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Methods 30 (2003) 142–151

METHODS

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Single-unit recording in the lateral geniculate nucleus of the awake behaving monkey

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Accepted 3 February 2003

Abstract

In recent years, recording neuronal activity in the awake, behaving primate brain has become established as one of the major tools available to study the neuronal specificity of the initiation and control of various behaviors. Primates have traditionally been used in these studies because of their ability to perform more complex behaviors closely akin to those of humans, a desirable prerequisite since our ultimate aim is to elucidate the neuronal correlates of human behaviors. A wealth of knowledge has accumulated on the sensory and motor systems such as vision, audition, and eye movements. For more demanding behaviors where the main focus has been on attention, recordings in awake primates have begun to yield valuable data on the centers of the brain that are reactive to different attributes of this behavior. As a result, various hypotheses of the origin and distribution of attentional effects have evolved. For instance, visual attentional effects have been described not only in the higher cortical area (V4) but also in areas earlier in the visual pathway which presumably involve a feedback mechanism in the latter region. Here we outline the ways in which we have successfully used these methods to make single-cell recordings in awake macaques to show how certain behavioral paradigms affect neurons of the thalamus (with emphasis on the lateral geniculate nucleus). As we have done with established techniques these methods can be readily adapted to incorporate most behaviors needed to be tested and allow recordings to be made in virtually any part of the brain.
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1. Introduction

The in vivo recordings of single neurons or small groups of neurons in a behaving animal allow the investigator an excellent opportunity to study the contribution that specific neurons and neuronal regions make to the animal's behavior. The underlying strategy is to correlate changes in neuronal responses with specific behaviors on the part of the animal. Although, many awake, behaving experiments have been performed in other mammals, primates have been the experimental animal of choice for many years and some notable studies have resulted in seminal findings.

In one of the earlier studies it was shown that the firing rate of a population of neurons of the motor cortex can be modulated by the extension/flexion of the wrist on the side contralateral to the recording site [1]. By designing appropriate experimental tasks for the monkey to perform it was possible to separate the effects of the position of the wrist from the effects of force, which was shown to have a direct relationship to the activity of these particular neurons. Some studies using the awake monkey preparation have demonstrated the effects of external sensory stimuli on neuronal activity. Visual stimuli have been used (e.g., [2,3]), as have cutaneous somatosensory stimuli (e.g., [4]), joint movement (e.g., [5]), and limb movement (e.g., [6]). Some hypothalamic neurons were shown to respond to the recognition of food [7]. Apart from the obvious knowledge we have gained from these studies they also demonstrate that a wide variety of behaviors can be studied and many locations in the brain can be used to make recordings.

In terms of the visual system, the use of the monkey preparation has provided important new insights into the functioning of many regions of this system such as the

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1046-2023/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S1046-2023(03)00075-6

cortex and the superior colliculus. However, the lateral geniculate nucleus has been largely neglected. The lateral geniculate body has long been thought of as just a simple, machine-like relay station for retinal signals. This concept has dramatically changed over the past decade. Convincing evidence now indicates that the lateral geniculate nucleus acts dynamically to affect how retinal signals are relayed to cortex, and this view has now been extrapolated to the rest of thalamus, which is no longer looked on as a passive, simple relay. It now appears that the animal's differing behavioral states, including differing levels of arousal and attention, dictate the nature of the geniculate relay. External, nonretinal inputs that influence the geniculate relay include large inputs from visual cortex, the brainstem, and the hypothalamus. Among other factors under control that affect the relay properties of thalamic cells are their intrinsic membrane properties, which are strongly influenced by nonretinal inputs (see review by Sherman and Guillery [8]).

Of these membrane properties, apart from those underlying the action potentials, the most important and interesting one is the low-threshold (LT) Ca^{2+} spike [9–11]. This is voltage dependent. When it is inactive, relay cells fire in *tonic mode*, responding to excitatory input with a barrage of unitary action potentials that lasts for the duration of the suprathreshold input. When it is activated they fire in *burst mode*, responding now with brief bursts of several action potentials with short interspike intervals. For further details of the cellular basis of this behavior, see Sherman and Guillery [8] and Sherman [12].

Our purpose here is to document how we have successfully used awake, behaving macaques (*Macaca mulatta*) initially to study the state-dependent (awake through drowsy to slow wave sleep) response of the lateral geniculate nucleus relay neurons. We have also used this primate preparation to study the influence of eye movements on the visual responses of the lateral geniculate nucleus. Our most recent studies, findings from which are as yet unpublished, involve the more complex behavior of attention. Studies using monkeys trained to perform tasks that require visual attention have demonstrated that this behavior elicits responses centrally in extrastriate visual cortex (e.g., [13]; see the review by Desimone and Duncan [14]). Attention has also been shown to affect striate cortex (e.g., [15]). We have therefore begun to look at the influence of attention on the firing of geniculate neurons.

The macaques we use are capable of performing tasks of a relatively high level of complexity and they also tolerate laboratory handling well, which is very important because it makes interactions with these animals very easy. Our institutional guidelines, which provide adequate environmental enrichment, result in animals that are very playful and exhibit behavioral traits of relaxed contentment. Such factors are of utmost importance because they determine how efficiently an animal performs the tasks. Prior to final training and recording, the monkeys undergo surgeries to chronically implant restraining head posts, eye coils, and recording cylinders. These animals have a robust constitution which allows quick and full recovery with minimal postsurgical trauma. In addition, they are relatively easy to train to perform specialized tasks, which engage particular attributes of behavior. Tasks can be made more complex or changed to accommodate other attributes of the same or other behavior using the same primate subject. One drawback is that earlier learned tasks are easily forgotten when a new task is taught. However, relearning a previous task is much less taxing to the animal than learning a novel one. A well-trained animal can be kept in use for several years provided the subject is made to perform the tasks regularly, even when experiments are not being performed. To determine the requisite level of proficiency in a given task(s) the investigator must be sure that the success rate is not simply due to chance. We routinely train animals to get in excess of 90% of trials correct within a 4-week training period. Under Methods which follows, we hope to give readers enough information to enable adaptation of our methods to accommodate their own requirements.

2. Methods

The methods we now describe are mostly routine for these kinds of studies of behaving monkeys. We have adapted them successfully to meet the requirements of our study (see Gnadt and Mays [16]).

2.1. Obtaining healthy animals

Animals can be obtained conveniently from commercial vendors or the Primate Clearinghouse. Before committing to buying monkeys we request that several tests be carried out so as to obtain healthy specimens. Before shipping, we request that monkeys be tested for tuberculosis (TB), simian B virus, filovirus, and bacterial and protozoal pathogens (*Shigella*, *Salmonella*, *Campylobacter*, and *Giardia*). We also request that complete blood counts and extensive blood chemistry be carried out on all animals. In addition, the animals are routinely checked by fecal float for parasites and general good health. If the animals do not attain the level of good health these tests demand, they are rejected. On arrival on campus, the subjects are quarantined for at least 1 month, then retested for simian B virus. Thereafter, TB tests are performed by the veterinary staff twice per year with full blood work annually. Any monkey that tests positive for TB, simian B virus, or filovirus is either rejected before shipping or isolated and terminated quickly for use in an acute experiment.

The testing is done before shipping, because they cannot be returned. Should an animal be found with a contagious disease, it could not be incorporated into our colony for fear of spreading the disease and would likely have to be destroyed. The preshipping testing varies from one institution to the next, and those listed above follow our own guidelines. They are part of standard practice in academic laboratories and "good veterinary care." After the quarantine period, the animals are moved to their permanent home cages in close proximity to the laboratory. The animals receive water and a balanced diet of dry food formula and fruit. To help the newcomers assimilate quickly, a battery of enrichment regimes is used. This includes special edible treats, toys, and human interactions. A special playpen is also used on a rotational basis, i.e., used by individual animals one day at a time. In some cases animals can be pair-housed. However, many if not most monkeys are incompatible because of behavioral dominance issues.

The fluid intake of animals in training or in experimental use is restricted 5 days a week to that obtained as rewards during training and recording sessions. On each of these days the animals are allowed to work to satiation. Water is made available to them ad libitum for the remaining 2 days a week. Prior to surgeries (see below) monkeys are pole-trained. They are fitted with a chain-link neck collar, a procedure done under ketamine sedation. A pole with a clipping mechanism attaches to a link of the chain, allowing the monkey to be removed from the cage in a controlled manner. They are walked around the room to familiarize them with pole handling. After several days of this kind of training they are taught to jump into a restraining chair. After they have mastered this we wheel them into the laboratory to familiarize them with this new environment. Edible treats are offered to the animals as rewards throughout these training sessions.

2.2. Surgeries

We generally perform three aseptic surgical procedures on each animal, for which we are aided by a staff veterinarian. Animals are sedated with ketamine and medicated presurgically with atropine. Once sedation is achieved, general anesthesia is maintained with isoflurane via an endotracheal tube. Lactated Ringer's solution is administered intravenously throughout the surgical procedure. Body temperature is maintained with a heated blanket and continuously monitored. Electrocardiogram, O_2 saturation, and pulse rate are also monitored during the surgical procedures.

2.2.1. Surgery 1: implantation of a head post, head cap, and scleral eye coil

In the first surgery we implant a scleral eye coil, for the purpose of measuring eye movements, and a restraining head post with head cap. After this surgery, an animal is basically ready for training in any of the visually based tasks we use. We routinely start this surgery with the eye coil placement. Our aim here is to implant a magnetic search coil on one eyeball. During experimental and training procedures, the animal in its restraining chair is positioned in a magnetic field that alternates at a high frequency [17,18]. The principle behind this method is based on the fact that the amplitude of the current induced in the coil is directly proportional to the sine of the angle between the axis of the eye coil and the magnetic field. The magnetic field in which the animal sits is generated by field coils, one producing a vertical field and a second one producing a horizontal field. The oscillating currents between the horizontal and the vertical are modulated in quadrature. As a result the currents produced by the eye coil by horizontal and vertical movement of the eye are 90° out of phase with each other. For eye position monitoring the magnetic field in which the monkey sits is generated by dual-power oscillators and the actual eye deflection signals are processed by phase-locked amplifiers and recorded at a resolution of 1 ms temporally and 0.2° spatially.

We start the eye coil surgery by retracting the eyelids with a springed retractor, using two weighted suture handles to move the globe around, attached near the limbus corneae at 12:00 and 6:00. The conjunctiva is cut near the limbus and the incision is continued around the diameter of the globe. The conjunctiva and Tenon's capsule are separated from the globe using blunt dissection. A 15- to 18-mm-diameter preformed scleral eye coil, made of three twists of Teflon-insulated stainless-steel wire, is positioned on the orbit with the lead at the temporal side of the eye. The coil is sutured to the globe with three or four sutures placed approximately equidistant apart around the eye. A loop of the coil is inserted into a pocket made between Tenon's capsule and the conjunctiva. The end of the coil is passed out at the base of the pocket laterally through a cut in the skin. The lead is taped to the skin while we proceed with the other parts of the surgery. The conjunctiva is sutured over the coil by making "tuck sutures" around the cut edge of the conjunctiva. At this point, we find it more convenient to move on to implantation of the restraining device and the head cap because the lead of the eye coil is later attached to a connector, then buried on the head cap near the head post.

For the restraining device the monkey is placed in a stereotaxic apparatus. The head is scrubbed with betadine solution and draped. The skull is revealed with two curved incisions, approximately 8 cm, to result in an oval-shaped midline skin flap and the periosteum and muscle are retracted laterally. After the bone is cleaned and dried, 10–12 stainless-steel bone screws are placed in small burr holes dispersed over the oval exposure.

A stainless-steel bar (~7 cm in height), which fits the head restraining device of the primate chair, is positioned stereotaxically toward the front and anchored by several bone screws and a mound of bone cement. To increase strength and stability, the base of the post has three prongs, each 90° to the other. It is of utmost importance that this restraining apparatus be anchored strongly because it has to prevent head movement of an awake animal. Much care must be taken to ensure an adequate number of bone screws and enough cement surround and cover the base of the post. Bone cement, approximately 1.5 cm thick, is spread over the remaining exposed calvarium, ensuring that the distributed bone screws are included in the cap. These screws form the main source of anchorage. A crescent-shaped head cap ~8 cm in diameter on the long axis and ~5 cm on the short axis should result. Once this is done, the lead of the eye coil can be attached.

The lead is threaded back through the skin cut and passed subdermally up to the head cap. A ~5-mm length of the end of the coil is stripped of its insulation and soldered to a "female" connector which is then inserted into a plastic casing. The entire connector assembly is buried in bone cement, ensuring the points of insertions of the connector are kept free of cement. The impedance of the coil is checked constantly while the connector is being fitted. Following all surgeries, animals are placed on a regimen of antibiotics (2.2 mg/kg Baytril, BID, for 7 days), analgesic (0.03 mg/kg Buprenex, BID, as needed) and a nonsteroidal anti-inflammatory (Banamine 1 mg/kg, SID, for 2 days) to reduce swelling.

We generally never insert eye coils in both eyes during a single surgery because we try to reduce the level of surgical stress on the animal. Furthermore, while the animal is in training, the recording cylinder is not used and the eventual buildup of fibrous tissue makes transdural penetration of the electrode increasingly difficult.

2.2.2. Surgery 2: implantation of a second eye coil and recording chamber

This surgery is begun with the placement of an eye coil on the second eye of the monkey. The procedures are exactly as described above. Once the coil surgery is over, the animal is placed in a stereotaxic apparatus for the implantation of recording cylinders. We use a line between the interaural bars as our anterior–posterior zero line. For recordings in the lateral geniculate nucleus, the center of the cylinder is placed 5.5 mm anterior to interaural zero and 12 mm lateral to the mediolateral midline. The diameter of the cylinder is 16 mm, which we have always found to be adequate to find and map the entire lateral geniculate nucleus. The acrylic bone cement overlying this area of the cranium is removed using dental burrs in a hand drill. A craniotomy of diameter slightly smaller than that of the cylinder is cut using dental burrs. Care is taken not to damage the *dura mater*. The stainless-steel cylinder is cemented over the craniotomy with the same dental cement used for the headcap. A threaded Teflon cap with a pressure venting center is used to securely cover the cylinder. The cylinder is kept filled with sterile saline and a few drops of an ophthalmic cocktail (polymixin B sulfates, neomycin, and dexamethasone) to stave off infection and scar buildup. These chambers are cleaned daily with saline flushes and kept filled with the solutions. At the end of this surgery, we ensure at least two bone screws are left exposed to allow later attachment of leads. These will be used to feed electroencephalogram (EEG) signals through amplifiers and filters to remove the interference of the magnetic fields via a data acquisition board to the runtime computer. In this way we can record EEGs as an index of state of consciousness simultaneously with eye movement, target, and spike data (see below). The postsurgical regimen of medication as described above is instituted. Occasionally, the animals are lightly anesthetized with ketamine (0.03 mg/kg) and supplemented with acepromazine (0.02 mg/kg) so that we can remove granular tissue overlying the *dura mater* (which makes electrode penetration increasingly difficult) every few months or so.

2.2.3. Surgery 3: implantation of a second recording chamber

We often need to record in the second lateral geniculate nucleus or some other thalamic nuclei, in which case we place a second recording cylinder over the adjacent thalamus. This is done in a third surgery and the procedures for cylinder implantation described above are adhered to in this surgery. On occasion, an eye coil may become nonfunctional and it can be replaced as part of this third surgery.

2.3. Training regimes

A pole-trained animal (see above) begins its behavioral training by being-fitted in a restraining chair that is individually adjusted to accommodate each monkey. The head is fixed by locking the head post to a bolt on the chair. Once the chair has been customized for a particular animal, it becomes dedicated to that animal and is never shared. The chaired animal is placed into the magnetic field, and, to track eye movements, leads connected to the phase detectors are plugged into the eye coil connectors mounted on the animal's head cap. A reward mouthpiece is positioned in the mouth just in front of the incisors to prevent the animal from chewing on the tubing. The dispensation of the fluid reward by gravity feed (usually a fruit cordial) is regulated by a solenoid whose control apparatus is connected to the runtime computer, which allows reward only at the completion of successful trials.

Approximately 60 cm in front of the monkey stands a large screen monitor whose surface subtends a $40° \times 30°$ viewable area. At this distance the magnetic field does not interfere with the images displayed on the screen. Animals are trained to fixate a small object (subtending 0.5°) displayed on the monitor, which is controlled by the Cambridge 2/5 Visual Stimulus generator system. This system is integrated into our customized runtime program to generate stimuli of various parameters (waveform, contrast, spatial and temporal frequency, luminance and drift direction, etc.) and to collect data that can be recorded simultaneously regarding eye movements and neuronal responses. We can generate several objects of various shapes, sizes, and colors, and their time of appearance and disappearance can be controlled individually. In the past, we have trained monkeys simply to hold fixation on an object and make saccades to, or smooth pursuit of, a second object. For more complex behaviors such as attention, we can train a monkey to make saccades to preferred areas as directed by the shape and color of the fixation object so they can attend to an area (which may or may not include the receptive field of the cell under study) specified by the fixation object. A subtle change in the attended area dictates when the monkey should saccade to the specified area. On any of these tasks, monkeys can reach a high level of competence and can satiate on its fluid intake.

2.4. Recording

Recording sessions differ from training sessions only in the additional attachment of an electrode/microdrive assembly to the recording chamber. For us, the electrode of choice is a parylene-coated tungsten electrode (Microprobe Corp.), which we customize to fit our assembly. This involves mounting the electrode first in polyimide tubing, then inserting it into a stainless-steel guide tube to increase rigidity. All the components are inserted into a 2-in., 21-gauge hypodermic needle. These electrodes have an impedance of 0.5–0.8 MΩ.

The electrode/guide tube is withdrawn into the hypodermic needle, which is then inserted through an opening in the electrode positioner mounted on the recording chamber, and it is pushed through the meninges. The electrode assembly can then be safely inserted manually into the brain. The upper portion of the electrode is fixed to a hydraulic sealed microdrive which is attached to the arm of the positioner. We can adjust the electrode positioner to comfortably get 10-mm movements in the anterior–posterior and medial–lateral axes. Advancing the electrode into the deeper parts of the brain is done remotely as is all other recording manipulations. At this point we have the option of attaching cables to bone screws on the cranium, some of which are left exposed during surgery, to record EEGs. After these preparatory measures, the animal area is darkened and closed off to maintain the integrity of visual-based paradigms and also to provide an undisturbed working environment for the animal. There are exceptions to this rule, because we sometimes need to enter the monkey area to test for ocular dominance of visual responses or to find the receptive fields of isolated units. These periods are usually brief, but these interruptions can significantly affect a monkey's behavior, and thus during training and before recordings are begun, it is important to enter the animal area and expose the subject to light flashes and the physical presence of someone in the room. Monkeys will in time learn to ignore these disturbances and continue to perform their appointed tasks.

Analog signals from the eye movement, spike recording, and EEG equipment are fed to a data acquisition board in the runtime computer where digital signals are generated and the data saved. This is all done in an adjacent room. We use standard electrophysiologic techniques to record neuronal activity. Signals are initially amplified by a high-impedance preamplifier and filtered between 100 Hz and 5 kHz. Individual action potentials recorded extracellularly, monitored on an oscilloscope and isolated through a time/voltage window discriminator, are recorded by the computer at a sampling frequency of 10 kHz.

The monkey is allowed to perform the appointed tasks while the electrode is advanced deeper into the brain. We generally test for visual responses at 500-μm increments by simply flashing a light into the monkey's eyes. The first sign of visual activity occurs at the level of the visual region of the internal capsule where a binocularly driven, multiunit visual response can be detected. This is a useful landmark indicating that the electrode is approaching the dorsal layer (layer 6) of the lateral geniculate nucleus. Once the electrode enters the lateral geniculate nucleus, there is usually an explosion of activity which is cleanly monocular and specific for the contralateral eye, confirming the location as layer 6. As the electrode is advanced, an orderly switch in ocular dominance occurs as the electrode passes through the successive layers. The monkey lateral geniculate nucleus has six layers: from ventral to dorsal these are numbered 1 to 6; the ventral two are magnocellular, and the dorsal four are parvocellular; layers 1, 4, and 6 are innervated by the contralateral eye, and the others, by the ipsilateral eye.

Once a neuron is isolated, we determine whether it is an on-center or an off-center cell, its ocularity, and the position of the receptive field on the visual display screen. At this point the testing of the experimental protocol can proceed. We describe below the effects of the state of consciousness of the animal and saccadic eye movements on the response properties of relay neurons of the lateral geniculate nucleus.

3. Experiments using the awake monkey preparation

3.1. The response of lateral geniculate nucleus neurons to state of consciousness of the monkey

Like all thalamic relay neurons, lateral geniculate nucleus cells fire in either tonic or burst mode depending on the inactivation state of voltage gated T-type Ca^{2+} channels, and these firing patterns differentially affect the relay of sensory information. However, in the behaving animal, it is less clear what behavioral traits trigger the predominance of one or the other of these modes. For many years it was assumed that there was a clear dichotomy where burst mode occurred only during deeper phases of sleep, anesthesia, and certain pathologic conditions (e.g., absence seizures), while tonic firing was the only pattern seen during awake, alert states [19]. Nonetheless, some reports have suggested that the story is not so clear-cut [20–25]. This awake monkey preparation is an ideal model to investigate this key issue further. For these relatively simple experiments, we recorded from isolated geniculate neurons while the monkey actively fixated a small target on the visual display screen or during periods of slow wave sleep. EEG was monitored during these states. Because the eye movement monitoring method based on eye coils dictates that the animal sit in a magnetic field, significant EEG interference results. Initially this was circumvented by simply switching between eye movement recording and EEG monitoring while the animal performed the alert state task. During sleep, it was not necessary to record eye movement so there was no problem during this latter state. We now overcome this problem by using amplifiers and low-pass filters in series to prevent the oscillating magnetic field from swamping the EEG signals.

The data presented in Fig. 1 are from a single geniculate relay neuron, representative of a population of 27 geniculate cells, 8 of which were magnocellular cells (M) and 19 parvocellular cells (P), during the alert state where the monkey actively fixated a target through to slow wave sleep [26].

Fig. 1 shows typical firing patterns during spontaneous activity for a geniculate cell. In the alert state (Fig. 1A), the animal was actively fixating a small visual target. During this state the EEG pattern was typically of high frequency and low amplitude where most of the firing seen is in tonic mode, but occasional burst firing is seen (*arrow*). When the animal was asleep, the EEG changed to a distinct slow ($\sim$3 Hz) high-amplitude trace (Fig. 1B), which was coupled with more bursting (*arrows*). This was, however, still interspersed with epochs of tonic firing. We noted that during sleep, cells would appear to switch intermittently between burst and brief epochs of tonic firing every few hundreds of milliseconds to several seconds. Unlike previous reports of state-dependent changes in firing patterns of thalamic neurons, we never observed a predominance of burst mode in any of the geniculate cells during any state of consciousness. Also unexpected was the finding that both modes of firing could be seen in all behavioral states tested.

Fig. 2 is an example of spontaneous firing of a lateral geniculate relay neuron when the animal was asleep and awake. As in Fig. 1, in the alert state the animal was made to perform a fixation task. The interspike interval was analyzed by plotting the interval before each spike on the abscissa and the interval after the spike on the ordinate (Figs. 2A and B). When the animal was asleep, the firing of the geniculate neuron resulted in several distinct spike clusters (Fig. 2A). The first cluster in the lower right corner consists of spikes with long prespike intervals of $\geqslant$100 ms (reflecting the

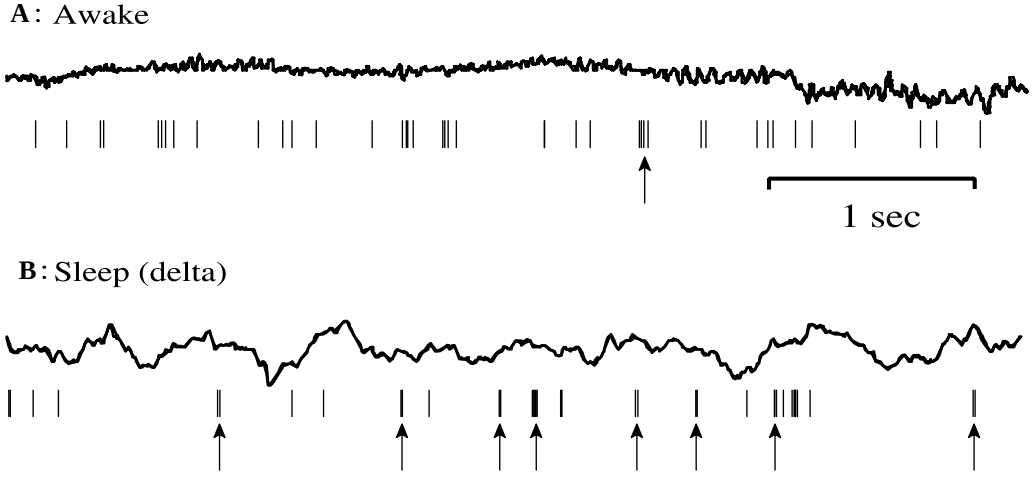

Fig. 1. Recordings from a single geniculate neuron during wakefulness and sleep. The EEG record appears above a spike raster, where a vertical line represents each action potential. The arrows depict bursts of action potentials. (A) Activity during wakefulness. (B) Activity during slow wave (delta) sleep.

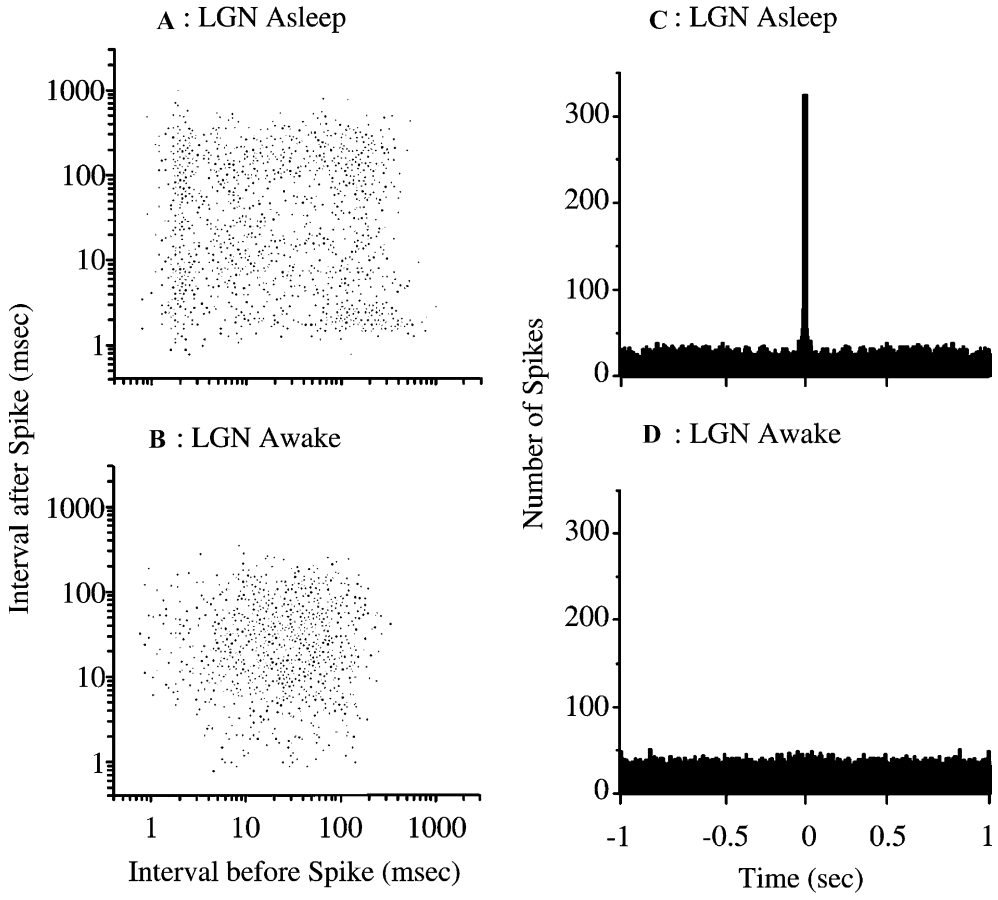

Fig. 2. Spontaneous activity of a typical geniculate cell during sleep and wakefulness. (A) Scatterplot of interspike intervals during sleep. For each action potential, this plots, the interval before versus the interval after. (B) Scatterplot of interspike intervals during wakefulness. (C) Autocorrelogram for the responses during sleep. (D) Autocorrelogram for the responses during wakefulness.

silent period preceding a burst, a requisite for the necessary cell hyperpolarization for this period to deinactivate I_T) and a short postspike interval of $\leqslant$4 ms, which is typical of the high-frequency burst. These are, therefore, the first spikes in the bursts. There was a band of spikes, along the left of the graph, with prespike intervals of $\leqslant$4 ms and postspike intervals of up to $\sim$300 ms. These are mostly the second or last spikes of bursts. All other spikes (i.e., the broad middle group) were part of tonic mode activity. Because there were far fewer bursts in this same cell during wakefulness, the burst mode clustering was not as apparent (Fig. 2B).

Because some recent studies suggested that thalamic firing patterns during sleep should be dominated by bursting that is rhythmic, we tested for rhythmicity by constructing autocorrelograms of the firing patterns and submitted these to Fourier analysis. For the geniculate cell illustrated, there was much more bursting during sleep (indicated by the large peak at −4 to +4 ms in Fig. 2C) than during wakefulness (Fig. 2D). However, there was no sign of rhythmic activity in either case (Figs. 2C, D). Thus, the bursting during sleep was arrhythmic, which is surprising in view of prior accounts. This was true for all geniculate cells studied during sleep and/or wakefulness: none showed any significant rhythmic bursting.

3.2. The effect of saccadic eye movement on the response of lateral geniculate nucleus neurons

Saccadic eye movements are high-velocity displacements of gaze made to foveate objects. Saccadic suppression is the reduced visibility that occurs during saccadic eye movements. Recent psychophysical studies have suggested that this is due to a reduction in responsiveness of magnocellular (M), but not parvocellular (P), cells of the lateral geniculate nucleus [27,28]. To address this and other phenomena of responsiveness during saccades, we recorded from geniculate neurons in the behaving monkey before, during, and after saccades. Specifically, we measured neuronal responses to a flashing, whole-field illumination [29].

For analytical purposes, each saccade trial was divided into five epochs, and the responses during each

epoch were separately analyzed (see Fig. 3A). *Epoch 1* extends from the beginning of the fixation on the target to the time when the target jumps 10° horizontally to the right; *epoch 2* extends from this point to the beginning of the eye movement; *epoch 3* extends for the duration of the saccade; *epoch 4* consists of a 50-ms period after termination of the saccade; *epoch 5* is the period from 50 ms following termination of the saccade to 300 ms. *Epochs 2–4* can be considered saccade-related, with the latter two *epochs* representing the saccade proper. *Epoch 4* is included with the saccade because responses of geniculate cells show visual latencies of ~50 ms. Thus, any responses in *epoch 4* are likely to be evoked by stimuli during the saccade. A final control consists of trials of fixation-only during which the target did not jump and so no saccades were generated. Responses during these five epochs plus the control (fixation-only) period were analyzed. We reasoned that the baseline response properties of the neurons should be similar during *epochs 1* and *5* and also during the fixation-only control periods. The cell responses per stimulus cycle were sorted into epochs, according to the epoch within which the onset of the excitatory phase landed. On-center cells responded to the bright phase of each cycle, and off-center cells to each dark phase. To simplify comparisons, we started each histogram with the excitatory phase of the stimulus (i.e., the bright cycle for on-center cells and the dark cycle for off-center cells).

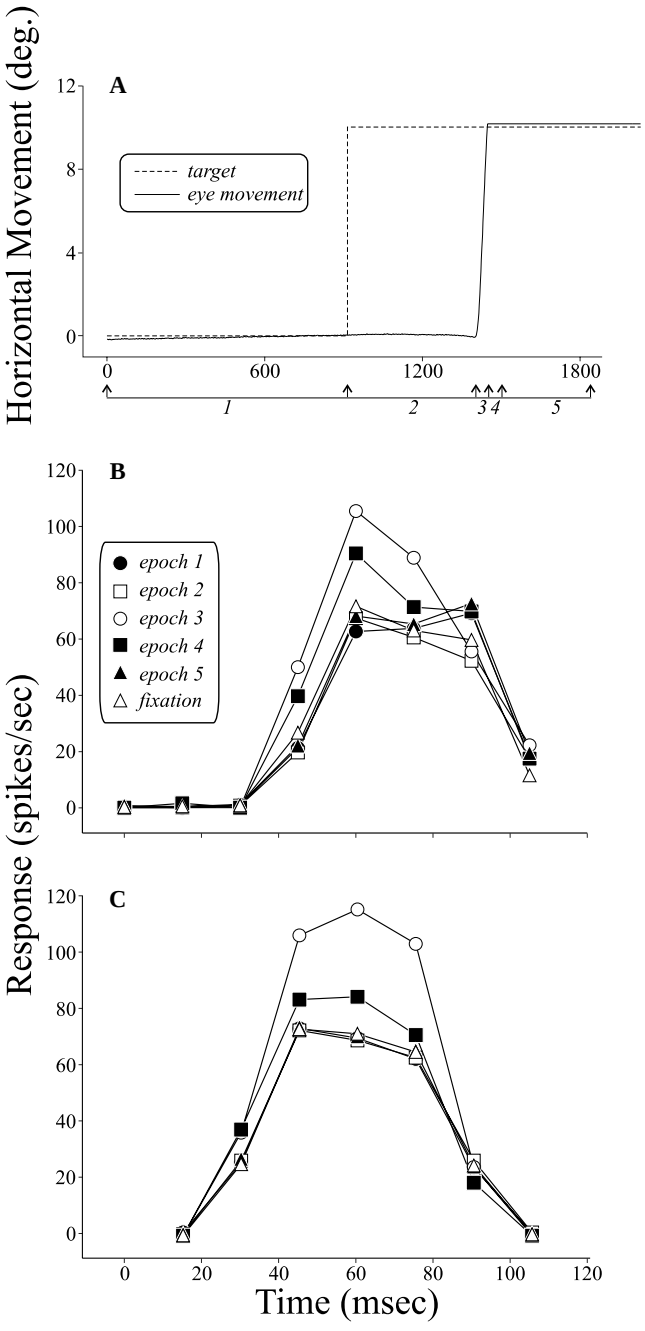

Fig. 3. Schematic of saccadic eye movement (A) plus responses of two magnocellular cells (B, C) to a full-field 8-Hz flashing stimulus. Below the schematic are shown the five *epochs* described more fully in the text, with arrows indicating boundaries between *epochs*. Analysis to a cycle of the stimulus. Each line on graphs (B) and (C) represents averaged histograms for stimulus onsets during each of the *epochs*.

Responses for all 10 M cells during simple fixation, involving *epochs 1*, *2*, *5*, and the fixation-only control period, were indistinguishable. This indicates that there was no obvious response change in *epoch 2* (i.e., the period between target movement and the beginning of the saccade). Five of the ten M cells also showed no significant response changes during the saccade itself or the period immediately afterward (*epochs 3* and *4*). However, the other five did show significant response elevation during one or both of these *epochs*. For two of these five neurons, the facilitation was significant only during *epoch 3* compared with the fixation periods ($P < 0.01$). Figs. 3B and C show the responses of these two cells. In these cases, it appears the facilitation peaked during the saccade (*epoch 3*) and tailed off in the 50-ms period after the saccade (*epoch 4*). For two others, the enhanced activity was significant in epoch 4, the period 50 ms after the eye movement ($P < 0.01$). Here it seems the enhancement in visual activity began during the eye movement and peaked during the 50 ms afterward. A fifth cell exhibited an increase in activity 50 ms after the saccade compared with the saccade ($P < 0.001$) and during periods of no eye movement ($P < 0.05$). In this case there was a small insignificant saccadic suppression followed by a large significant postsaccadic facilitation. Of the remaining five cells that did not show any significant changes between the various episodes, three showed some small tendency toward enhanced visual activity both during the saccade and extending to the earlier period after the saccade. The activity during saccades and the short period after saccades was also enhanced compared with the activity during fixation tasks. These findings are in agreement with Reppas et al. [30], who reported that monkey magnocellular geniculate neurons exhibited saccade-related facilitation.

We found no difference in the visual responsiveness between on and off magnocellular neurons. In contrast to the effects on M cells, saccades had no discernible effect on the responsiveness to the visual stimuli for P cells.

3.3. Summary

We have successfully used the awake monkey preparation to address salient questions in visual behavioral neurophysiology. Most of the procedures we used stem from earlier studies in other laboratories of cortical unit recording in primates. The cortical procedures were simply adapted to make recordings in the deeper thalamic structures of the brain in addition to designing behavioral tasks pertinent to our studies, thus proving that this preparation can be easily adapted to the requirements of most behavioral studies of a more complex nature requiring the use of primates. At present, our studies are at the stage where we have animals that are highly trained to perform relatively complex tasks recruiting attentional demands. Although attention seems an intuitively abstract concept, the tasks needed as logical controls and those that truly engage attention and can prove demanding on the monkey and on task designing. Our success with this method leads us to believe it can be readily used in other laboratories.

Appendix A. Equipment and vendors

A.1. Visual display

Mitsubishi Megaview Monitor PRO 37″ (No. 79208)
NECX Direct
4 Technology Drive
Peabody, MA 01960, USA

VSG 2/5Visual Stimulus Generator
VSGDSP Intelligent Waveform generator
Cambridge Research Unit
80 Riverside Estate
Sir Thomas Langley Road
Rochester, Kent ME2 4BH, UK

Runtime Computer $3000
Commercial vendor

A.2. Recording

Electrodes (WE 5003XXA: xx-0.8-1.0 MEG)
Polyimide tubing (MIL-33)
Micro Probe Inc.
11715 Tifton Drive
Potomac, MD 20854, USA

MDA-2 differential amplifier
DDIS-1 Dual Window Discriminator
RP-1 rack mount power supply
IMP-1 Electrode Impedance tester
Bak Electronics: P.O. Box 1600, Germantown, MD 20875, USA

Eye coil system
Riverbend Electronics
5292 Riverbend Trail
Hoover, AL 35244, USA

Remote motor drive (50-11-8B)
Hydraulic probe drive (50-12-1C)
Remote control (50-11-6)

Frederick Haer & Company 9 Main Street
Bowdoinham, ME 04008, USA

EEG DP-301 differential amplifier
Warner Instrument Corporation
1125 Dixwell Avenue Hamden, CT 06514, USA

Positioner: Model 608 chronic adapter
David Kopf Instruments
7324 Elmo Street
Tujunga, CA 91042, USA

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