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Neuronal Responses in Rabbit Cingulate Cortex Linked to Quick-Phase Eye Movements During Nystagmus

ROBERT W. SIKES, BRENT A. VOGT, AND HARVEY A. SWADLOW

Departments of Anatomy and Physiology, Boston University School of Medicine, Boston, 02118; Veterans Administration Hospital, Bedford, Massachussetts, 01730; and Department of Psychology, University of Connecticut, Storrs, Connecticut, 06268

SUMMARY AND CONCLUSIONS

1. Responses of single units in area 29 of cingulate cortex were examined in alert rabbits during vestibular and optokinetic nystagmus. Eye movements were measured by optically detecting the position of an infrared light-emitting diode attached to the cornea.

2. Fourteen percent of cingulate cells (68 of 477 isolated units) had responses that were correlated to the occurrence of quick phases. Latencies ranged from 60 ms before to 220 ms after the onset of the quick phase with a mean of 70 ms and standard deviation of 58 ms. Most units responded during or following quick phases, although four units had responses that preceded the quick-phase onset.

3. Unitary responses during quick phases were not due to visual field movement, since these responses occurred in the dark as well as the light. The responses were not dependent upon vestibular stimulation, since responses related to spontaneous saccadelike eye movements were observed in cingulate quick-phase neurons.

4. The majority (37 of 52) of the quick-phase neurons had a directional preference. Approximately equal numbers of directional units responded to quick phases directed ipsilaterally and contralaterally with respect to the recording site.

5. About one-fourth of the quick-phase units were bidirectional (15 of 52) with virtually equal responses to ipsilaterally and contralaterally directed quick phases.

6. Auditory and/or somatosensory responses were observed in only five of the quick-phase cells. All such multimodal units were bidirectional.

7. The quick-phase units were histologically confirmed to be primarily in area 29d of cingulate cortex. Although most cells were located in layer V, some were isolated in layer II-III.

8. Cingulate cortex has reciprocal connections with visual cortex and oculomotor-related thalamic nuclei and projects to the layers of the superior colliculus that are involved in oculomotor control. Responses to quick phases in cingulate neurons may synchronize cingulate cortex responsiveness with the arrival of new, and potentially significant, visual information.

INTRODUCTION

Cingulate cortex occupies much of the medial surface of the cerebral hemispheres in all mammals and, as a major component of the limbic system, is the primary cortical target of the anterior thalamic nuclei (16, 27, 29, 30, 33, 47). Cingulate cortex is divisible into distinct anterior and posterior regions through cytoarchitectural, connectional, and functional criteria.

The agranular anterior cingulate cortex is composed of areas 24 and 25 and has strong connections with the anteromedial and mediodorsal thalamic nuclei in rat and rabbit (4, 5, 14, 33, 44, 46). Stimulation and lesion studies have described several visceromotor and behavioral functions of anterior cingulate cortex including changes in respiration, blood pressure, vocalization, and complex

movements of the hands, face, and eyes (1, 8, 9, 23, 24, 38, 42).

The granular posterior cingulate cortex is composed of subdivisions of area 29, which receive thalamic input from the anterodorsal and anteroventral nuclei of the anterior thalamus, the laterodorsal and lateroposterior nuclei of the lateral thalamus, and the centrolateral nucleus of the intralaminar thalamus (30, 33, 44, 46, 47). In addition, area 29 has direct and reciprocal connections with visual cortex in rat and rabbit (45, 48).

Physiological relations between posterior cingulate cortex and sensory or motor systems have not been clearly established. Stimulation of posterior cingulate cortex evoked movements of the head, eyes, and face with little of the autonomic effects seen with anterior cingulate cortex stimulation (38). Lesions involving posterior cingulate cortex resulted in increased locomotion in both monkeys and rabbits (8, 38), with little or no autonomic effects. Thus it appears that posterior cingulate cortex is functionally distinct from anterior cingulate cortex and may be more closely related to sensory and somatomotor systems than visceral systems.

Previous reports of neuronal activity in posterior cingulate cortex, however, have not demonstrated a tight link to sensory stimuli or any link to specific motor responses. Evoked potentials and unit activity related to diffuse light flashes have been described (12, 43), but these responses were not rigorously related to stimulus presentation and did not appear to code specific stimulus parameters. In a recent study (48), posterior cingulate cells did not discharge to any of a variety of discrete visual stimuli (stationary and moving spots, bars, and edges of many different sizes) which were effective in the adjacent visual cortex. Responses to auditory stimuli were variable and nonspecific in untrained animals (18).

The present work describes a class of cingulate neurons with responses that are associated with quick-phase eye movements. These responses occur in the presence or absence of visual feedback and constitute the first demonstration of a consistent and nonhabituating physiological relation between cingulate neuronal activity and a specific motor response. A preliminary report of these findings has been presented (39).

METHODS

Recordings were made in 10 alert and unanesthetized Dutch-belted rabbits (2–3 kg). The animals were painlessly immobilized during recording by attaching a steel bar, previously cemented to the skull, to a frame designed to maximize the rabbit's field of view. All surgical procedures were conducted under full anesthesia and in aseptic conditions. A minimum of 3 days were allowed for recovery prior to recording.

Surgical procedures

The rabbits were anesthetized with either a mixture of chloral hydrate and pentobarbital (Chloropent, Fort Dodge Laboratories, Fort Dodge, IA; 3.5 ml/kg) or a mixture of ketamine and xylazine (Bristol Laboratories, Syracuse, NY; 35 mg ketamine and 5 mg xylazine/kg; 50). The scalp was additionally locally anesthetized with xylocaine (Astra Pharmaceutical Products, Worcester, MA). The dorsal cranium was exposed, and stainless steel screws were implanted in each bone plate of the skull. The screws were bonded together with dental acrylic, and a 6-cm steel bar was cemented to the skull with the acrylic. A small (1 × 4 mm) window was opened in the skull with care taken not to damage the underlying dura mater. In all of the cases in this study the opening was made over the left hemisphere. Between recording sessions, the opening was filled with a plug of sterile cotton moistened with saline containing an antibiotic and covered by a thin layer of dental acrylic. The cut edges of the skin were treated with an antibiotic and sutured around the opening. With these procedures, the scalp always healed with no complications. A minimum of 3 days were allowed for recovery prior to recording. Recordings were made in 1- to 3-h daily sessions for 2–8 wk. After recordings were completed, the animal was killed, and its brain was prepared for histological processing.

Recording techniques and eye movement production

The recording techniques used in this study have been previously described (41). Rabbits generally sit quietly when their head is restrained and consequently do not have to be adapted to the recording apparatus prior to recording. In these experiments each rabbit was placed in a stocking bag to limit the range of its movements, and its skull bar was attached to a rigid frame anchored to a thick steel base plate. The rabbit rested on a foam pad and appeared to be comfortable. The animal's head was adjusted so that the optic streak was oriented in the horizontal plane by observing the band of myelinated optic nerve fibers and the arteries entering the eye with an opthalmoscope. Care was taken to adjust the apparatus so that

there was no strain on the neck. Under such conditions, the rabbit would usually sit quietly for several hours. Although the head was fully restrained and body movements were somewhat limited, the rabbit could indicate displeasure or boredom by fidgeting. The experiment was suspended if this occurred.

Glass-coated, tungsten steel electrodes (5–10 μm tips) were lowered through intact dura mater to isolate single units in cingulate cortex. To enhance the probability of locating eye movement-related units, the electrode was advanced in small 10- to 20-μm steps, and quick phases were elicited with vestibular stimulation after each step. The presence of a quick-phase-related unit could often be detected first by listening to the multicellular activity on the audio monitor. The electrode was then advanced and withdrawn while the animal was continuously oscillated until the unit was maximally isolated. Only well-isolated units with qualitatively clear changes in activity associated with quick phases were stored on FM magnetic tape for subsequent analysis. Units with no apparent response associated with eye movements were qualitatively evaluated for responses to auditory, visual, and somatosensory stimuli (see below). The location of the physiological border with adjacent visual cortex was determined by mapping receptive fields in visual cortex and locating the transition to the visually nonresponsive cingulate cortex as previously described (48). All recordings reported in this article were made medial to this border.

Eye movements were recorded with a photosensitive X-Y position detector (United Detector Technology, SC-50), which detected the position of a narrow beam infrared light-emitting diode attached with a suction cup to the eye ipsilateral to the recording site (3). The cornea of this eye was anesthetized with proparacaine (Alcon Laboratories, Fort Worth, TX). The cup was frequently removed and the cornea was moistened and reanesthetized during each experiment. This procedure appeared to block nociceptive input from the cornea, since the rabbits did not blink excessively during the recording sessions. The eye movement measurements were calibrated by rotating the eye through a known angle and observing the resulting electrical signal. The accuracy of this system was approximately 1°, which was adequate for detecting quick phases. Eye movement signals were amplified with direct coupling and stored on FM magnetic tape. Since only eye movement was recorded, the relation of cingulate unit activity to other movements such as jaw or neck movement could not be determined.

Eye movements were elicited by either sinusoidal oscillation of the animal at amplitudes from 5 to 30° at rates of 1–20°/s to elicit the vestibular nystagmus (VN) in both dim light and darkness or by rotating a 1 m optokinetic drum about the animal at rates of from 0.1 to 10°/s to produce optokinetic nystagmus (OKN). Although rabbits make spontaneous saccadelike eye movements infrequently when the head is fixed (41), these movements were occasionally observed, and unit responses related to these movements were studied.

The isolated units were also tested for responses to sensory stimuli. Visual stimulation consisted of diffuse light flashes, stationary and moving spots, or bars of light in various sizes that were projected onto a tangent screen and whole visual field movement produced by step movements of the optokinetic drum. Auditory stimuli were noise bursts of varying amplitude and duration. Somatosensory responses were elicited by touching or stroking the rabbit with cotton applicator sticks. No attempt was made to quantify the force of the pressure applied. The stimulus was applied to the eyelids, regions of the face and ears, and generally to the limbs and body. The latter areas, however, were covered by a stocking, and precise localization could not be determined. It was not possible to passively manipulate the eye in these alert rabbits without disturbing them. Therefore, the effects of proprioceptive afferents in the orbit could not be evaluated.

Data analysis

Unitary responses were amplified and discriminated by voltage amplitude. Quick-phase onset and offset were automatically detected by a computer program which located the abrupt change in eye velocity that occurs at the beginning or end of a quick phase. The eye position voltages were first smoothed with a 12-ms moving average to reduce noise. The onset was defined as the moment when average eye velocity exceeded 25°/s (sampled at 500 Hz). The offset was the moment at which the average velocity fell below 25°/s. Artifacts due to eye blinks were manually rejected. These onsets and offsets were accurate to within 10 ms.

One-second peri-quick-phase records of unitary responses and eye movement measurements were saved for 10–50 quick phases in each direction for units under three main conditions: VN in dim light, VN in total darkness, and OKN in the light. Some cells, however, were tested in only one or two of these conditions. A minimum of 10 quick phases in each direction, during at least one of the above conditions, were recorded for all quantitatively analyzed units.

Neuronal responses were displayed as rasters and histograms of the number of spikes per 10-ms bin. Descriptive statistics were calculated using all data collected from each unit. Latency of the response was measured as the time from quick-

phase onset or offset to the first bin that contained a number of spikes statistically greater than base line. This was accomplished by first calculating base line as the average spike per 10-ms bin in the initial 300 ms of the raster for all collected sweeps. Next, the number of spikes per bin corresponding to the 99% level of a Poisson distribution with a mean equal to the base-line average was determined. The first bin that exceeded this number was found, and latency was defined as the time from quick-phase onset to this bin. In cases where the base-line average was less than one, a threshold of three spikes was required.

The strength (S) of the response to quick phases in each direction was measured as the number of spikes in the 300-ms period following the initiation of unit responses divided by the number of spikes during the base-line period. Since many cells were found to have a directional preference, the degree of the directionality of the response was quantified by calculating a directionality index (DI) from the strengths of the responses when the quick phase is directed contralaterally (C) and ipsilaterally (I)

$$DI = \frac{S_c - S_i}{S_c + S_i}$$

The DI of units with at least a moderate spontaneous rate of discharge agreed well with qualitative assessments of directionality in three classes: bidirectional (i.e., no preferred direction) $|DI| \leq 0.15$; weak contralateral (positive) or ipsilateral (negative) directional response $0.15 < |DI| \leq 0.35$; increasingly strong and specific directional responses $|DI| > 0.35$.

Histological analysis

After testing was completed, the location of some quick-phase units was marked with a small (50–300 μm in diameter) electrolytic lesion (−10 μA, 10–20 s). At the end of the experiment larger lesions (−50 μA, 10–30 s) were made to mark the border with visual cortex. The animal was killed with an overdose of Chloropent and perfused with physiological saline followed by 10% Formalin. Following several days of postfixation in Formalin, the brain was removed from the skull with care taken to keep the dorsal skull bones intact. The location of the opening in the skull could thus be precisely located in terms of the skull sutures. The blocks of the brain beneath the opening were removed and embedded in celloidin. The blocks were serially sectioned at a 30 μm thickness, and every section was saved and stained with cresyl violet. The sections were mounted on slides, and the location of all lesions and many electrode tracks was determined.

Cytoarchitecture

Posterior cingulate cortex in rabbit is composed of five subdivisions of area 29 (48) of which three (areas 29d, 29c and 29b) were systematically explored for neurons with discharges associated with eye movements. See Fig. 8 for the topographical distribution of these areas.

Area 29d shares a border with area 17 of visual cortex. This border lies just medial to the splenial sulcus and has been characterized both cytoarchitecturally and electrophysiologically (48). Of particular note is the fact that layers II–IV are much more condensed in area 29d than in the medial parts of area 17. Area 29c lies medial and ventral to area 29d and, like areas 29b, 29a and 29e, is granular cortex. Thus area 29c has a densely packed layer II–III, which is composed mainly of small and fusiform pyramids, and a layer IV containing less densely packed small and fusiform pyramids. Area 29b is distinguished from area 29c by its broader layer II–III and by its homogeneous and more cell dense layer V.

RESULTS

I. Classification of units

Discharges were studied in 477 isolated units of which 417 were histologically verified to have been in area 29 of posterior cingulate cortex. The remaining 60 cells were localized to cingulate cortex based on their position medial to the splenial sulcus and the medial border of visual cortex as defined by receptive-field mapping. The topographic distribution of posterior cingulate areas is briefly described in METHODS. Of the sampled cells, 68 or ~14% were found to have responses closely linked with quick-phase eye movements generated by either vestibular and/or optokinetic nystagmus. None of the responses of units in area 29 were influenced by the velocity or direction of slow-phase eye movements or position of the eye in the orbit.

II. Quick-phase responses

Examples of neurons with responses linked to quick phases are shown in Fig. 1. The first of these three units responded with a marked increase in action-potential frequency following contralaterally directed quick phases (left panel). This unit, however, had only a weak and variable response to ipsilateral eye movements. The second cell reliably responded during contralateral quick phases with no responses to ipsilateral movements. The cell in Fig. 1*C* responded during quick phases in each direction with a slightly more vigorous response in the ipsilateral direction.

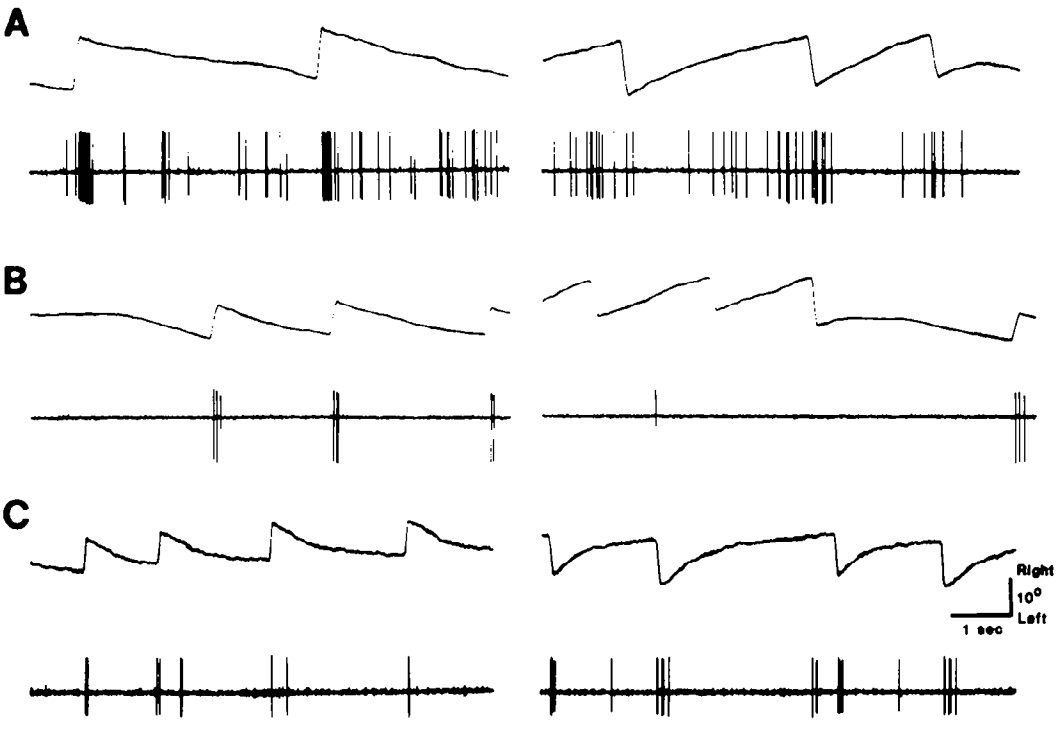

FIG. 1. Examples of 3 units in cingulate cortex with responses linked to quick-phase eye movements during vestibular stimulation in total darkness. The *top trace* in each section shows eye position, and the *lower trace* shows unit activity. Quick phases directed contralaterally (directed to the right; upward movement of the *top trace*) and ipsilaterally (directed to the left) are shown. *A*: a directional unit with a moderate preference for contralaterally directed quick phases. *B*: another directional unit with a reliable response to contralateral quick phases and no response to ipsilateral quick phases. *C*: a bidirectional unit with reliable responses to quick phases in each direction but a slight increase in response strength following ipsilateral quick phases.

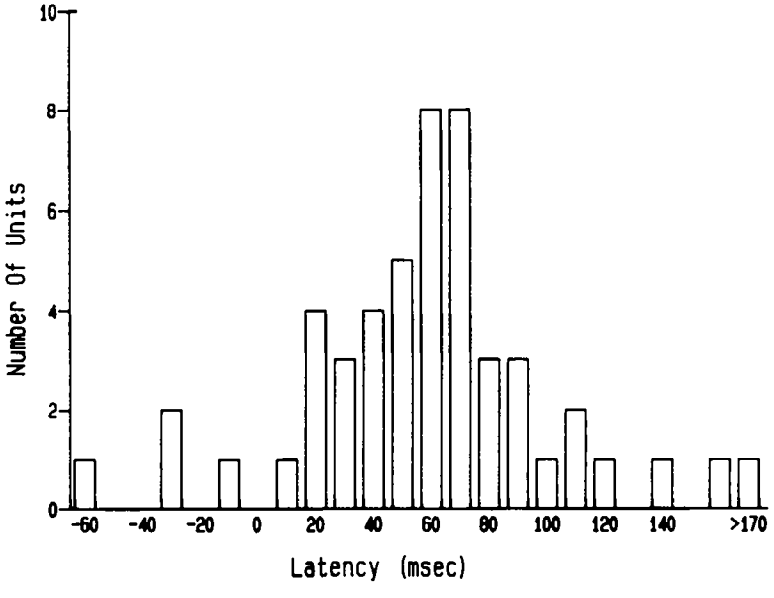

FIG. 2. Frequency histogram of unit response latencies related to quick-phase onset. Zero represents quick-phase onset.

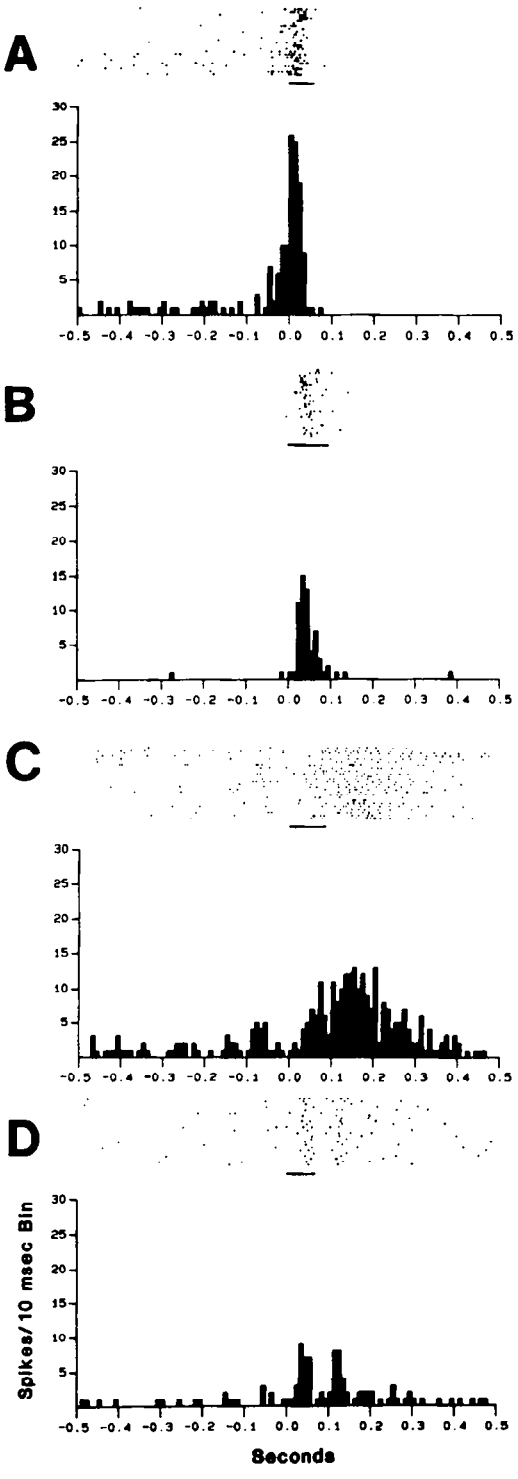

FIG. 3. Examples of units with different latencies. Peri-quick-phase rasters of unit responses and histograms showing the number of spikes/10-ms bin associated with 25 quick phases. The beginning of *bar* in center shows quick-phase onset, and its length indicates average quick-phase duration across all quick phases. *A*: a unit with an increase in response rate prior to quick phase. *B*: a unit with an intermediate latency and a short burst during the quick phase. *C*: a longer-latency neuron with sustained burst. *D*: a cell with a biphasic response.

Quantitative analysis was performed on 52 units for which 10 or more quick phases were recorded in each direction. The units were evaluated on the basis of the latency of the neuronal response to quick-phase onset and offset and in terms of a directionality index (see METHODS).

A. LATENCY. A frequency histogram of unit latencies to quick-phase onset in the preferred direction is presented in Fig. 2. These cells had latencies ranging between −60 and 220 ms with a mean of 70 ± 58 (SD) ms. The distribution was essentially unimodal and approximately normal.

The majority of units responded with an increase in activity, which lasted between 100 and 200 ms (mean 163 ms). There was considerable variability, however, in the duration of the response [85 (SD) ms]. Three units responded with only one or two discharges after each quick phase, whereas three other units responded with bursts lasting >300 ms. Units with shorter latencies tended to have longer bursts. The correlation between latency and burst duration (measured in the preferred direction) was −0.583, which was statistically significant at the $P = 0.05$ level. The frequency of the bursts was low when compared with oculomotor units in the brain stem and was quite variable [mean 67 ± 40 (SD) impulses/s].

Examples of rasters from units that responded before, during, and after the quick phase are in Fig. 3. The cell in Fig. 3*A* increased its activity 40 ms prior to the quick phase, reaching peak activity within the first 10 ms after quick-phase onset. Its rate of discharging then quickly fell below base line: the cell fired only once during the 500-ms periods following the quick phase. Unit responses that preceded quick-phase onset were rarely observed in cingulate cortex. Indeed, only four cells had a statistically significant increase in discharge rate prior to quick-phase onset.

An example of a more typical response is in Fig. 3*B*, where the cell increased its activity 40 ms after quick-phase onset. This unit was virtually silent during the slow phase of vestibular nystagmus but discharged briskly during the quick phase, reaching a peak response at 50 ms and falling back to its low base-line level at quick-phase offset. Approximately one-third of the units (15 units) had responses similar to this cell.

The majority of cingulate quick-phase neurons (33 units) had longer duration responses to quick phases similar to the cell presented in Fig. 3*C*. This response began at 60 ms and gradually increased so that the burst did not have the abrupt onset of the previous units. The response reached a peak 60 ms after quick-phase offset and slowly decayed to base line.

The cell in Fig. 3*D* had a biphasic response, which was occasionally observed (10 units) particularly in weakly responding units or in units that were stimulated in the nonpreferred direction. This cell had an initial latency of 50 ms and a second period of increased rate of discharge at 120 ms.

Three units (not illustrated) were observed with a pure decrease in activity. The spontaneous activity fell to virtually zero during the quick phase and remained at this level for 100–500 ms. No obvious rebound excitation was observed in these neurons.

There was no statistically significant relation between the unit latency or burst duration and the size of the quick phase (r = 0.076 and r = −0.071, respectively, in the preferred direction). The quick phases were usually close in size, however, making these relations difficult to test. In a few cases, quick phases of markedly different sizes were observed, and the latency and strength of the unit's burst tended to increase with the quick-phase amplitude.

B. DIRECTIONALITY. Quick-phase units varied considerably in terms of their directional specificity. The majority of units responded to quick phases made to both the contralateral and ipsilateral direction, but there was often an imbalance in the strength of the response. To quantify the degree of directionality, DI was calculated for each cell (see METHODS). A frequency histogram of these indices is in Figure 4. Fifteen of the units had virtually identical responses in each direction of eye movement ($|DI| \leq +0.15$) and were considered to be bidirectional. An additional 19 units had a slight

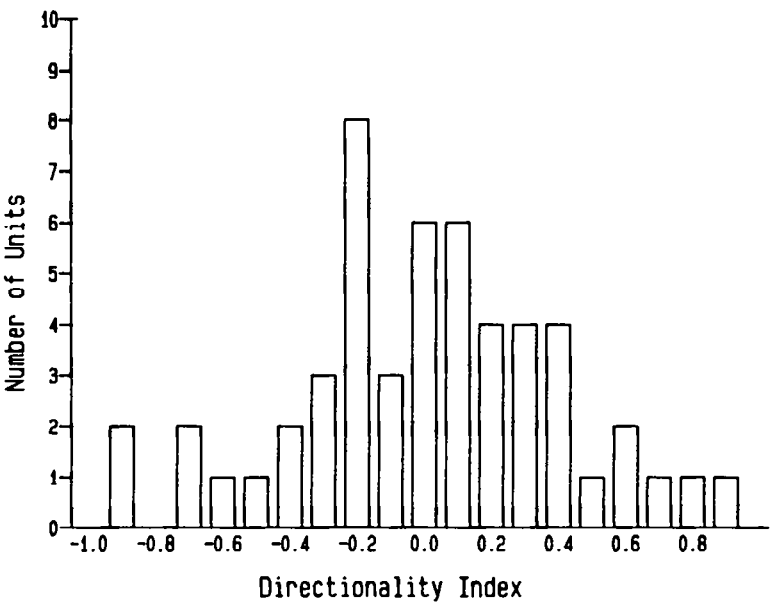

FIG. 4. Frequency histogram of directionality of units. The degree of directional preference is quantified in the magnitude of a directionality index in which negative values denote ipsilateral preference.

directional preference ($0.15 < |DI| \leq 0.35$), whereas the remaining 18 units had a clear directional preference ($|DI| > 0.35$). Contralateral and ipsilateral directional units were present in approximately equal numbers (18 contralateral, 19 ipsilateral).

Examples of directional and bidirectional neurons are shown in Fig. 5. These cells represent the extremes of each category. Ipsilateral and contralateral direction-sensitive neurons (Fig. 5, *A* and *C*, respectively) had large-magnitude DIs of −0.87 and +0.75, re-

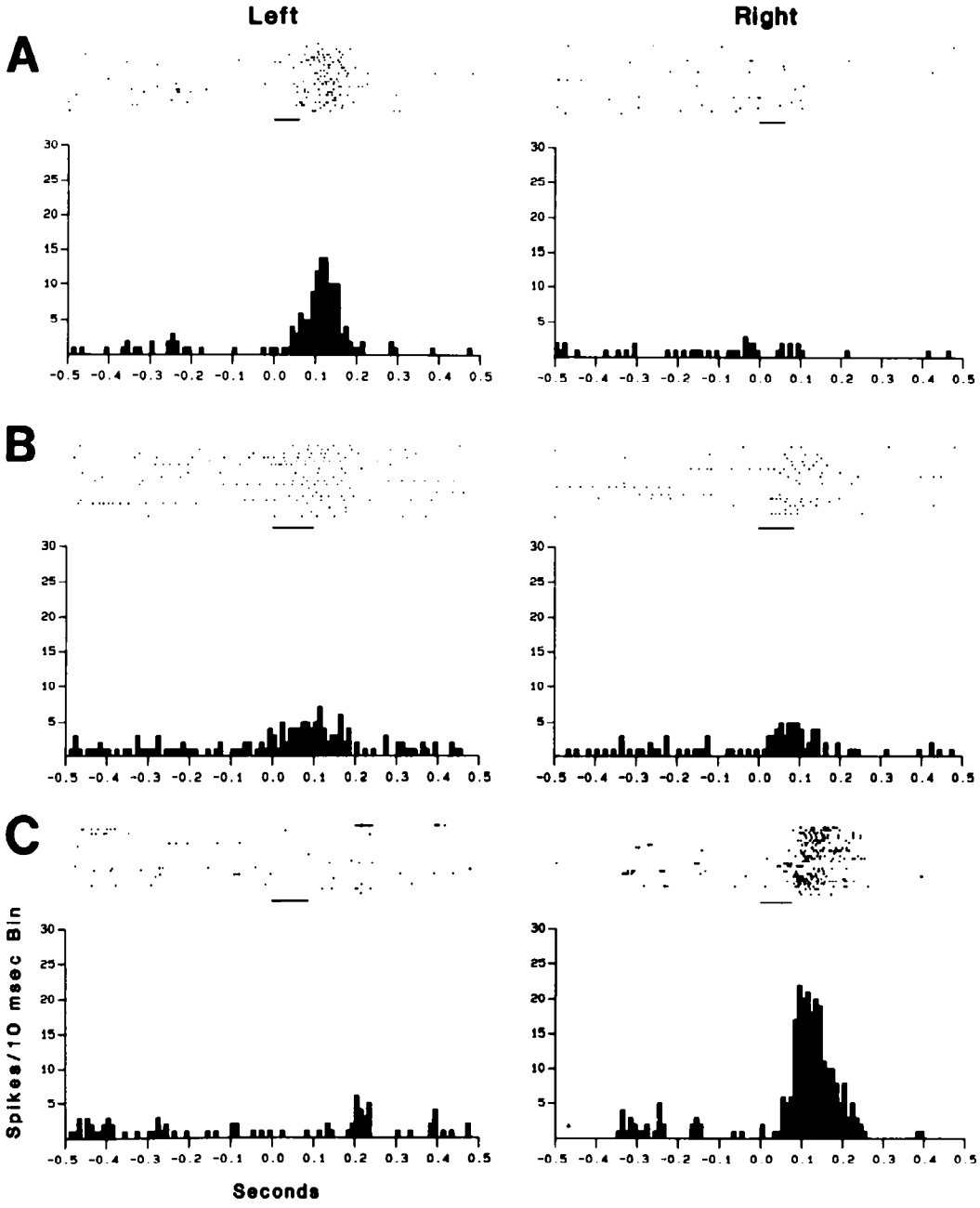

FIG. 5. Examples of units that differ in directionality (DI). *A*: ipsilateral (*left*) directional unit (DI = −0.87). *B*: bidirectional unit (DI = −0.03). *C*: contralateral (*right*) directional unit (DI = 0.75).

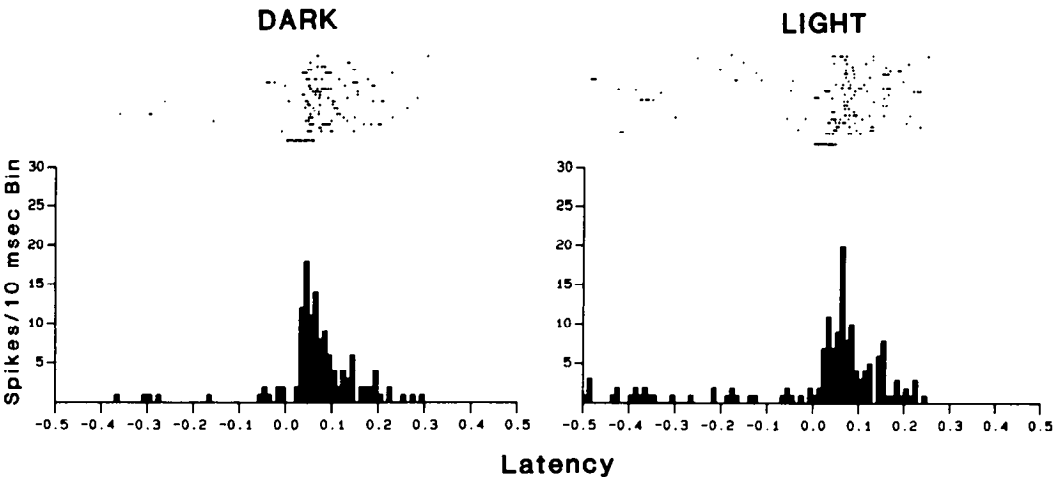

FIG. 6. Comparison of neuronal responses to vestibular nystagmus-generated quick phases in the dark vs. light.

spectively, whereas the bidirectional neuron (Fig. 5*B*) had a DI of only −0.03. As was generally the case, the responses of the directional neurons were more vigorous than those of the bidirectional neurons.

III. Responses to other sensory modalities

Although no visual responses were observed in any of the quick-phase units, it seemed possible that responses to eye movements might be altered by visual field movement. Consequently, in order to discount the role of such visual stimuli, units were often tested in the dark as well as light. Sixteen units were fully tested in dark and light using vestibular stimulation. No significant differences were observed in the latency of the response. The mean difference between dark and light latency was 0 ± 10.1 (SE) ms (paired t test; $t = 0.66$, $P > 0.05$). Directionality was similarly equivalent in both light and dark. The mean difference and standard error between dark and light DI was 0.004 ± 0.126 ms ($t = 0.98$, $P > 0.05$).

Figure 6 shows responses of a unit to VN-elicited quick phases in both dark and light. The unit responses in the dark were clearly correlated with the occurrence of the quick phase and were similar in latency to the responses in the light. The slight increase in spontaneous discharging in the light was not consistently observed in all 16 units.

Unit responses to quick phases did not depend on vestibular input, since unit responses occurred in association with quick phases elicited by nonvestibular stimulation. Optokinetic stimulation in 11 units produced equivalent responses to those generated by VN. Furthermore, responses from six units were recorded during spontaneous saccadelike eye movements without head movement. A response to the saccade was always observed when it was in the unit's preferred direction for quick phases. No units were observed to respond in phase with low-amplitude (<5°) sinusoidal vestibular stimulation that might be expected to produce a response in units which detect a vestibular signal.

TABLE 1. *Responses of quick-phase and non-quick-phase units*

Quick-phase units (n = 52)	*Bidirectional*	*Directional*
Auditory	2	0
Somatosensory	2	0
Auditory and somatosensory	1	0
Visual	0	0
Total	5	0
Non-quick-phase units (n = 360)		
Auditory	8	
Somatosensory	3	
Auditory and somatosensory	9	
Visual	0	
Total	20	

n, No. of units; n = 15 for bidirectional and 37 for directional units.

Some bidirectional quick-phase neurons responded to auditory and/or somatosensory stimuli (5 of 15 bidirectional units; Table 1). Two quick-phase units were located with clear responses to auditory stimuli (clicks) and two others had somatosensory responses. One other cell responded to both modalities. Stimulation of the face, eyelids, vibrissae, or regions near the head seemed to be particularly powerful, although responses could be elicited by stimulation of the body. Since we were not able to directly manipulate the eye in these alert rabbits, the effects of proprioceptive input from the orbit could not be evaluated.

None of the directional units responded to these sensory modalities (38 units tested). This difference between directional and bidirectional units was statistically significant ($\chi^2 = 5.0$, df = 1; $P < 0.05$).

Comparing the quick-phase with non-quick-phase units, 5 of the 52 quick-phase units versus 20 of the 360 non-quick-phase units had sensory responses (Table 1). This difference, however, is not statistically significant ($\chi^2 = 1.13$, df = 1; $P > 0.05$).

An example of a multimodal cell is in Fig. 7. The first trace (Fig. 7*A*) is the audio channel showing the amplitude and duration of the click, whereas the second trace is the unit response. Although no eye movements were evoked by the auditory stimulus, the unit discharged 70 ms following auditory stimulus onset with a 470-ms duration burst. With vestibular stimulation, the cell responded following quick phases in both directions (Fig. 7, *B* and *C*, respectively).

Approximately half of the cingulate units had irregular bursts of activity that were not linked to eye movements (23 of 52 units). An example of these bursts can be seen in Fig. 1, *A* and *B*, and 5*C*. In these examples, the bursts were rather rare, but in some units the bursts were much more frequent, occurring once or twice during each sweep. Although the source of this activity was not determined, it did not appear that the bursts resulted from stray auditory, somatosensory, or visual stimuli, because these modalities were controlled during the experiment. Similar bursts were observed in other thalamic and cortical eye movement units (see DISCUSSION).

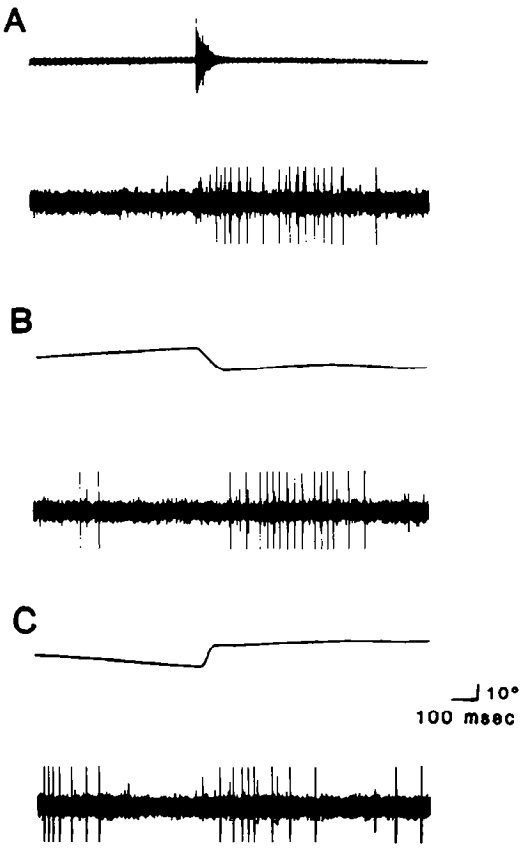

FIG. 7. Multimodal unit in cingulate cortex. *A*: *top trace*; amplitude and duration of a click. *Bottom trace*: unit response to click. Eyes did not move during sweep. *B* and *C*: *top trace*; eye position. *Bottom trace*: unit response during ipsi- and contralaterally directed quick phases, respectively.

IV. Histologic localization of units

Of the 65 quick-phase-related units, 16 were located precisely by microlesions and 39 were localized approximately by their electrode track or an adjacent track. A summary diagram of all microlesions is in Fig. 8. All of these lesions were in area 29d except for one in area 29c (Fig. 8*F*). It should be noted, however, that areas 29d and 29c were most frequently explored. Fewer probes extended into area 29b and no probes were made into areas 29a or 29e. Lesion sites were most frequent in deep layer V. Fewer lesions were in layer II–III and only one was clearly localized to layer VI. The functional classes of quick-phase neurons did not appear to be

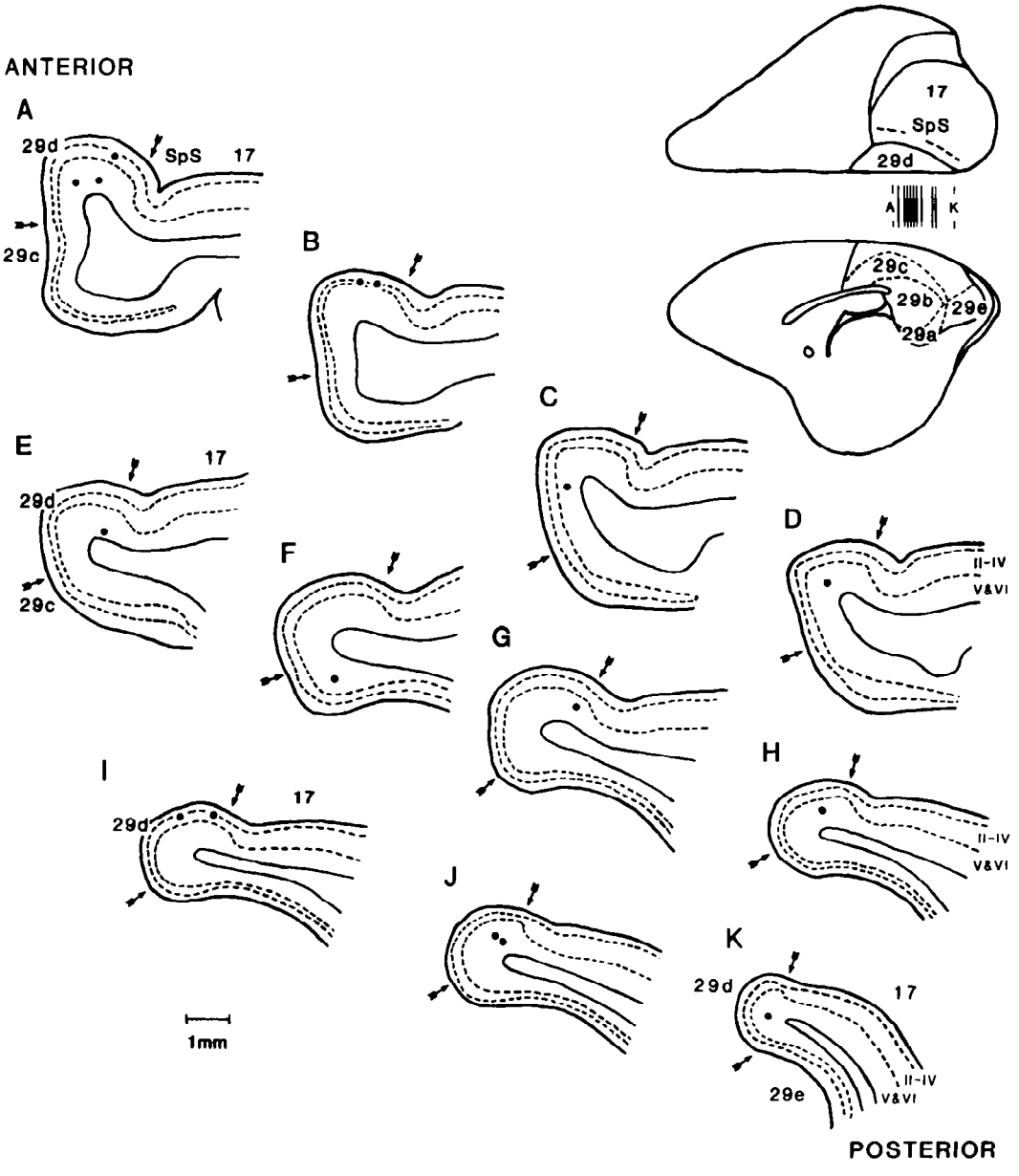

FIG. 8. Distribution of quick-phase units which were verified with microlesions. —— on brain surface drawings, cytoarchitectural areas of posterior cortex; ---- labeled SpS indicates the location of the splenial sulcus and subdivisions of area 29. Levels *A* to *K* indicate anterior-posterior levels at which coronal sections are illustrated. †, Location of border of area 29d with visual cortex (area 17) and area 29c. •, Location of microlesions.

segregated into different layers, since cells with both short and long latencies and cells in each directional class (e.g., ipsilateral directional, contralateral directional, and bidirectional) were observed in both layers II–III and layer V.

DISCUSSION

Previous investigations of the response properties of cingulate cortex neurons have described only desultory relations with sensory systems and no relations to particular

motor responses. The present study demonstrates for the first time that a significant population of cingulate cortical neurons have response properties that are clearly correlated to a specific motor response.

The responses of many of these cingulate neurons (47 of 52) were specifically related to the occurrence of quick-phase eye movements and were not generalized responses to sensory or motor events. In all of the directional quick-phase units and most of the bidirectional quick-phase units, the only observed event that predicted an increase in unit activity was the occurrence of the quick phase. These units were unresponsive to visual, auditory, and somatosensory stimulation. Eye movement-related discharges were reliably observed across quick phases and did not habituate as did the responses of some cingulate neurons to nonreinforced sensory stimuli (18).

It should be noted, however, that these responses were somewhat variable when compared with the responses seen in oculomotor neurons of the brain stem, where neuronal responses accurately encode the magnitude and velocity of eye movements (see 17 and 31, for review). In some cingulate neurons, a single burst did not always indicate the occurrence of a quick phase, because these neurons produced spontaneous bursts of irregular duration and latency while the eye was stationary. In such cases it was necessary to view the unit's activity across several quick phases in order to detect a clear relation between neuronal activity and eye movement. Nevertheless, these neurons allow cingulate cortex to accurately detect quick phases, since the summed output of a small pool of these cells would produce a clear quick-phase signal. Spontaneous bursts of irregular duration and latency can also be seen in the saccade-related neurons of the thalamus (37) and parietal cortex (32). In the frontal eye fields, a dissociation between presaccadic neuron activity and saccade initiation was observed in that the cells would fire when the animal failed to make a learned saccade, as well as when it did make the movement (7). Thus, in the main oculomotor areas of the thalamus and cortex, neuronal activity is highly correlated with saccadic eye movements, but not invariably linked to it.

Most cingulate neurons responded to the quick phase after its onset (mean = 70 ms). In this respect, cingulate neuronal responses were similar to frontal cortex units, which also responded primarily after quick phases and spontaneous saccades (6, 7) and were unlike frontal and parietal cortex units, which responded prior to learned-saccades (7, 26, 32). Although frontal and parietal eye movement-related units had visual responses in monkeys (7, 32), none of the cingulate neurons could be driven with any of a variety of visual stimuli.

Cingulate neurons appeared to encode a specific characteristic of the quick phases: direction. In many cases, units responded exclusively to movements in one direction, and most other neurons had a definite directional preference. Similar directional specificity has been reported in the parietal and frontal cortex saccade-related units. Forty-four percent of parietal saccade units were found to be unidirectional and another 36% of these cells show some directional preference (26). In the frontal eye fields, presaccade units were broadly tuned with respect to direction but each cell had a preferred direction (7). Since only horizontal stimulation was used in this study, units with vertical or oblique preference could not be detected, which may have resulted in a low estimate of the size and response strengths of the directional units.

These observations clearly show that posterior cingulate cortex receives an eye movement signal related to quick phases. The source of this signal is unknown, however, a likely source is the dorsal thalamus. The intralaminar and lateral thalamic nuclei have strong connections with cingulate cortex (2, 22, 30, 47) and, in cat and monkey, contain neurons with eye movement-related responses (36, 37). The physiological properties of neurons in these nuclei have not been examined in rabbit, but they receive input from the deep layers of the superior colliculus and nucleus of the optic tract (21) and may, therefore, have neurons that respond during eye movements.

In cat and monkey the intralaminar and lateral thalamus contain three types of units:

burst, pause-rebound, and eye position units (37). The cingulate neurons were similar to the burst neurons in that most cingulate units responded with a fairly low-frequency burst of spikes before or during quick phases, and the majority of these units had a clear on-direction. However, fewer cingulate cell responses preceded the quick phase (only 4 of 52 with a clear increase prior to the quick phase vs. 63% in thalamus; 36). Since the thalamic burst units typically reached peak activity after saccade onset, the longer latency of cingulate neurons may indicate a high threshold to thalamic input. A higher proportion of bidirectional responses were observed in cingulate cortex than in the thalamus (28 vs. 7%, respectively; 37). Since the previous study evaluated directionality qualitatively, it is not clear whether or not these measurements can be compared directly. In both the cingulate cortex and thalamus, ~10% of the eye movement-related neurons responded to sensory stimuli (36). It was not reported whether or not the thalamic "complex" neurons were more likely to be bidirectional.

Should cingulate cortex be considered a limbic component of the oculomotor system? Although the data necessary to support this hypothesis is sparse, anatomical studies indicate that cingulate cortex has direct connections with the oculomotor system. Both anterior (13, 25, 34, 51) and posterior (13, 39) cingulate cortex project to the deep layers of the superior colliculus where neurons that project to oculomotor control areas in the thalamus and brain stem are located (15, 21). Electrical stimulation of these layers of the superior colliculus produces eye movements in many species (10, 19, 28). Cingulate cortex might further influence eye movements through its direct and topographically organized projections to the ventral pons (13, 51).

Although few physiological studies have examined the role of cingulate cortex in eye movement, electrical stimulation of posterior cingulate cortex in monkeys produces movements of the eyes and head (38). In humans, electrical stimulation of anterior cingulate cortex occasionally produces saccadic eye movements (42). The effects of cingulate cortex lesions on eye movements have not been determined; however, cingulate lesions produce a contralateral neglect syndrome in monkeys (49). These ablations were thought to disrupt attentional mechanisms mediated through cingulate projections to the brain stem. The sensory neglect, observed in these cases, might result in decreases in contralaterally directed saccadic eye movements and produce deficits similar to those seen following frontal eye-field lesions (35).

What might be the function of cingulate cortex in oculomotor-related responses? It is unlikely that cingulate cortex plays a role in stabilization of the retina for this appears to be reflexive and controlled in the brain stem. Indeed, total cortical ablations did not effect OKN in rabbits (20). In the afoveate rabbit, cingulate cortex would not be expected to be involved in targeting or tracking eye movements, since these movements are rarely, if ever, made (11). Indeed, the majority of cingulate cells responded after the quick-phase onset, so this activity is not likely to represent a motor control signal.

Instead, the activity in cingulate neurons may represent the arrival of a quick-phase-related corollary discharge signal in the cortex. This signal might modify the response of cells to sensory stimuli. Although cingulate cortex receives direct input from visual cortex (45, 48), cingulate neurons in rabbits do not discharge spikes to discrete visual stimuli when the eye is stationary (48). Since the occurrence of a quick phase predicts that a new visual field will arrive at the end of the quick phase (40), cingulate visual responses might be enhanced immediately after the movement; allowing each new visual field to be quickly scanned for objects with behavioral significance. As mentioned above, enhancement of visual responses in relation to saccades have been reported in the parietal and frontal cortical regions (7, 32), and this enhancement has been interpreted as a component of visual attention (32). If additional studies demonstrate a quick-phase enhancement of cingulate visual responses, this enhancement may represent a phylogenetically primitive anlage of visual attentional mechanisms.

Due to the lack of basic behavioral information relating cingulate cortex and eye movement, it is presently difficult to ascertain the function of the quick-phase neurons in cingulate cortex. Our finding of a relation between cingulate neuronal responses and quick-phase eye movements combined with studies that demonstrate anatomical connections between cingulate and both visual and oculomotor areas of the brain suggest that cingulate cortex may link the limbic system with the visual and oculomotor systems.

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Reprint requests to: B. A. Vogt, Dept. of Anatomy, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118.

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