Nonhuman Primate Models of Visually Based Cognition

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Introduction

Perhaps the deepest mysteries facing the natural sciences concern the higher functions of the central nervous system. Scientists in the field of systems and cognitive neuroscience address these issues with an interdisciplinary mixture of techniques derived from cognitive psychology, neurophysiology, computational modeling, and animal behavior. For investigators in this field, the ultimate goal is to understand the neural processes underlying perception, memory, learning, emotion, decision making, communication, and planning—in short, to understand the biological basis of mental life. Success in this effort will illuminate the sources of normal cognition and behavior, as well as provide an essential base of information for understanding and treating the many neurological and psychiatric diseases that devastate the mental and behavioral capacities of afflicted persons.

An essential approach to understanding the brain and its cognitive abilities is the conduct of neurophysiological experiments in awake, behaving animals. The neural mechanisms underlying high-level cognitive functions are investigated most inclusively in animals as they actually perform mental operations. Many basic problems in cell biology can be investigated in strains of cells grown in tissue culture. Higher brain functions such as perception, memory, and planning do not occur in tissue culture, however, and must therefore be investigated in functioning organisms. Another popular approach to investigating complex systems, computational modeling, can contribute to the study of brain function by clarifying the sorts of computational algorithms that may be implemented in networks of neuron-like elements. Eventually, however, many models can be proposed to account for any given mental phenomenon, and the only way to determine which (if any) is correct is to obtain experimental data from the functioning central nervous system. Ultimately, then, we must be able to study signal processing inside the brain while the brain produces intelligent behavior.

Of the experimental preparations currently available for studying cognitive function in alert animals, the most powerful and versatile is the awake, behaving monkey, used originally in the laboratory of Herbert Jasper (Jasper and others 1960) and developed extensively by Edward Evarts and his colleagues at the National Institute of Mental Health (Evarts 1966, 1968). Macaque monkeys can be trained with operant conditioning techniques to perform a wide variety of simple cognitive tasks such as perceptual discrimination, object recognition, short-term memory, attentional priming, and sequential motor planning, among many others. Furthermore, macaques will perform these tasks for several hours each day, in exchange for positive rewards, while experimenters monitor the electrical activity of individual nerve cells by means of small microelectrodes positioned at known locations within the brain. The tips of the microelectrodes are only a few microns in diameter and are invisible to the naked eye. The electrodes are made of metal, usually tungsten or platinum, and insulated to within 10 to 20 μm of the electrode tip. By advancing the electrode tip very slowly through the brain using a hydraulic or motor-driven microdrive, an experimenter can pick up electrical signals from one neuron after the other, allowing the experimenter to study the activity of individual cells while the monkey performs a behavioral task of interest. Typically, electrical activity as well as records of the animal's behavior are monitored automatically by a computer during the experiment and stored on a magnetic disk for subsequent off-line analysis. Microelectrodes in the brain cause the animals no discomfort because the brain lacks primary pain sensors ("nociceptive" fiber endings). At any given time, in fact, recording electrodes are implanted in thousands of humans around the world as a conjunct to therapy for epilepsy, Parkinson's disease, or chronic pain (Behrens and others 1994; Bushnell and others 1991; Gross and others 1997; Krack and others 1998; Long 1998; Young and Chambi 1987). (Practical issues concerning animal health and care are discussed in detail below.)

The ability to record neural activity simultaneously with behavior presents a rich opportunity for experimental analysis of the neural basis of mental function. A task can be made progressively more difficult or easy, and the experimenter can observe accompanying changes in activity at the neural level. Similarly, the experimenter can modify electrical activity in local groups of neurons and observe the behavioral effects of the manipulation. (Neural activity is modified either by inactivating local groups of cells with injected pharmacological agents or by exciting local groups of cells with electrical microstimulation.) In this manner, the investigator can explore the interplay between neural activity and behavior in an attempt to understand how cognitive functions arise from the coordinated activity of systems of neurons.

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A large proportion of the experimental investigations involving awake, behaving monkeys focus on the visual system. Vision is of paramount importance in primate behavior, and the visual system of the macaque is strikingly similar, both anatomically and functionally, to that of humans (DeValois and others 1974; Engel and others 1997; Gekht and others 1941; Harwerth and Smith 1985; Myerson and others 1981; Robinson 1968; Shipp and others 1995; Tootell and others 1996). Thus, studies of the macaque visual system have generated important new information that is directly relevant to understanding the human visual system. Importantly, our extensive knowledge base concerning the visual system makes it the best available experimental system for investigating higher cognitive functions such as attention, memory, and motor planning. As we shall see in subsequent sections, the most revealing experiments concerning the neural basis of such functions employ manipulation of visually based attention, visually based memory, or visually based motor planning.

While the awake, behaving monkey preparation is powerful, its applicability to understanding human cognition is not nearly universal. Humans have many advanced cognitive capacities that are, by comparison, primitive or totally absent in monkeys: language, abstract representation and association, planning far into the future, self-awareness, and number manipulation, to name only a few. Ultimately, then, some distinctly human cognitive functions can be studied only in our own species.

In this article, we review the use of nonhuman primates in studies of simple forms of visually based cognition. To illustrate the general strategy of such studies, we first consider a classic experimental analysis of the effects of visual attention on the activity of parietal lobe neurons. We next present studies from our own laboratory that explore the link between motion perception and the electrical activity of motion-sensitive neurons in the visual cortex. Finally, we discuss several practical issues related to the preparation, training, experimental study, and care of the monkeys involved in these studies.

Visual Attention

In most work with awake, behaving monkeys, the central experimental strategy is to train animals on a simple, well-controlled behavioral paradigm that taps the cognitive system of interest and then record neural activity while the animals perform the task. In some situations, neural activity can be monitored noninvasively from outside the head using electroencephalographic or evoked potential techniques. These techniques, however, are of limited utility because they sum electrical activity over a substantial area of the brain that typically contains neural circuitry devoted to diverse functions. In other words, the spatial resolution of the noninvasive techniques is quite poor and yields relatively little information concerning exactly which brain circuits are active during any particular task. Recording neural activity with a microelectrode, however, permits excellent spatial resolution (down to the level of the single cell) with minimal risk of significant damage to the brain. The data one actually records with a microelectrode are brief electrical impulses (about 1-msec duration), or action potentials, that are generated by neurons in response to chemical messages from earlier cells in the processing chain. Once generated, action potentials travel long distances down the cell’s outgoing fiber, or axon, to relay information to the next cells in the processing chain. Thus, action potentials are the currency of information transfer within the brain, and a physiological understanding of how the brain processes information and how that information is related to behavior must involve the analysis of trains of action potentials generated by single neurons.

In experiments of this nature, the monkey’s daily routine begins with transfer from its home cage to a primate chair in which it can sit comfortably in a natural posture. The primate chair is transported to the laboratory and positioned in front of a television screen on which visual stimuli are displayed. The primate chair is surrounded by a magnetic search coil apparatus that provides a continuous measurement of the direction in which the animal’s eyes are pointing; this “eye position” signal is monitored continuously by a laboratory computer that controls all aspects of stimulus presentation, behavioral control, and data acquisition. The animal works on a designated task to receive liquid rewards through a lick tube mounted on the primate chair. The monkey receives no water overnight and is therefore thirsty at the beginning of each experiment; the monkey will work diligently for fluid rewards until achieving satiation. When the animal is no longer motivated to work, the experiment is ended and the monkey returned to its home cage. The length of the daily work session is quite variable from animal to animal but typically lasts between 2 and 5 hr. In most laboratories, the animals engage in work sessions 5 days per week. (All procedures are described in more detail below in Practical Issues.)

Figure 1 illustrates a behavioral paradigm for studying visual attention in awake, behaving monkeys that was developed at the National Eye Institute by Robert Wurtz, Michael Goldberg, David Robinson, and their colleagues (Wurtz and others 1982). The figure also depicts example trains of action potentials that are observed in such experiments during microelectrode recordings from the parietal lobe (Figure 2). This region of the brain was first implicated in attentional phenomena through observation of human patients with parietal lobe damage resulting from traumatic brain injury or stroke. The three panels on the left side of Figure 1 illustrate three different behavioral tasks these investigators used to assess the effect of visual attention on the responses of single parietal lobe neurons. In the experiment depicted in the top left panel, the monkey was required to look directly toward a point of light presented on a television screen and maintain its gaze at this location for a few seconds until the light vanished. As usual, the monkey was rewarded with a few drops of water or juice for successful completion of a trial. If
Figure 1  Three behavioral tasks for the study of visual attention (left panels) and an illustration of the pattern of data typically obtained in each task (right panels). Top row, left panel: The monkey is rewarded for gazing steadily at the fixation point while a behaviorally irrelevant visual stimulus is presented within the receptive field of the neuron under study. Top row, right panel: neural responses, visual stimulus onset, and a record of the monkey's eye position during a single trial of the fixation task. Vertical tic-marks in the cell activity trace indicate the time of occurrence of individual action potentials. The vertical offset in the visual stimulus trace indicates the time of onset of the stimulus. The flat eye position trace confirms that the monkey maintained steady gaze on the fixation point for the duration of the trial. Middle row: The monkey gazes at the fixation point but must make a saccadic eye movement to the visual stimulus when it appears in the receptive field. All conventions as in the upper row. The saccadic eye movement is evident as a downward deflection in the eye position trace. Bottom row: The monkey maintains gaze on the fixation point but reaches to the visual stimulus when it appears in the receptive field. All conventions as in the upper row. Again, the flat eye position trace confirms that the monkey maintained its gaze on the fixation point for the entire duration of the trial. Adapted from Wurtz and others (1982).
the monkey broke fixation prematurely, the trial was aborted immediately and no reward was given (recall that the computer monitors the monkey’s eye position continuously throughout all experiments). The monkey was therefore highly motivated to complete each trial successfully.

While the animal fixated on the point of light in the center of the screen, a second visual stimulus was flashed to the right of the fixation point, in a region of the visual field that is “analyzed” by the single neuron under study. This region is called the neuron’s receptive field, and stimuli flashed in the receptive field elicit a brief burst of action potentials from the neuron as illustrated in the top right panel of Figure 1. Note that this stimulus was entirely irrelevant to the behavioral task; the monkey was rewarded only for maintaining its gaze on the fixation target at the center of the screen. Nevertheless, the neuron responded weakly to the irrelevant stimulus, consistent with the fact that we can see irrelevant stimuli even though we typically ignore them.

In the new experiment illustrated in the middle left panel of Figure 1, an important twist was introduced to the behavioral task. As before, the trial began with the onset of a fixation point, and the monkey was required to direct its gaze toward this location. As before, a visual stimulus was flashed to the right of the fixation point within the receptive field of the (same) neuron under study. In the new task, however, the monkey was required to make a quick movement of its eyes (a saccade) to gaze at the new visual stimulus as soon as it appeared on the screen. Only in this manner could the animal obtain a reward. As shown in the middle right panel of Figure 1, the monkey moved its eyes to the new location about 250 msec after onset of the new stimulus. Note, however, that until the monkey actually executed the eye movement, the sequence of visual stimuli falling on the retina was identical to the experiment illustrated in the top left panel. The only difference is that the visual stimulus to the monkey’s right was behaviorally irrelevant in the top left panel but was highly relevant in the middle left panel. In the middle left panel, the monkey must pay attention to the new stimulus to make a quick eye movement, thereby obtaining the reward.

Interestingly, the parietal lobe neuron appeared to reflect this difference in the behavioral significance of the peripheral stimulus. In the middle right panel of the figure, we see that the neuron responded to the onset of the visual stimulus with a much more vigorous burst of action potentials than it did in response to the same visual stimulus in the top right panel. In other words, the response of this parietal lobe neuron to the visual stimulus was amplified, or enhanced, when the stimulus was important for the monkey’s impending behavior. Because clinical studies had previously implicated the parietal lobe in mediating visual attention, Wurtz and his colleagues suggested that the neural modulation they discovered reflected the influence of attention on visually responsive parietal lobe neurons. From other experiments, we know that neurons at earlier stages in the visual pathway respond in a stereotyped manner to the pattern of light falling on the retina, irrespective of the behavioral significance of the stimuli. Wurtz and colleagues suggested, however, that attention acts as a “regulator” on the flow of visual information from “low” to “high” levels of the cerebral cortex, ensuring that only the most important information reaches the highest processing levels of the system. In this manner, valuable neural resources can be allocated efficiently; the most sophisticated processing stages are called into action only for the most significant stimuli appearing in the visual field.

Although attractive, the attentional explanation proposed by Wurtz and colleagues suffered from a potential flaw. While the sequence of visual stimuli preceding the eye movement was identical in the top and middle rows of Figure 1, the motor plans developing in the monkey’s brain were vastly different. In the top row, the monkey planned only to maintain its gaze on the fixation point, whereas in the middle row, the monkey developed a plan to make a saccadic eye movement. Thus the possibility exists that the enhanced visual response in the middle row of the figure reflects the development of a plan to move the eyes rather than visual attention per se.

To test this possibility, Wurtz and colleagues modified the task yet again as illustrated in the bottom left panel of Figure 1. In this version of the task, all events were again identical through the onset of the visual stimulus to the right
of the fixation point. In the new experiment, however, the monkey was required to continue maintaining its gaze on the fixation point while moving its hand to touch the visual stimulus to obtain a reward. This clever manipulation ensured that the visual stimulus would again be relevant to the animal's behavior but removed the necessity for an eye movement. If the enhanced visual response in the middle right panel resulted from an eye movement, it should disappear in the new experiment. As shown in the bottom right panel, however, the enhanced response remained intact under the new experimental conditions. In this manner, Wurtz and colleagues obtained strong evidence that the enhanced visual response actually resulted from visual attention rather than from a specific motor act.

The study illustrated in Figure 1 is a classic in the history of cognitive neuroscience because it demonstrated that a sophisticated phenomenon such as attention could be brought under the scrutiny of rigorous neurophysiological techniques. For the first time, we were able to gain mechanistic insight into how attention might arise from the physiological functioning of the brain. Attentional phenomena have now been explored in numerous regions of the cerebral cortex (for reviews see Colby 1991; Desimone and Duncan 1995; Maunsell 1995), and our views of the neural systems underlying attention are considerably more sophisticated than when Wurtz and colleagues performed their pioneering experiments. Many critical questions, however, remain to be answered. For example, the visual responses illustrated in Figure 1 are clearly modulated by an attentional signal; but where in the brain, and by what mechanisms, does the attentional signal itself arise? Answers to questions of this nature are most likely to emerge from progressively more sophisticated analyses of visual attention in experiments with awake, behaving monkeys.

Visual Motion Perception

Studies in our laboratory focus on the processing of motion information by specific circuits of neurons in the visual cortex of monkeys. Figure 2 is a lateral view of a cerebral hemisphere of a macaque monkey. The primary cortical visual area (V1) resides at the back of the brain and is the major recipient of visual information flowing from the retina to the cerebral cortex. As first discovered by David Hubel and Torsten Wiesel nearly 40 yr ago, the first stages of visual processing in the cortex are characterized by neurons that respond selectively to specific features of the visual environment (see Hubel 1988). Figure 3 illustrates the pattern of responses that define a directionally selective neuron, the class of neurons studied most intensively in our laboratory. Such a neuron responds well to a visual stimulus that moves through its receptive field in a particular range of directions, but it responds little or not at all to motion of the same stimulus in the opposite range of directions. The hypothetical neuron illustrated in Figure 3 responds optimally to upward motion, but other neurons prefer alternative directions so that all directions are well represented.

In primates, directionally selective neurons first appear in V1 (they have not been observed in the retina or in the main visual relay nucleus of the thalamus) but are substantially more numerous, percentage-wise, in several extrastriate visual areas that receive anatomical projections from V1. The best studied of these is the middle temporal visual area (MT) (see Figure 2), in which more than 90% of the neurons are directionally selective (Zeki 1974). Thus, MT appears to be specialized for processing motion information, and this inference is reinforced by the fact that neurons that prefer a particular direction of motion are clustered together into "cortical columns," as illustrated in Figure 4 (Albright and others 1984). This illustration depicts a small section of cortex excised from MT. A microelectrode that passes through MT on a trajectory perpendicular to the cortical surface typically encounters a sequence of direction-selective neurons that respond optimally to the same direction of motion, illustrated by the small arrow at the top of each column. If the electrode passes tangentially through MT, the direction of motion preferred by single neurons changes systematically as the electrode moves from column to column. Roughly speaking, a complete set of direction columns exists for each receptive field location represented in MT, so that each direction of motion can be encoded at each point in the visual field.

Although our laboratory is concerned primarily with directionally selective neurons, other neurons in the visual cortex respond selectively to different features of the visual environment. Most V1 neurons, for example, respond selectively to the orientation of the edges of a stimulus, whereas other neurons appear to encode the color of a stimulus or the distance of a stimulus from the animal. Since the late 1950s, physiologists have operated on the assumption that the properties of these neurons revealed by microelectrode recordings provide reliable clues about the roles the neurons play in visual perception. Thus, directionally selective neurons are thought to play an important role in motion perception, orientation-selective neurons in form perception, and so on. Note, however, that this is a vast gulf to cross merely by assumption. These assumptions actually constitute a set of hypotheses, frequently tacit, concerning the relationship of cortical physiology to perceptual psychology. The central hypothesis is that neuronal activity contributes to the subjective experience of perception in a manner that can be inferred from the properties of individual neurons studied one at a time. Although this hypothesis is highly appealing to the neurophysiologist, it is imperative to find ways to test the notion empirically. Fortunately, the roles played by particular classes of neurons in perception can be tested using awake, behaving monkeys trained to perform appropriate perceptual tasks. Our laboratory, for example, has had con-

1Abbreviations used in this paper: CHV1, Cercopithecine herpesvirus 1; fMRI, functional magnetic resonance imaging; MT, middle temporal visual area; PET, positron emission tomography; V1, primary cortical visual area.
Figure 3  Method for analyzing direction selectivity in single neurons and an illustration of the pattern of data typically obtained in such studies. The monkey is required to gaze steadily at the fixation point (+) while visual stimuli move through the visual receptive field (circle) in four different directions (arrows). The four boxes depict example neural responses to each of the four directions of motion. This hypothetical neuron discharged vigorously (vertical tic-marks) while the upward-moving visual stimulus was on the receptive field. In contrast, the neuron fired very weakly to downward motion. Responses were intermediate for the two orthogonal directions of motion. This neuron, therefore, responded optimally to upward motion.

Figure 4  Columnar organization of directional neurons in the middle temporal visual area. Cortical columns run perpendicularly from the surface of the cortex to the boundary between cortical gray matter and the underlying white matter. Each column contains a preponderance of neurons that respond optimally to a specific direction of motion, as indicated by the arrow at the top of each column. Each column is approximately 100 μm wide; the thickness of the cerebral cortex in the middle temporal visual area is typically 1.5 mm. In this illustration, the columns are depicted in a rectilinear “ice cube” model. The actual arrangement of columns in the cortex is substantially more irregular than this. Adapted from Albright and others (1984).
siderable success in using this preparation to analyze the role of directionally selective MT neurons in motion perception.

Using standard operant conditioning techniques, we trained several monkeys to discriminate the direction of motion in a family of stochastic motion stimuli illustrated in Figure 5. The visual stimuli are flickering random dot patterns designed specifically to activate direction-selective neurons in the brain. The random dot display can take several forms in which the strength of the motion signal in the display is varied. At one extreme (left panel), the direction of motion of individual dots is entirely random and there is no coherent motion signal in the display; this form of the display looks like the visual noise on a domestic television tuned between stations. Coherent motion can be introduced to the display by specifying a proportion of the dots to move in a particular direction (50% in the middle panel) while the remaining dots continue to move randomly. At the other extreme, of course, all dots move coherently (right panel), and the direction of coherent motion is easily perceived. The difficulty of the discrimination can be smoothly varied by manipulating the percentage of dots in coherent motion. After sufficient training, monkeys typically perform as well as humans at discriminating the direction of motion in these displays.

We performed several types of experiments, including pharmacological inactivation (Newsome and Paré 1988) and single neuron recording (Britten and others 1992, 1996; Newsome and others 1989) to examine the role of MT in performance of this task. All results pointed toward the conclusion that the direction columns in MT actually provide the signals used by the monkey in performing the direction discrimination task. To test this notion more rigorously, we carried out a series of electrical microstimulation experiments while monkeys performed the task (Salzman and Newsome 1994; Salzman and others 1990, 1992). In these experiments, we positioned our microelectrode in a column of directionally selective neurons and activated the column artificially with a train of weak (10-μA) electrical currents while the monkey attempted to discriminate the direction of motion in the display. If the monkey actually uses that direction column in judging the direction of motion in the display, we should be able to influence its judgment by stimulating the column. Furthermore, we should be able to predict the direction the monkey will choose based on the direction preferred by the column of neurons that we stimulate.

Our experimental paradigm is illustrated in Figure 6A. On each trial, the monkey was required to fixate on a small point of light (labeled FP) while viewing the random dot display for 1 sec. The coherent motion signal could occur in any of eight directions equally spaced around the clock at 45° intervals. The dot display was presented within a circular aperture (solid circle in Figure 6A), which was superimposed on the receptive field of the neurons at the stimulation site. Thus, the monkey attended eccentrically to the display while maintaining its gaze on the fixation point. At the end of the 1-sec viewing interval, the monkey reported the direction of the coherent motion signal by moving its eyes from the fixation point to one of eight small targets (light emitting diodes, or “LEDs”) that corresponded to the eight possible directions of motion. As usual, the monkey sat within a magnetic search coil apparatus that provided a precise measure of eye position throughout the experiment. Thus, correct answers could be detected by the computer and reinforced with a liquid reward. The reward contingencies were

![Figure 5 Stochastic motion stimuli used in our studies of visual motion perception. The stimuli are dynamic random dot patterns plotted rapidly (6.67 thousand dots/sec) on a television screen under computer control. At one extreme (left panel), all dots are plotted transiently at randomly chosen locations. This version of the stimulus (0% coherence) has no net motion in any single direction, because all directions and speeds are present in equal amounts due to random pairings of the dots in space and time. To a human observer, this stimulus looks like the noise on a household television tuned between stations. At the other extreme (right panel) (100% coherence), each transient dot is replaced by a partner dot with a specified offset in space and time. Thus, each dot in the display moves at a constant speed in the specified direction. At 50% coherence (middle panel), half of the dots are randomly plotted “noise” dots, and the remaining half move coherently in a specified direction. The direction discrimination task can be made easy or difficult by varying the proportion of dots in coherent motion. Adapted from Newsome and Paré (1988).](https://example.com/image-url)
the same whether or not microstimulation was applied on a given trial.

Data were obtained in blocks of trials in which motion occurred in any of the eight directions, and over a range of dot coherences, with equal probability. All trial conditions were presented in random order; the monkey had no basis for predicting the direction or strength of the motion signal on a given trial. Microstimulation was applied on half of the trials for each condition (chosen randomly), and the stimulating pulses (10-μA, 200-Hz, biphasic pulses) began and ended simultaneously with the onset and offset of the random dot display. In this manner, we attempted to influence the monkey's perceptual judgments by activating a specific direction column artificially. We examined the effect of microstimulation by contrasting the monkey's choices on "stimulated" and "nonstimulated" trials.

Data from one experiment are depicted in Figure 6, B and C. The polar plot in Figure 6B shows the visual responses of neurons in the MT column. The visual responses were elicited using 100% coherent random dot patterns that moved across the receptive field in each of eight possible directions. The angle of each data point on the polar plot indicates the direction of motion of the visual stimulus. The distance of each data point from the center of the polar plot shows the average number of action potentials recorded for that direction of motion. Thus, the "tuning curve" illustrated in Figure 6B shows that the electrode was positioned within a column whose preferred direction was up and to the left.

In Figure 6C, the behavioral data obtained during the microstimulation phase of the experiment are illustrated. Plots in the figure represent the increase in the number of judgments on stimulated trials (compared with nonstimulated trials) for each of the eight possible choices. Directions with no increase are plotted at zero (the origin). The result of the experiment is clear-cut: Microstimulation caused the monkey to increase dramatically its decisions favoring motion up and to the left but caused little or no increase in favor of any other direction. The beautiful part of this result, of course, is that the direction of increased behavioral choices is matched perfectly to the direction of motion encoded by the stimulated column (compare Figure 6, B and C). Thus, the outcome of the experiment conforms nicely to our intuition: Artificial activation of a column with a particular preferred direction causes an increase in behavioral choices toward that direction. Close matches between physiological responses and perceptual choices were typical of the data obtained in this experiment (Salzman and Newsome 1994).

This result shows that the microstimulation-induced changes in a monkey's choice behavior can be predicted from the visual tuning properties of the stimulated neurons. Thus, the microstimulation signal is interpreted in a meaningful fashion by the monkey as it performs the discrimination task. This result therefore establishes a causal link between the activity of direction-selective neurons in the cortex and a monkey's perceptual decisions on a direction-discrimination task. Clearly, our success in this particular experiment raises the hope that similarly compelling links
between physiology and performance can be established for other classes of cortical neurons as well (for example, DeAngelis and others 1998).

Like the attention experiments reviewed in the preceding section, the visual motion studies illustrate the power of the awake, behaving monkey preparation for illuminating the relationship between brain activity and intelligent behavior. In all experiments of this nature, the investigators rely critically on the active cooperation of the monkey in the experiment. Thus, great care and preparation are required to create a working situation in which the monkey can achieve its goal (to acquire a steady stream of rewards) and the investigator can achieve his/her goal (to acquire a steady stream of informative data). Many practical aspects of preparing and training the animals, caring for animal health, and executing the experiments require careful planning to create a successful research program. We consider several of these issues in detail below.

**Practical Issues**

In this section, we concentrate on procedures used in our own laboratory because a comprehensive review is beyond the scope of this article. Procedures can vary from laboratory to laboratory, depending on the exact nature of the research being performed.

**Surgical Preparation of the Animals**

Each animal must be prepared surgically to have (1) a fine wire search coil implanted around the eye, which enables measurement of eye position; (2) a stainless steel post attached to the skull, which allows the head to be stabilized during recording experiments; and (3) a craniotomy, which is then covered by a stainless steel recording cylinder and plastic cap, allowing microelectrodes to be introduced into the brain for electrophysiological experiments. These three procedures may be performed during a single surgery, but the craniotomy and cylinder implant are frequently performed in a second surgery several months after installation of the eye coil and head-stabilizing post. The latter two procedures are necessary for behavioral training, whereas the former is needed only for physiological experiments after the animal has been well trained. Because of the potential for infection and regrowth of bone, it is unwise to implant a recording cylinder that will not be used during several months of training.

All surgical procedures are performed under aseptic conditions and general anesthesia. Isoflurane gas is the most common anesthetic in current use. After implanting the necessary hardware, the head-stabilizing post and recording cylinder are melded into a single “cranial implant” by means of a small, elliptically shaped mound of bone cement, which ensures stability, uniformity, and cleanliness of the implant. The shape of the implant should be convex and smooth at all points, if possible, so that the skin will adhere to the implant. Small irregularities or concavities at the edge of the implant increase the likelihood of infection at these sites. The implant is affixed to the skull with small orthopedic bone screws. The animals adjust quickly to their implants, manually exploring them during the first few postoperative days and virtually ignoring them after that.

The purpose of the head-stabilizing post and eye coil is to provide a means for controlling head and eye position. In vision experiments of the kind described in this paper, it is essential that the visual stimulus be presented to the same location on the retina (that is, in the neuron’s receptive field) during each block of trials. This becomes impossible, however, if the monkey is allowed to make uncontrolled head and eye movements throughout the experiment. The head-stabilizing post connects to a mating post on the primate chair, holding the monkey’s head in a stable position during the experiment. The eye coil is implanted beneath the conjunctival tissue, anterior to the insertions of the extracocular muscles. The insulated leads from the eye coil travel subcutaneously to the cranial implant where they are soldered to a tiny electrical plug that is then joined to the cranial implant using bone cement. By means of an electromagnetic induction technique, the eye coil provides a sensitive measure of the monkey’s eye position throughout the experiment, allowing the investigators to train the animal to fixate as described in earlier sections (Judge and others 1980; Robinson 1963). (The conjunctiva reattaches to the sclera—the hard globe of the eye ball—several days after implantation of the eye coil. In particularly good surgical outcomes, it is almost impossible for a nonspecialist to tell which eye contains the coil.)

Additional surgeries are sometimes necessary and are permitted if they are essential to the scientific goals of the study. If, for example, the thin eye coil wire breaks, it must be replaced for experiments to continue. In addition, some studies require electrophysiological recordings at multiple locations in the brain to determine how information is processed from stage to stage in the central visual pathways. Thus, a short surgery may be required to change the position of the recording cylinder.

**Training and Daily Routine**

Awake animals are generally handled with a pole and collar technique, avoiding direct contact between the animal and the handler. An initial training period, usually of 2 to 4 wk, is required to acclimate the animal fully to the pole and collar handling method and to the primate chair. After learning that the chair signifies imminent treats such as juice, fruit, and nuts, most monkeys become quite cooperative with the entire process. After becoming comfortable with the chairing process, the chaired animal is moved to the laboratory each day for extended training on specific behavioral tasks. This phase of the training requires as little as 2 to 3 wk for the simplest tasks but can take the better part of a year for sophisticated tasks involving perceptual threshold measure-
ments or intricate manipulations of attention and memory. Each animal may engage in experimental protocols for 1 to 4 yr after being completely trained. Plainly, intense effort is expended on each animal, and awake monkey laboratories generally use very few animals as a result. In our laboratory, for example, eight to 10 animals are in the colony at any one time, and we typically euthanize only two per year.

Daily training sessions generally last 1 to 3 hr; session length is typically determined by the monkey's willingness to perform the task. When the monkey ceases working, the session ends and the animal is returned to its home cage. All training is accomplished using standard operant conditioning techniques with positive rewards for desired behavior.

After an animal has been fully trained, experimental procedures begin. Daily electrophysiological recording sessions typically last somewhat longer than training sessions (3 to 6 hr) because the animal is willing to work longer after weeks or months of acclimation to the laboratory routine. The major difference introduced during recording sessions, of course, is insertion of a microelectrode into the brain at the beginning of each session. Since there are no nociceptors (pain fibers) in the brain, passage of microelectrodes through the brain causes the animal no discomfort. The membranes covering the brain (the dura mater) are richly innervated, however, and the insertion of electrodes or transdural canulae can cause minor discomfort. Evident discomfort on the part of the animal can generally be relieved by topical application of a local anesthetic such as lidocaine. At the conclusion of the recording session, the electrode is removed, the recording cylinder is cleaned, and a plastic cap is affixed over the open end of the cylinder. The animal is returned to its home cage and given fruit, water, or food supplements as needed.

**Controlled Water Intake**

As indicated above, behavioral control is always accomplished with standard operant techniques, using fluid as a positive reward for desired behavior. To ensure adequate motivation for each session, the animal is maintained on a carefully controlled fluid intake schedule, established in consultation with veterinary staff and with prior approval of the institutional animal care and use committee. The desired outcome, of course, is to provide the animal with a sufficient volume of fluid each day to maintain good health, while establishing proper motivation for a few hours of task performance. Water is used most frequently as a reward, but fruit juice solutions are sometimes used if the animal is more highly motivated to work for juice rewards. Identifying a highly desired fluid (such as fruit juice of a particular flavor) for a given monkey is advantageous because the animal may maintain good work habits on a more relaxed water intake schedule and because the animal presumably enjoys it more. In our experience, the relative efficacy of water versus fruit juice varies greatly from animal to animal.

Some monkeys receive their entire daily allotment of fluid during experimental sessions, and others receive supplemental fluids to bring the total up to the required allotment. The allotment is established individually for each animal depending on body size, age, the specific behavioral task to be performed (difficult tasks require more motivation), and physiological factors that are idiosyncratic to each animal (like humans, different monkeys appear to regulate hydration more or less efficiently, leading to substantial variation in amount of fluid intake required each day). We have never found a particular formula that works well for all, or even a substantial majority, of monkeys.

Because a tremendous amount of time is invested in each animal, investigators who employ awake, behaving monkeys are particularly attentive to animal health issues. The primary health concern associated with the controlled water intake paradigm is the potential for dehydration or, in some cases, for the animal to eat less solid food when water intake is being controlled. Hydration levels are monitored daily by measurement of body weight, examination of the moisture content of fresh feces, assessment of skin turgor (elasticity), as well as general demeanor and activity level of the animal. The animals are examined daily by their handlers and by the animal husbandry staff, and daily records are kept of body weight, fluid intake volume, behavioral appearance of the animal, and any other factors deemed relevant to the animal's health. In addition, each animal is inspected periodically by the veterinary staff. Any decrease in appetite for solid food can be ameliorated by providing specially prepared chow with extra moisture content (such as Jello-soaked biscuits) or with fruit and nuts blended into the chow. Extra fluids are provided on weekends or on other days when no experiments take place.

The controlled water intake paradigm (or controlled food intake paradigm) has been criticized in some circles as unnecessary or inhumane (for example, Orleans 1991). The general arguments are that monkeys will continue to work for highly desired treats while receiving food and water ad libitum, and that any form of controlled intake causes unacceptable suffering of the animal. Over 15 yr of behavioral work with monkeys, our experience is unequivocal: The vast majority of monkeys will not work at challenging tasks like those described in earlier sections of this paper without the incentive supplied by controlled water intake. Even for easy tasks, most monkeys will not work steadily for sustained periods without the added motivation provided by controlled water intake. Thus, our experience is consonant with that of Desimone and colleagues, as described in their response to the article by Orleans cited above (Desimone and others 1992). We also concur with Desimone and others that controlled water intake, as practiced in most alert-monkey laboratories, mirrors conditions sometimes encountered by monkeys in the wild, who may travel long distances and for extended periods of time, between visits to watering stations (see citations in Desimone and others 1992). Thus, we believe that controlled water intake is both scientifically necessary and possible to apply humanely within the laboratory.

In judging the necessity of the controlled water intake
paradigm, it is important to remember that the scientists who employ it have no interest in, or fondness for, controlled water intake per se; it is simply a method for gaining control of behavior for a few hours each day. A rich variety of behavioral paradigms have been explored across tens of thousands of person-hours in dozens of laboratories, both in the United States and abroad. In the great majority of cases, controlled water (or food) intake has proven essential for the science to be accomplished. If there were a practical alternative, most scientists would embrace it readily.

Psychological Well-being

Psychological well-being of nonhuman primates, although poorly defined, has become an increasingly important concern over the past several years; psychological enrichment for nonhuman primates in laboratory environments is in fact mandated by federal law (NRC 1998). We rotate simple, durable toys through each animal’s home cage (a toy that is constantly present is soon ignored), wherein mobile mirrors are available to each animal. The animals are generally housed individually so that we can carefully monitor food and water intake as well as excreta for each animal. The housing arrangement allows for social interaction, however, because all animals have visual, olfactory, and auditory contact with other animals. Animals may be pair-housed during periods in which they are not actively involved in training or experimental sessions, increasing the opportunity for social interaction. Substantial care must be given, however, to pairing socially compatible animals. Pair-housing of “on-study” animals is more difficult because of the problems mentioned above, but this type of housing is being attempted in some laboratories.

All animals interact daily with laboratory staff and animal husbandry staff, who offer nuts, fruits, or other treats on a nearly daily basis. Animals on study, in particular, interact extensively with a familiar human handler and spend a portion of each day solving challenging problems for positive rewards. All of these activities are reasonable steps to promote psychological well-being, but objective assessment of their actual impact on psychological state remains an elusive goal. Most experienced primate handlers continue to rely on the traditional bright, alert, responsive (“BAR”) criteria to gain a daily sense of an animal’s overall level of well-being, both physical and psychological.

Safety Issues

A variety of pathogens can be exchanged between humans and monkeys, creating potential hazards for both. In the laboratory environment, the pathogen of most concern by far is the Cercopithecine herpesvirus (CHV1, also known as monkey B virus). This virus is endemic in macaque monkeys in the wild, with a prevalence of 80 to 90% in adults (Holmes and others 1995). It produces mild to asymptomatic disease in macaques, with oral lesions and/or conjunctivitis (similar to herpes simplex lesions in humans) being the most common indicators. In humans, however, CHV1 causes a rapidly progressive encephalopathy with a fatality rate of roughly 70% (CDC 1998; Holmes and others 1995; Krugner-Higby and Schultz 1995). In actively shedding animals, the virus can appear in saliva and in mucosal or genital secretions. In the majority of human cases, CHV1 infection has resulted from a bite or scratch from an infected animal. Infection of humans through exposed mucosal membranes has also been documented, indicating that protection of the eyes and mouth is a necessary precaution (CDC 1998). The frequency of transmission from monkeys to humans appears to be very low: Roughly 50 cases have been confirmed since the virus was discovered in the 1930s. Although the actual risk to laboratory personnel is small, the downside of an infection is very steep. Many awake-monkey laboratories, therefore, attempt to maintain a colony that is free of CHV1. CHV1-free animals can usually be obtained with prepurchase serological testing. After arrival in our laboratory, each animal is tested for CHV1 once a year. Maintenance of a CHV1-free monkey colony may be impractical in some institutions where larger numbers of animals being used for very different purposes must be housed in a common facility.

Despite such precautions, nominally CHV1-free animals can sometimes convert to CHV1-positive status. Thus, although we attempt to maintain a CHV1-free colony, our handling procedures are designed as though all animals were CHV1-positive. The golden rule is “no exposure to bodily fluids.” Personal protective equipment is required at all times of close contact with a monkey; latex gloves, laboratory coat, protective eye glasses, and a face mask are standard gear. When handling monkeys using the pole and collar, laboratory personnel wear a hard face shield (akin to a welder’s hat) and supplie leather gloves. Before independent work with monkeys, laboratory personnel receive extensive training. One-to-one training in daily handling procedures by an experienced handler is essential. In addition, everyone participates in more formal training sessions provided by the Stanford University Department of Comparative Medicine. This training session covers the biohazards associated with nonhuman primate work as well as first-aid and follow-up procedures that must be implemented if a potential exposure occurs. Laboratory personnel are also familiarized with relevant laws, policies, and regulations concerning the care and use of nonhuman primates. Finally, all laboratory staff participate in basic health surveillance supervised by Stanford’s Occupational Health and Safety Program.

Although communication of CHV1 is the primary health concern of scientists who work with monkeys, it should be remembered that dangerous pathogens can also be transmitted from humans to monkeys. Tuberculosis, in particular, is extremely dangerous to some monkey species, and regular tuberculosis screening should be required for all persons working in close contact with monkeys.
Role of the Veterinarian

A competent, knowledgeable veterinary staff is a great boon to laboratories involved in awake-monkey research. Unlike many animal users, awake-monkey researchers invest immense resources in individual animals and are therefore vitally concerned with the health of each animal. The veterinary staff performs several critical roles in quality care, including consultation on a variety of animal health issues, supervision of diagnostic laboratory work, dispensing appropriate medications, supervision of quarantine rooms, and education of laboratory personnel concerning animal care standards mandated by various regulatory and accrediting agencies. Veterinarians, however, frequently assist our research program in ways that are less obvious. Our staff has provided useful advice concerning surgical procedures, psychological welfare issues, handling of individual animals during experiments or training, and preparation of animal care and use protocols. Importantly, the veterinarian provides an independent, objective voice in animal care issues since he or she is not involved in the ongoing research program.

Summary

Clearly, the practical issues involved in establishing and maintaining an awake-monkey laboratory are substantial. The enterprise, however, is not as daunting as it might first appear. Typically, investigators who employ this preparation are trained for several years as graduate students and postdoctoral fellows in existing awake-monkey laboratories. The new investigator therefore brings a substantial reservoir of knowledge and experience to the task of establishing a laboratory. The daily routines of animal husbandry, surgery, experimental procedures, management of controlled water intake, care for psychological well-being, and safety of laboratory personnel are numerous, and all must be performed according to high professional standards. In a smoothly functioning laboratory, these procedures are built into all laboratory routines and are easily maintained as long as new laboratory members are appropriately trained and educated concerning the reasons for the procedures and the importance of compliance.

Conclusion

In this article, we have reviewed experiments aimed at understanding the neural basis of visual attention and motion perception. Although these studies demonstrate the power of the awake, behaving monkey preparation for illuminating the relation between brain activity and cognition, they are merely illustrative of the diverse work being performed in a remarkably fecund field. The awake, behaving monkey preparation is currently in use worldwide to explore the neural underpinnings of a broad range of behavioral and cognitive phenomena such as learning, memory, motor planning, motivation and reward, object recognition, spatial coordinate transformations, and many others (Andersen 1987; Chen and Wise 1995; Fuster and Jervey 1982; Georgopoulos and others 1993; Goldman-Rakic 1987; Logothetis and Sheinberg 1996; Miller and others 1996; Miyashita and others 1996; Raymond and others 1996; Schultz and others 1993; Sparks and Groh 1995). These investigations are of extraordinary interest because they promise to shed light on the biological basis of our mental life—those phenomena that define our very existence (cogito ergo sum!). The brightest of our young scientists are being attracted to this field in increasing numbers, and we can certainly expect steady progress on all of these fronts in the years ahead.

In recent years, many investigators in the field of cognitive neuroscience have been excited by new experimental techniques that permit functional imaging of the human brain: functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) (for example, Corbetta and others 1993; Engel and others 1997; Shipp and others 1995; Tootell and others 1996). Some investigators have even wondered whether imaging techniques might one day replace invasive microelectrode recordings as our main physiological tool for studying brain function. This outcome would be salutary in many ways. Humans can learn and master complex cognitive tasks much more quickly than non-human primates, permitting more rapid progress in scientific investigation. Ultimately, the human brain is the system we most want to understand.

Imaging techniques, however, are severely limited in their spatial and temporal resolution and are unlikely to replace microelectrode recording at any time in the foreseeable future. Imaging techniques rely on the fact that the flow of blood to each region of the brain is regulated from moment to moment in rough proportion to the overall level of electrical activity in that region of the brain. By monitoring the blood flow to specific regions of the brain while human subjects perform cognitive tasks, the investigator can acquire information about what parts of the brain are most active during performance of a given task. PET, however, cannot resolve blood flow differences between regions separated by less than 1 cm in space nor resolve events separated by less than 40 to 60 sec. fMRI is better, with spatial resolution on the order of a few millimeters and temporal resolution on the order of a few seconds. However, the dimensions of the cortical columns described earlier in this article are 100 to 200 μm, and the electrical activity of neurons occurs on the temporal scale of milliseconds. Without major new advances, then, even fMRI is unlikely to enable analysis of the central nervous system at spatial and temporal levels, where the most important events occur. For the near future, our best hope is that imaging techniques and traditional neurophysiological techniques can complement each other in a broad-based approach to understanding the neural basis of cognitive function.

The questions pursued in experiments with awake, behaving monkeys are among the most fundamental in all of neuroscience: How is information processed within the brain,
and how does this information processing result in organized, purposeful behavior? Although we have emphasized visually based cognition in this review, the intellectual thrust of the research is directed toward fundamental issues in neural information processing that are central to understanding virtually all mental function.

Ultimately, biomedical research is justified primarily by its contributions to the health of the citizens who support the research. The study of the visual system in experimental animals has already yielded substantial benefits. For example, amblyopia is a common form of blindness in which the victim loses useful vision in one eye due to misalignment of the eyes during early life (squint or “crossed-eyes”). Nobel Prize-winning work in cats and monkeys, performed by Torsten Wiesel and David Hubel at Harvard Medical School, revealed the neural basis of this problem and showed that normal vision can be saved in animals with squint if the condition is surgically corrected very early in life. Since this discovery, vision has been saved in thousands of children by ophthalmologists who, taking these lessons from basic research to heart, provided corrective eye surgery for the victims during infancy.

Many other health-related applications will surely follow from an understanding of the biological basis of mental function. Mental health disorders take a massive toll on the health and well-being of our citizenry; these disorders are particularly insidious because they rob the afflicted person of the very essence of personal identity. Understanding how brain activity mediates mental function will inevitably provide a deeper understanding of the pathophysiology of these disease states and suggest new therapeutic approaches for treating such diseases.

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