figure 6). They can be used to determine whether simple cells receive their input from one eye or from both eyes (adapt subjects with one eye, and determine whether the effect can be detected with the other eye). This effect can also be used to ascertain which simple cells are compared in determining the color of a line (by recording the perceived illusory color following adaptation to stimuli of various colors). Other questions that can be investigated include how the brain interprets a signal where simple cells responding to similar orientation but complementary colors are exhausted simultaneously (a subject adapts to a stimulus consisting of bands of similar orientation but complementary colors – is an effect reported?). The effect can also be used to determine the range of orientations to which simple cells respond, by monitoring how much rotation of the final stimulus is tolerated while the effect is still reported. Students may also wish to investigate whether simple cells are sensitive to lines of particular width (adapting subjects with lines of specific width, and testing them with a stimulus containing lines of a different width).

**Research Project 4: Complex Cells of the Visual Cortex**

Complex cells receive input from simple cells. These cells process the visual information and detect the presence of lines of particular orientation, moving in a specific direction. This is the first step in motion processing. Many complex cells receive input from both eyes.

The waterfall illusion, or motion aftereffect, can be used to study the complex cells of the visual cortex (Addams, 1834). The waterfall illusion occurs when a subject adapts by watching lines moving in a fixed direction for several minutes. Upon viewing an immobile scene, the subject will report perceiving motion in the opposite direction. It is thought to result from overstimulation of complex cells. When the stimulus is removed, the fatigued complex cells are inhibited during their recovery. Comparing the level of activity of these cells with that of other complex cells responsive to lines of similar orientation but moving in the opposite direction, the brain interprets the signal to mean that the lines are moving in the opposite direction. There are several stimuli that can be used for this experiment. Addams (1834) first noticed the effect upon staring at a waterfall for several minutes. If waterfalls are not easily accessible, there are several online sites that can be used for this purpose (http://www.neave.com/strobe/ or http://www.michaelbach.de/ot/mot_adaptSpiral/index.html). Please note that subjects prone to epilepsy should refrain from viewing these stimuli, as they may trigger a seizure. The students should interview their subjects before testing them with these stimuli.

One of the properties of complex cells that can be assessed with this optical illusion is the length of adaptation required to exhaust a complex cell and see the effect (Figure 7). Students may wish to investigate whether complex cells receive their input from one eye or from both eyes. If they have the technical knowledge to construct their own
they may wish to test whether complex cells respond optimally to specific colors. Students may also wish to investigate whether caffeine, nicotine, age, or sex is correlated with the recovery time of subjects (how long the aftereffect lasts, or how long the complex cells are inhibited).

Classroom Management

To perform this activity, it is imperative that the students have sufficient background to understand, design, and interpret an experiment. At the beginning of the course, the students were introduced to the elements of experimental design, and they received a primer in statistical analysis. The differences between controlled and correlative studies were explained. The students were also introduced to the elements of a scientific paper: the writing style and the contents of each section. As this course included student-designed experiments every week, the students were given 9 hours of instructions on experimental design before delving into the specifics of developing their own experiment.

To ensure familiarity with the materials, the students were required to read a chapter in their textbook on visual processing in the human brain and were given a 2-hour lecture on this subject. They were then introduced to the neuropsychological tools available to them. Each optical illusion was described, and the neurological basis for its effect was explained. The students were also given sample ideas for questions they might wish to investigate. They were directed to the library, where relevant research articles were placed on reserve for their perusal.

The students gathered in groups of three to five and reached a consensus on their experimental design. To aid in their discussion, they were given a form on which to record their ideas. The information requested included:

- type of cell investigated
- optical illusion to use
- specific question to investigate and hypothesis
- detailed description of materials required
- description of controls
- description of experimental condition(s) (dependent and independent variables)
- number and description of subjects
- how the data will be collected
- how to ensure that the data are reproducible
- statistical analysis expected to be used (chi-square, t-test, ANOVA)
- what sort of tables and graphics they anticipate incorporating in their paper
- what data they expect and how this relates to the property of the visual cells under investigation

Once the students completed the form, each group met with the instructor to discuss the protocol suggested, receive feedback, and make corrections if necessary.

The students then prepared and conducted the experiment as designed. This was done as an out-of-class assignment, and their report was due at the start of the next class. The students reported their findings in the form of a research paper with the same format as an article in a scientific journal. Each student in a team wrote one section (Abstract and Introduction, Materials and Methods, Results, or Discussion). The students were assessed only on the section of the paper that they wrote, but the whole article had to be internally consistent. There were many experiments on other topics throughout this course, and the students had a chance to write each section of an article in turn.

Suggested Adaptations for High School Students

The first research project (mapping of the retina) requires some simple background information on the anatomy of the human eye and virtually no statistical analysis. It may therefore be the most appropriate for use with senior high school students. Note that visual processing in the brain is quite modular and takes place in a stepwise fashion. It is therefore possible to teach only a portion of the whole process. Each project described above relies on a portion of knowledge. An instructor could cover visual processing from retina to thalamus and use projects 1 and 2 only.

Alternatively, with some adaptations, these inquiry-based laboratories could serve as an introduction to experimental design. The instructor might select one of the research projects, present the relevant background, and propose a question to investigate. The instructor can then facilitate a whole-class, step-by-step discussion of an appropriate experimental design to study the proposed question. The students then break into groups and use the agreed-upon protocol to collect data. The data from all groups are pooled. The collated data can be analyzed by the whole class, in small groups, or as an individual assignment. This method of proceeding allows the students to guide the design process but enables the instructor to maintain control and may be more appropriate for novice experiment-designers.

The optical illusions for the Hermann grid, McCollough effect, and waterfall illusion could also be used to teach younger high school students about experimental design. To do this, the illusions could be used without mentioning the underlying biology. For example, students could investigate whether the inverted Hermann grid produces an optical illusion. Optical illusions tend to interest students, serving as a motivator to teach about experimental design. Buttemer (2006) and Flannagan and McMillan (2009) suggest models for introducing students to experimental design that are simpler to use than the experimental design form suggested above and adapted to a younger audience.

Evaluation of This Activity (Lessons Learned)

Most student groups are fairly adept at devising sound experiments. The meetings in which each group received feedback from the instructor on...
their experimental design before conducting the experiment were invaluable in ensuring sound design. The most common mistake was to alter more than one variable in each experimental condition compared with the control. While this is a serious experimental design flaw, it can serve as an opportunity to discuss the importance of testing for one thing at a time and is easily corrected. Most groups had considerable revisions to make following this meeting, but most incorporated the suggestions into a sound plan.

Students should be encouraged to test an appropriate number of subjects. Some experiments (particularly those utilizing the McCollough effect, which requires 10 minutes per subject) are time intensive. Students are often tempted to reduce the number of subjects. However, this strategy poses many challenges in detecting statistically significant differences between control and experimental groups.

Roughly half the groups reported their data but failed to infer the implications for the properties of the visual system. Individual team-instructor meetings following data collection should be conducted to ensure that the students understand how to analyze their data and to ensure that they link their results to properties of visual cells.

The majority of papers showed no evidence of research beyond the materials presented in textbook and in lectures. In the future, in-class time will be provided to explore the published literature for data relevant to the experiment.

Seventy-eight percent of the students felt that it was “exciting and motivating” to engage in a true research experience in which the results were uncertain, and only 20% felt frustrated because they couldn’t check their results against expected or known figures and data.

○ Conclusions
This activity meets the objectives of introducing students to genuine research on the properties of human brain cells using inexpensive and safe equipment. Students seem to appreciate engaging in genuine research and were not overly frustrated by the fact that there was not a “set way to proceed,” nor that their data could not be compared with previously established results. In the future, this assignment will be broken down into steps. On the first day, the students will be asked to survey the literature and begin to write the introduction of their paper. On the second day, the students will design their experiment and get approval from the instructor. On the third day, the students will bring their collected data to class, where they will proceed with data analysis and figure-generation. By providing more time and guidance, most of the difficulties encountered in this activity should be addressed and it should be even more effective at meeting its goals.

References

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