

RESEARCH METHODS

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RESEARCH METHODS

Animal Research

Most of our knowledge of the brain is based on scientific animal research. This CD describes findings obtained from animal experiments and presents a number of research methods commonly used to study the brain and behavior. The use of animals for research raises a number of ethical questions, yet at the same time such research has made enormous contributions to our knowledge of the anatomy and physiology of the brain. Animal research has helped, among other things, to discover the possible causes of mental and neurological diseases (such as manic-depressive disorder, drug addiction, and Parkinson's disease). Scientific discoveries made by animal experimentation have helped in developing treatments and drugs that alleviate human suffering, as well as the suffering of other animals.

Ethical issues of animal research are very complex. Researchers engaged in animal research are not oblivious to these issues. In most research institutions and federal agencies, animal research is highly regulated, performed under restrictions and supervision. Efforts are made to minimize the suffering of animals and to carefully consider the scientific benefit expected from each experiment.

Magnetic Resonance Imaging

MRI scans are based on the magnetic properties of the organic tissue. The *nuclei* of certain atoms in the brain (such as hydrogen atoms) behave like small magnets under the influence of a strong magnetic field and become aligned in the direction parallel to the force of the magnetic field. Radio waves are then passed through the magnetized tissue, which makes the atoms rotate in a uniform fashion. When the radio waves are turned off, the nuclei return to their initial magnetized alignment and emit radio waves of their own. The reflected radio waves differ from one tissue to another, according to the chemical composition of the tissues (for example, the amount of water in the tissue). The reflected radio waves are picked up by a magnetic detector surrounding the tissue and are translated by the computer into high-resolution brain

sections. Unlike Computerized Tomography (CT) sections, which are limited to the horizontal plane, MRI sections can also be obtained in the sagittal or coronal planes. MRI scans can generate extremely detailed brain images with a precision close to that of actual brain sections. This scanner is used for studying the structure of the brain, and for identifying brain tumors and *lesions*. In certain cases, an MRI scan can help reach a decision regarding surgical or chemotherapeutic brain intervention. This scanning method is noninvasive, does not involve the introduction of external markings (such as stains or radioactive elements), and does not cause the subject any damage.

Sagittal Sections

The video clip presents a sequence of two-dimensional sagittal brain sections obtained by an MRI scan of a human brain. The sequence begins at the left side of the head (left ear) and proceeds toward the right side (right ear), showing the head and the brain. In a midsagittal section (which splits the brain into two symmetrical hemispheres), one can clearly see the contours of a face, the cortex, *corpus callosum*, *brain stem*, *cerebellum*, and part of the *spinal cord*.

(Sequence of sections taken from Sundsten, J.W., Ph.D,(1994), *The Digital Anatomist: Interactive Brain Atlas*. University of Washington, Seattle, Washington.)

Coronal Sections

The video clip presents a sequence of two-dimensional coronal brain sections obtained by an MRI scan of a human brain. The sequence begins at the back of the head and proceeds toward the forehead, showing the head and the brain. First seen are the cortex and *cerebellum*. Two black regions then appear in the cortex—these are the *dorsal* horns of the lateral brain *ventricles*. Toward the forehead, the third ventricle appears in the midline below the lateral ventricles. From this point forward, the lateral ventricles draw further apart. Finally, we see the eye orbits and eyeballs.

(Sequence of sections taken from Sundsten, J.W., Ph.D,(1994), *The Digital Anatomist: Interactive Brain Atlas*. University of Washington, Seattle, Washington.)

Midsagittal Section

The image presents a midsagittal section of the human brain obtained by an MRI scan. The section shows medial structures, including the corpus callosum (a white semi-crescent shaped strip), fornix (another thin strip linked to the corpus callosum), and *thalamus* (ventral to the fornix). In lower areas of the brain, one can clearly see the cerebellum, brain stem (particularly the pons), and the beginning of the spinal cord.

(Sequence of sections taken from: Sundsten, J.W., Ph.D,(1994), *The Digital Anatomist: Interactive Brain Atlas*. University of Washington, Seattle, Washington.)

MRI Apparatus

The MRI apparatus comprises a series of cylinders surrounded by magnetic rings that generate a powerful electromagnetic field (60,000 times that of Earth). The subject lies on a table and is placed into the inner cylinder (the CP body coil). When the subject's head is introduced into the strong magnetic field, certain atomic *nuclei* in the head are magnetized and become aligned in the direction parallel to the force of the magnetic field. At this stage, the inner cylinder sends a radio frequency signal through the head and the external cylinders (the gradient coils) to direct the radio waves according to the desired section plane—*horizontal*, *sagittal*, or *coronal*. When the radio waves are turned off, the nuclei return to their initial magnetized alignment and emit radio waves of their own. The reflected radio waves are picked up by a magnetic detector surrounding the tissue and are translated by the computer into high-resolution brain sections. The scanner is usually tuned to detect the radiation (radio waves) of hydrogen atoms, as these atoms produce magnetic resonance very easily and are widely spread in the brain tissue as part of the ubiquitous water molecule. Since the concentration of water differs from one tissue to another, the MRI scanner can distinguish among different types of tissues. For example, it can distinguish between the brain [gray matter](#) and white matter.

(Illustration courtesy of MRI Division, Elscint Inc., Haifa, Israel.)

Functional Magnetic Resonance Imaging—fMRI

The functional MRI (fMRI) technique is based on the fact that brain activity increases the blood flow and oxygen consumption in the active tissues. Consequently, the level of blood oxygenated hemoglobin rises in such tissues. The MRI apparatus detects this change, as the paramagnetic properties of oxygenated hemoglobin differ from those of deoxygenated hemoglobin (hemoglobin that is not linked to oxygen). The fMRI technique is noninvasive, does not involve the introduction of external markings (such as stains or radioactive elements), and does not cause the subject any damage. It provides precise imaging, both in spatial resolution (within a range of a few millimeters) and in temporal resolution (within seconds), and is therefore currently considered to be the leading technique in brain imaging.

The video clip demonstrates how associative areas within the *ventral* visual pathway participate in the identification of complex figures. A subject's brain is scanned by an MRI scanner while the subject is looking at visual stimuli presented on a computer screen. The coronal MRI section shows the posterior part of the brain. In the lower part of this section one can see the *cerebellum*; at the center are the primary visual areas, while on both sides are the ventral associative visual areas in the lateral [occipital lobe](#). Superimposed on the MRI section are fMRI scans. The increased oxygen consumption in the activated tissue is represented in the clip by blotches of color that appear across the brain section (the brighter, white-yellow hues denote the highest level of brain activity). The subject is presented with two alternate types of stimuli: a grid of black and white lines in different orientations or a figure-like image at the center of the screen. Looking at the grid of lines mainly activates the central areas of the occipital lobe (areas V1, V2, and V3), while looking at the figure-like image mainly activates the lateral and inferior parts of the occipital lobes (areas V4 and LO). Thus, primary and secondary visual areas mainly react to simple lines with sharp contrast, while higher associative areas mainly respond to visual stimuli such as faces, figures, objects, and parts of objects.

(Imaging clip based on research data provided by Malach, R., & Grill-Spector, K., Weizmann Institute of Science, Rehovot, Israel; and by Kushnir, T., and Yaakov, I., The Chaim Sheba Medical Center, Israel.)

Computerized Tomography (CT)

CT (Computerized Tomography) scans are x-ray images that measure fine differences in the density of brain tissues. The scan produces a series of two-dimensional horizontal brain sections called tomograms. The scan allows the researcher to differentiate between brain areas of differing density, such as gray matter and *white matter*, and to identify brain convolutions and subcortical structures at a resolution of 1 mm.

CT scans are often used in cases where pathological brain conditions are suspected, such as tumors, lesions, blood clots, hydrocephalus, and degenerative diseases. CT scans allow physicians to determine if brain surgery is required. This method is noninvasive and does not cause damage to the subject, as the exposure to x-ray radiation is relatively small and short.

Horizontal CT Scans

The video clip presents a sequence of two-dimensional horizontal brain sections obtained by a CT scan of a human brain. The subject is facing the bottom of the screen. The sequence begins at the top of the head and proceeds toward the neck, showing the head and the brain. As the scan progresses downward, one can see the cortical folds and the lateral brain *ventricles* (resembling a dark butterfly shape). In the last section one can see the nose and eyes (at the bottom of the image), and part of the *cerebellum* (at the top of the image).

(Series of brain sections courtesy of CT Division, Elscint Inc., Haifa, Israel.)

CT Apparatus

This video clip shows the table on which the patient lies, surrounded by a large ring into which the patient's head (or body) is introduced. A source in the tube projects x-rays that pass through the head and are picked up by detectors at the opposite side of the scanner. The amount of radiation that passes through the patient's head varies according to the density of the brain tissue. Thus, CT scans are based on

measurements of fine differences in the density of brain tissues by the x-ray detectors. After the brain has been scanned at one position, the radiation source and the detectors are rotated by a few degrees, and the radiation passing through the head is measured again. This process is repeated again and again until the entire brain is scanned. The information recorded by the detectors is translated by a computer into a two-dimensional image at the horizontal plane. After this image has been generated, the patient's head (or body) is moved in or out through the ring and a different horizontal brain section is generated. In this way, a series of horizontal sections is obtained for the entire brain.

(Simulation clip courtesy of CT Division, Elscint Inc., Haifa, Israel.)

Positron Emission Tomography (PET)

Positron Emission Tomography (PET) is an imaging technique used to identify metabolic changes correlated with neural activity. A small and harmless dose of radioactive substance, such as 2-DG, is injected into the subject's circulation. The radioactive substance is carried by the blood into the brain and accumulates in active brain areas. Because the 2-deoxyglucose (2-DG) molecule resembles glucose (the primary metabolic fuel of the brain), it is taken up by active neurons – the more active neurons, require more glucose, and thus will take up more 2-DG. But unlike glucose, 2-DG cannot be metabolized, therefore it stays in the cell. As a result, the more active areas become more radioactive. Since the radioactive material is unstable, it rapidly decays by emitting a positron from its atomic *nucleus*. When a positron collides with an electron two photons or gamma-rays are emitted, and this energy is recorded by special detectors. A computer determines the radiation level in every brain region and translates it into a color-coded image of the *horizontal* section. Areas of high activity appear as red or yellow patches, while areas with lower activity appear blue or purple.

The PET technique allows for spatial resolution of approximately 4 to 8 mm. However, its temporal resolution is lower in comparison to the fMRI (functional Magnetic Resonance Imaging) and EEG (Electroencephalogram) imaging techniques. The PET scan produces images of brain activities, such as sensory perception, motor planning, verbal behavior, learning, and decision making. The PET scan also aids clinical diagnosis as it can help in detecting lesions and tumors in the brain. In

addition, this technique is used to identify neurological diseases such as Parkinson's and Alzheimer's diseases.

Data Analysis

The PET technique isolates brain activities related to a particular experimental task (such as reading) from background brain activities. To accomplish this, PET scans are carried out during the experimental condition, as well as during a control condition. The control condition is identical to the experimental condition in all possible aspects, except for the experimental task itself. In the illustration shown, brain activity was examined in response to presentation of visual stimuli. Under the experimental condition, subjects were asked to focus their gaze on a certain point, where the visual stimuli (flickering checkerboards) were presented. Under the control condition, subjects focused their gaze on the same point, but were not presented with any visual stimuli. In order to isolate brain areas showing activity associated with visual perception, a computer program is used to subtract the control scan from the experimental scan (see top of illustration). The resulting brain image indicates brain activity in the occipital lobes, which include the visual cortex (red-yellow patches in the posterior part of the brain scan). The scans of the control condition were subtracted from the scans of the experimental condition for every subject (see middle row). Finally, the mean difference image for all subjects was calculated (bottom row). (Picture courtesy of Posner & Raichle (1994). "Image of Mind." Scientific American Library. Page 65. By kind permission of Prof. Marcus E. Raichle, Washington University School of Medicine)

Verbal Activity

The illustration presents PET scans of active brain areas during various verbal tasks. The images are presented on a schematic diagram of the cortex, as viewed from the side. As can be seen in the images, passively viewing words activates the primary visual areas in the *occipital lobe*. Listening to words activates the primary auditory areas and Wernicke's area in the *temporal lobe*. Speaking words (repeating words that were presented to the subject) activates motor areas in the frontal lobe, such as the

primary motor cortex and the associative motor cortex. Finally, generating verbs associated with the experimental stimuli activates [Broca's area](#) in the frontal cortex, which is important for verbal production, and Wernicke's area in the temporal lobe, which is associated with comprehension of verbal language.

(Picture courtesy of Posner & Raichle (1994). "Image of Mind." Scientific American Library. Page 115. By kind permission of Prof. Marcus E. Raichle, Washington University School of Medicine.)

PET Apparatus

Positron Emission Tomography (PET) is an imaging technique used to identify metabolic changes correlated with neural activity. The PET technique is based on the injection of a radioactive substance, such as 2-deoxyglucose (2DG), into the subject's circulation. It should be noted that the amount of radioactive 2-DG used during the scan is not harmful. After the material is injected, the subject is laid on a table and his/her head is introduced into the PET scanner. The radioactive substance is carried by the blood into the brain and accumulates in active brain areas, where the metabolic rate is higher. As a result, these areas become more radioactive. Since the radioactive material is unstable, it rapidly decays by emitting a positron from its atomic *nucleus*. When a positron collides with an electron two photons or gamma-rays are emitted, and this energy is recorded by special detectors. A computer determines the radiation level in every brain region and translates it into a color-coded image of the horizontal section. Areas of high activity appear as red or yellow patches, while areas with lower activity appear blue or purple.

Electroencephalogram (EEG)

The EEG technique records the electrical activity of thousands or millions of neurons in the [cortex](#) by placing macroelectrodes over the subject's head. A number of recording [electrodes](#) are placed over the scalp, while a reference electrode is placed at some distance from the recording electrodes, on the nose or forehead. The EEG waves are recorded by a pen oscillograph (a polygraph) or presented on a computer screen. Each recording represents the potential differences between the recording electrode

and the reference electrode. The EEG technique is used for recording various mental states, such as wakefulness, drowsiness, and stages of sleep. It is also used for identifying brain tumors or for clinical diagnosis of epilepsy and coma. The EEG is a noninvasive technique, and it is characterized by high temporal resolution but low spatial resolution.

Brain Electrical Activity Mapping Technique

The EEG technique is used to record event-related potentials (ERPs), which are changes in brain electrical activity related to specific stimuli, such as a sound or a flash of light. A significant feature of ERPs is that they provide the precise temporal record of underlying neural activity.

In recent years, a number of computerized methods have been developed to improve the spatial resolution of the EEG technique. One such method is the Brain Electrical Activity Mapping technique (BEAM), which combines a three-dimensional model of the scalp (obtained by MRI) with recordings of ERPs.

The illustration examines cortical activity during performance of different memory tasks. During the high load, or difficult, memory task, subjects were asked to decide if the stimulus on each trial matched either the verbal identity or the spatial location of a stimulus occurring three trials previously. During the low load, or easier, memory task, only the verbal or spatial location of the first stimulus had to be remembered. Increased activation of the left frontal cortex was observed during a difficult memory task (right) in comparison to the easier memory task (left).

(Picture taken from Toga & Mazziotta (Eds.) (1995). "Brain Mapping Methods."
Figure 7. By permission of Academic Press, Inc., Orlando, FL.)

EEG Recording During Wakefulness and Sleep

Based on characteristic EEG activity, it is customary to distinguish between two states of wakefulness (alertness and rest) and five stages of sleep (stages 1–4 and REM

sleep). The illustration presents characteristic EEG activity during the various states of wakefulness and sleep:

1. States of Wakefulness

Alertness – When a person is alert and attentive, usually with open eyes, the typical EEG is characterized by beta activity, which is characterized by high frequency, greater than 15 Hz (cycles per second) low voltage (low amplitude) waves.

Rest – When a person is relaxed, resting quietly with closed eyes, the typical EEG records alpha activity, which is characterized by medium frequency (8–12 Hz) and medium voltage waves.

2. Stages of Sleep

Stage 1 – As a person goes from drowsiness to sleep, alpha waves give way to theta waves, which are slower (4–7 Hz).

Stage 2 – This stage is characterized by theta waves, K complexes, and sleep spindles. The K complexes and sleep spindles appear to decrease the brain sensitivity to sensory input during sleep.

Stages 3 and 4 – As a person goes from light sleep to deep sleep, the typical EEG is characterized by delta waves, which are characterized by slow frequency (less than 4 Hz) and very high voltage.

Rapid Eye Movements (REM) Stage – During REM sleep the EEG is characterized by beta waves in combination with alpha waves. There are also bursts of theta wave activity. This stage is also characterized by fast movements of the eyeballs underneath the closed lids, by a significant decrease of muscle tonus, by autonomic arousal, and by dreaming.

Optical Imaging

Optical imaging is based on a video recording of changes in cortical blood flow in the exposed cortex of a living organism. High brain activity is associated with increased blood flow, expanded blood vessels, and an elevated cellular oxygen consumption.

These changes are recorded by a video camera and are converted into “activity maps.” The map shows the areas of increased brain activity as dark blotches on the surface of the brain cortex (see the “Orientation Pinwheels” video clip). It is also possible to produce a color-coded activity map, where each color represents increased brain activity in response to a different stimulus (see “Orientation Pinwheels” illustration)

Optical imaging is used to investigate cortical activity in experimental animals. It provides the greatest level of spatial resolution (down to 30 microns). The technique has recently been used to scan functional activity in the human cortex during brain surgery.

Orientation Columns

This experiment demonstrates the existence of orientation columns in the primary visual cortex. The images are based on a camera recording of changes in blood flow reflected from the cortex when the monkey is watching a line in various orientations on a computer screen (represented by a purple line in the upper-right-hand corner of the screen). When the monkey observe the line, neurons sensitive to its orientation are activated. This activity is associated with dilation of blood vessels and increased oxygen supply, which appears in the clip as dark blotches over the surface of the cortex. When the orientation of the line is slightly changed, adjacent cells sensitive to the new orientation are activated, and a new pattern of dark patches is seen over the cortex. During the experiment, the orientation of the line is changed in a circle. The results show that each orientation of the line activates a group of cells. These groups are spatially arranged in a pinwheel pattern. The video clip presents three such pinwheels (delineated by three purple circles) that rotate around their centers (purple points).

(Video clip provided by Grinvald, A., and Bonhoeffer, T. Weizmann Institute of Science, Rehovot, Israel.)

Orientation Pinwheels (image)

This illustration presents an optical imaging recording of the primary visual cortex of a monkey. The imaging is based on a camera recording of changes in blood flow reflected from the cortex while the monkey is watching lines in various orientations. Cells that respond to a particular orientation are recorded by the camera and are then assigned a certain color. Each color represents a cluster of cells sensitive to a specific line orientation (see legend along the side of the picture). The different colors are

arranged in a pinwheel pattern. Each of the pinwheels contains cells for all possible line orientations, and adjacent segments in each pinwheel react to adjacent line orientations (see magnification). Note that each orientation is represented only once within a pinwheel.

(Image courtesy of Grinvald, A., Weizmann Institute of Science, Rehovot, Israel.)

Confocal Microscope

The confocal microscope allows detailed scanning of cells and cellular structures at a very high level of spatial resolution. Unlike the optical microscope and the electron microscope, which are limited to a two-dimensional inspection of thin tissue samples, the confocal microscope makes it possible to inspect tissue slices more than 100 microns thick. The advantage of this microscope is in its ability to produce optical sections at various tissue depths, thereby providing a three-dimensional image that can be used to study the spatial and functional connections between individual neurons. The confocal microscope makes it possible to observe the structures of *axon* branches, [synaptic clefts](#), and [dendrite](#) branching. Long-term scanning (in living tissues preserved under special conditions) can even show dynamic cellular processes, such as the growth of axon branches or the migration of cells at early stages of brain development.

Single Cell

The following is a three-dimensional video clip of an astrocyte (star-like) cell, stained in fluorescent yellow and scanned by a confocal microscope. Astrocytes are a type of *glia cell* that provide neurons with physical support within the central nervous system. Astrocytes remove brain waste products and produce a number of chemicals that are required for neural functioning. They are thought to scavenge excess neurotransmitters and ions from the spaces between neurons. They may also contribute glucose to very active neurons and redirect blood flow to especially active regions. Some astrocytes participate in the decomposition and removal of damaged neural tissues, and in the formation of scar tissue surrounding the damaged area.

(Scans courtesy of Chiaia, N.L., & Rhoades R.W., Medical College of Ohio, Toledo, OH. Used by permission of E.D. Feijter, Meridian Instruments, Inc.)

Groups of Cells

Shown here is a three-dimensional video clip of a group of astrocyte cells. Astrocytes are a type of *glia cell* that provide neurons in the central nervous system with physical support. Thanks to the ability of the confocal microscope to focus on layers at various depths in the sample tissue, it is possible to visualize spatial relationships among the astrocytes.

(Photograph courtesy of Chiaia, N.L., & Rhoades R.W, Medical College of Ohio, Toledo, OH. Used by permission of E.D. Feijter, Meridian Instruments, Inc.)

Confocal Microscope Apparatus

To prepare for examination with the confocal microscope, the sample tissue is stained with fluorescent markers. A laser beam is then aimed at the tissue and focused on some depth of the sample by the objective lens of the microscope. The point of focus defines a confocal plane. The laser beam strikes the tissue, causing it to reflect fluorescent light of different wavelengths. This light goes through the lens and strikes the beam splitter, which directs the light toward a light detector. The light passes through a pinhole, which allows only the rays that arrive from the confocal plane (continuous lines) to pass through. Rays reflected from other depths within the tissue or from out-of-focus points (broken lines) do not reach the detector. This mechanism allows examination of specific depth layers of tissue in a series of confocal planes. Computer image analysis is then used to compose these sections, producing a three-dimensional reconstructed image of the tissue.

Stereotaxic Apparatus

Stereotaxic surgery is used to insert an [electrode](#) or cannula (a thin tube) into a specific brain region, deep in subcortical brain areas, avoiding considerable damage to

other brain tissues. This procedure is performed by means of a stereotaxic apparatus and a stereotaxic atlas.

The stereotaxic apparatus includes a head holder, which keeps the animal's head in a certain position. It also includes a holder for the electrode or cannula, and a mechanism that moves the electrode holder in measured distances along the three axes: anterior-posterior, *dorsal-ventral*, and lateral-medial. Stereotaxic surgery is employed for many procedures, including recording electrical activity, electrical stimulation, administration of chemical substances, and sampling of extracellular fluid in specific subcortical areas. Stereotaxic surgery is also used in human patients when surgical intervention at a precise subcortical region becomes necessary. For example in Parkinson's disease, [lesions](#) of a certain subcortical area (globus pallidus of the *basal ganglia*) can reduce the severe tremors associated with the disease. A stereotaxic atlas is used in order to determine the coordinates of the brain area under investigation. Such surgery is performed in humans using a stereotaxic apparatus, and only after the location of the inserted electrode has been verified by a CT scan.

Stereotaxic Atlas

The following illustrations were taken from a stereotaxic atlas. The illustration on the left presents a rat skull, in which the "sutures" of the cranial bones are clearly visible. These sutures are characteristic of every species, and their points of intersection are used as reference points for the stereotaxic atlas. This illustration presents a certain reference point known as the bregma.

The illustrations on the right describe a [coronal](#) section of a rat brain, located 2.3 mm posterior to the bregma. The lower illustration is a coronal section, while the upper illustration portrays a schematic representation of the section, on which the main brain areas are marked. An area known as the lateral hypothalamus (LH) is indicated with orange in both illustrations. Electrical stimulation of the LH activates the reward system (see the "Self-Stimulation" video clip in the "Electrical Brain Stimulation"). In order to insert an electrode into the LH, the electrode holder must be placed 2.3 mm posterior to the bregma, and 1.8 mm to the right of the midline (see the orange

plus sign on the rat skull in the left illustration). The electrode is then lowered to a depth of 8.8 mm from the cranium surface, into the LH area.

(Images taken from Paxinos & Watson (1986) “The Rat Brain in Stereotaxic Coordinates., 2nd ed., Academic Press. Plate 27 and Figure 27 (Interaural 6.70 mm, Bregma - 2.30mm). By permission of Academic Press, Inc., Orlando, FL.)

Electrical Brain Stimulation

Electrical stimulation involves the passing of a weak electrical current through an *electrode* inserted into the brain. Stimulation of an awake animal, moving freely in the cage, often induces behavioral changes in response to the activation of certain brain area. For example, the reward system can be experimentally excited by electrical stimulation of certain brain areas, including the *nucleus accumbens*, *ventral* tegmental area, and lateral hypothalamus (LH). Activating the reward system provides the animal with a sense of pleasure, and plays an important role in learning and motivational processes. The system is naturally activated by motivational behaviors, such as eating, drinking, or copulating. It is also called the reinforcement system because performing a certain behavior (e.g., eating) in a certain state (hunger) can activate the reward system, increasing the probability of performing the same behavior in similar states in the future.

The reward system can be experimentally activated by electrical stimulation of certain brain areas. In a certain experimental procedure the animal triggers the brain stimulation by pressing a lever, causing a weak electrical current to flow into the electrode implanted in its brain. This procedure is called self-stimulation (see the “Self-Stimulation” video clip).

Self-Stimulation

The video clip shows a rat with an electrode implanted in the lateral hypothalamus (LH). The electrode is connected to an electrical cable, which is connected on the other side to the stimulation apparatus. The animal can freely move about the cage. By accident, the animal presses the lever at the far end of the cage, which activates

the stimulation apparatus and stimulates the LH. Each time the lever is pressed and a current is passed, a red light goes on in the stimulation apparatus. The electrical current excites the LH area and activates the reward system, which in turn reinforces the rat's behavior. The rat presses the lever continuously at a constant rate, sometimes at the expense of other vital behaviors, such as eating and drinking.

Microelectrode

A microelectrode is a fine conductive element that makes it possible to record the activity of a single neuron (or a number of neurons) in a laboratory animal.

Microelectrodes consist of fine metal wires or a very thin glass tube that contains a conductive liquid such as potassium chloride. The fine electrode tip is inserted into the target brain area, while the other end is connected to an amplifier. Another instrument converts the neural signals into visual signals and displays them on an oscilloscope, a computer screen, or a paper strip. The neural signals can also be converted into an auditory output, which makes it possible to hear the firing rate of a cell.

Using microelectrodes is important for the study of how the single neurons respond to the presentation of specific stimuli. In addition, microelectrodes make it possible to record neural activities in brain tissues removed from the brain and maintained in a nutrient medium, where they can survive for a few hours. For example, it is possible to isolate a particularly large *axon* (such as may be found in squids) and measure its electrical activity.

Complex Cell

The video clip presents a recording of the activity of a complex cell in the primary visual cortex of a cat. The cell's response to a moving bar of light is recorded by means of a microelectrode. The neural activity (action potentials) is translated into auditory signals, so that each action potential is heard as a single shot, while a sequence of action potentials sounds like a barrage.

In the first stage of the experiment, the receptive field of a complex cell is identified and marked. A bar of light with a given orientation is swept across the receptive field, and the cell's response is recorded. The area where the cell responds to the visual stimulus is delineated with lines forming a rectangle. In the second stage, an arrow marks the selective direction to which the cell responds maximally. Note that the cell is totally unresponsive to movement in the opposite direction. Following this, the reaction of the cell to various lengths of the light bar is examined. The cell does not respond to light bars with the preferred orientation extending over the boundaries of its receptive field (end-stopping). This may be explained in the following way: Three complex cells with similar receptive field properties lined up one below the other converge on another complex cell (the recorded end-stopping cell). The central cell (of the three) is excitatory, while the two on both sides are inhibitory. Activation of all three cells inhibits the recorded cell. However, when at least one end of the light bar is within the receptive field, the inhibition is reduced and the cell responds once again. Thus, the recorded complex cell is sensitive to orientation, specific movement direction, and to ends of the line within its receptive field (end-stopped).

(Clip courtesy of Hubel, D. H., Harvard Medical School, Boston, MA; and Wiesel, T. N., Rockefeller University, NY.)

Histology

The brain's soft, grayish tissue makes it difficult to discern details. In order to investigate the structure of the brain tissue, it must be preserved and stained. The tissue is usually fixed with formalin in order to prevent decomposition by bacteria or molds. After preservation, the brain is either frozen or immersed in another stabilizing material such as paraffin, making it easier to cut the tissue into thin slices. The slices are placed into culture dishes under a chemical hood, as shown on the screen. The chemical hood protects the researcher from exposure to harmful substances, such as certain staining agents. The air in the hood is circulated through a special filter that absorbs the harmful substances. The brain slices are placed on microscope slides, dried, and prepared for staining. Depending upon the purpose of the staining (viewing cell bodies, *Myelin sheaths*, neural connections, etc.) different histological stains are used.

Golgi-Cox Stain

The Golgi-Cox stain selectively stains individual cells. Using this stain, which contains silver metal salts, it is possible to track neural *dendrites* and *axons*, as well as their connections with other neurons. The photograph shows a pyramidal neuron from the primary visual cortex of a monkey viewed through a confocal microscope. (Images provided by Malach, R. & Harel, M., Weizmann Institute of Science, Rehovot, Israel.)

Myelin Stain

The myelin stain is achieved by the accumulation of stain pigments in the myelin sheath. Using this stain, it is possible to identify tracts of large *axons*. This technique, however, does not enable the researcher to trace specific axon fibers. The photograph shows part of the primary visual cortex of a monkey (1x1 mm). The neural fibers (darker areas) were stained by the myelin glyas pigment. (Images provided by Malach, R.,& Harel, M., Weizmann Institute of Science, Rehovot, Israel.)

Biocytin Stain

The biocytin stain is injected into the target area while the organism is still alive. The stain is absorbed in the dendrites and transported by fast anterograde axoplasmic transport to the *terminal boutons*. After the stain has spread through the neurons, the animal is sacrificed and the stained tissue examined. Using biocytin it is possible to trace the connections made by an individual neuron with other neurons. The photograph shows interconnections in layers II–III of the primary visual cortex of a monkey. (Images provided by Malach, R., & Harel, M., Weizmann Institute of Science, Rehovot, Israel.)

Nissl Stain

The Nissl stain is used to stain cell *somas*, especially nucleic acids (DNA, RNA) and related proteins within the soma and cytoplasm. Nissl stains make it possible to identify clusters of cell bodies (nuclei) in the brain. The drawback of this method is that it is not selective for neuron cell bodies, but rather stains all cell somas of neurons and *glia cells*. The investigator must determine which cell is which, according to size, shape, and location. The photograph shows a section of a monkey's primary visual cortex. Cell bodies of cortical cells can easily be seen as purple areas adjacent to brighter areas of unstained *axon* fibers.

(Images provided by Malach, R., & Harel, M., Weizmann Institute of Science, Rehovot, Israel.)

Horseradish Peroxidase Stain (HRP)

HRP stain is an *enzyme* produced from horseradish that is injected into the target tissue while the organism is still alive. This stain is absorbed by the *terminal boutons* and is transported by retrograde axoplasmic transport to the cell **somas**. After a few days, the HRP is oxidized into certain colors, ranging from black to orange. The animal is then sacrificed, and the stained tissue is sliced and examined. Using this technique, it is possible to trace neural pathways. The picture shows a frontal section of a cat's brain. The purple stain designates the injection site into the primary somatosensory cortex. From this area, the stain was transported to the secondary somatosensory cortex on the same side (lower branch on the right), *thalamus* (lower branches in the center), and through the *corpus callosum* (which looks like a thin white line), to the secondary somatosensory cortex in the **contralateral hemisphere**.

(Images provided by Malach, R., & Harel, M., Weizmann Institute of Science, Rehovot, Israel.)

Fluorogold Stain

Fluorogold stain is injected into a living organism. The stain is absorbed by the *terminal boutons* and is transported by retrograde axoplasmic transport, back into the

cell *somas*. The animal is subsequently sacrificed and its brain sliced thin. When exposed to ultraviolet light the fluorogold stain in the cell somas emits light. The photograph shows a horizontal section of the primary visual cortex of a rat. Neuron somas are clearly visible as white blotches connected by an axonal network.

(Images provided by Malach, R., & Harel, M., Weizmann Institute of Science, Rehovot, Israel.)