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RESEARCH ARTICLE

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Influence of the superior colliculus on the primate blink reflex

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Abstract In this study we used microstimulation to investigate the influence of the superior colliculus on the trigeminal blink reflex. We report that stimulation in the intermediate to deep layers of the tectum produced inhibition of reflex blinks at a latency of approximately 26 ms. We considered the hypothesis that the blink inhibition was mediated via the omnipause neurons (OPNs) of the eye movement control system in the brainstem. Our results show that the least effective sites for suppression were in the rostral colliculus. This is inconsistent with the prediction that OPNs should be maximally recruited from the rostral tectum near the "fixation zone." From these points and other considerations, we conclude that the reflex blink suppression from the superior colliculus is not directly mediated by the OPNs or the saccadic eye movement circuits.

Key words Trigeminal · Omnipause · OPN · Saccade · Monkey

Introduction

The superior colliculus is an anatomically and physiologically complex structure involved in mulitmodal sensorimotor functions (Cusick 1988; Grantyn 1989; Sparks and Mays 1990; Galiana and Guitton 1992; Peck et al. 1993; Stein et al. 1993). Recent work from Basso et al. (1996) has shown that microstimulation of the superior colliculus transiently suppresses trigeminal reflex blinks in rats. Conversely, increasing inhibition of superior colliculus with microinjections of muscimol increases the excitability of trigeminal reflex blinks. These observations in rodents show that the superior colliculus excites tonically active neurons that inhibit reflex blink circuits. Nevertheless, the neuronal elements in the colliculus that are responsible for these effects are unclear. Collicular function is perhaps best understood for its involvement in oculomotor control (e.g., Sparks and Mays 1990), especially in primates, where behavioral control is optimal. Thus, we sought to investigate the involvement of the superior colliculus in the blink reflex of primates, where we could address specific hypotheses about the relation of reflex blink suppression to the known physiology and anatomy of the primate collicular and oculomotor systems.

One possible substrate for mediating the collicular suppression of reflex blinks is a class of tonic, inhibitory cells in the pons: the omnipause neurons (OPNs). Most OPNs are contained within the nucleus raphe interpositus (rip; Strassman et al. 1987; Büttner-Ennever et al. 1988), a putative glycinergic (Horn et al. 1994) cell group at the midline in the caudal pons. The superior colliculus directly excites these neurons with short latency (Raybourn and Keller 1977; King et al. 1980; Kaneko and Fuchs 1982; Paré and Guitton 1994). In turn, the OPNs inhibit another class of neurons necessary for saccadic eye movements, burst neurons in the brainstem reticular formation (Nakao et al. 1980, 1988; Evinger et al. 1982; Furuya and Markham 1982; Curthoys et al. 1984). Consistent with this role for reflex blinks, the tonic activity of OPNs has been shown to pause during blinks in primates (Cohen and Henn 1972; Fuchs et al. 1991; Mays and Morrisse 1994). Furthermore, microstimulation of OPNs suppresses reflex blinks in both monkeys (Mays and Morrisse 1995) and rats (C. Evinger, unpublished observations). Thus, OPNs could

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cross-link the blink and oculomotor circuits during natural behavior.

Clinical studies provide additional evidence for interaction of the saccadic and blink motor systems through the OPNs. For example, voluntary blinks can accelerate pathologically slowed saccades (Leigh et al. 1983; Zee et al. 1983) and blinks can initiate certain types of saccadic oscillations (Hain et al. 1986; Ashe et al. 1991). Consideration of these behavioral, anatomical, and physiological data have led a number of investigators to argue that OPNs serve as a shared element in both oculomotor and blink subsystems (Zee and Robinson 1979; Zee et al. 1983; Haine et al. 1986; Ashe et al. 1991; Evinger et al. 1994; Yee et al. 1994; Mays and Morrisse 1995).

The relationship of the superior colliculus to OPNs offers an opportunity to investigate the possible role of OPNs in mediating collicular suppression of reflex blinks. In cats, Paré and Guitton (1994) have shown a gradient of excitatory influence from the intermediate layers of the tectum to OPNs, such that the rostral colliculus provides the most robust excitatory effects. This result is consistent with the hypothesis that the rostral superior colliculus specializes in the process of active fixation (Munoz and Wurtz 1992). In primates, Munoz and Wurtz (1993a) have found cells in the intermediate layers of the rostral colliculus that exhibit tonic activity during fixation and pause during most saccadic movements. In contrast to the rostral fixation neurons, neurons in the intermediate layers of the caudal superior colliculus are silent during fixation and burst for saccadic movements of specific metrics (e.g., Wurtz and Goldberg 1972; Sparks et al. 1976; Sparks and Mays 1980; Ottes et al. 1986). Moreover, stimulation in the caudal colliculus recruits the same brainstem burst neurons (Raybourn and Keller 1977) that the OPNs inhibit (Nakao et al. 1980, 1988; Evinger et al. 1982; Furuya and Markham 1982; Curthoys et al. 1984). In addition, Raybourn and Keller (1977) demonstrated that the predominant effect on OPNs from stimulation in the caudal colliculus is to produce a long-lasting inhibition that follows an initial excitatory effect.

Based on these data, we predicted that, if OPNs link the superior colliculus to the reflex blink circuits, microstimulation of the tectal "fixation zone" should maximally suppress reflex blinks. On the other hand, more caudal microstimulation should exert minimal effects or perhaps even facilitate blinks. We tested this hypothesis directly in the current study. Preliminary versions of these data have been presented previously in abstract form (Lu et al. 1994).

Materials and methods

Two monkeys (*Macaca mulatta*) were trained to fixate and follow small visual targets on a tangent screen at a distance of 64 cm. All experimental protocols were performed according to the NIH guide for the care and use of laboratory animals and were consistent with the principles approved by the Council of the American Physiological Society.

The visual display and behavioral control

The visual targets were 0.5° *X*'s made of arrays of dots back-projected from a vector-plotting oscilloscope. The run-time computer presented the targets as a randomly interleaved series of the behavioral tasks while continuously recording analog and digital inputs. We sampled four channels of eye and lid position at 500 Hz from search coils attached to the subjects' eyes and lids (Robinson 1963). Eye coils were permanently implanted according to the method of Judge et al. (1980). The lid coil was made of 30 turns of teflon-coated stainless steel wire (0.0076 cm diameter) at 4 mm diameter. Monkeys were rewarded with a sip of fruit juice for correctly following the visual targets. Trials were aborted without reward when the subjects failed to track the targets appropriately. To motivate the subjects adequately, for 5 days/week they received their daily fluids as rewards for proper behavior. This restricted their fluid intake to the time of daily experiments and handling, but they were allowed to acquire fluids until they satiated. For 2 days/week, the subjects were allowed water *ad lib* in the home cage.

Neurophysiological methods

Head movements were restrained using a surgically implanted head cap of bone cement and bone screws. The cap included a stainless steel restraining post and a stainless steel recording cylinder sealed with a sterile, threaded plug of Delrin. Microelectrodes were introduced into the superior colliculus through craniotomies below the recording cylinders. To approach the superior colliculus roughly perpendicular to its surface, the recording cylinders were positioned 13 mm off midline with a 15° lateral-to-medial angle. Stimulating electrodes were parylene-insulated tungsten microelectrodes (15–150 kΩ) mounted in a guide tube of hypodermic tubing. Transdural penetrations were made with a custom-designed microdrive. Recording of the stimulus pulses was made by isolating stimulus artifacts from the electrode using a time/voltage window discriminator along with eye- and target-position parameters. Recording sites were reconstructed by interpolation from microdrive coordinates (corrected for histological shrinkage) and compared with histologically identified injection sites (histological tracers were injected for other studies not reported here) made at known coordinates during the last 2 weeks of recording. Estimates were corrected in the reconstruction to conform to neurophysiological landmarks noted during recording, such as transitions in activity levels between the supraquadrigeminal space and the top of the tectum.

Multiunit neuronal activity was used to identify superior colliculus with its typical burst of activity preceding a saccade. In most cases, we could estimate the top of the superior colliculus by the abrupt transition from quiescence in the ventricular space to multiunit visual activity in the superficial layers. Furthermore, we could confirm this transition to within 0.2 mm by noting the electrode depth above which it became impossible to elicit saccades at 100 μA. Thus, we express the depth of each stimulation site according to its depth relative to the top of the colliculus. Occasionally, we had to rely on comparison of the microdrive depth with surrounding penetrations and from identification of the lowest threshold zone for producing saccades. The low-threshold zone for eliciting saccadic movement was identified by microstimulation (Robinson 1972) using trains of biphasic, constant-current electrical pulses (0.15 ms at 250–300 Hz). For each electrode tract, we compared the amplitude of normal, reflex blinks with the amplitude during trains of collicular stimulation using current intensities of 10–75 μA at several depths throughout the tectum.

For each electrode penetration, we used a stimulus current of ~1.2 times threshold for producing saccades to determine the location of the electrode in the systematic motor map of saccades for the superior colliculus (Robinson 1972).

Reflex blink procedures

We monitored blink amplitude in one monkey (monkey 530) with a lid coil (e.g., Evinger et al. 1991) and with the eye movements accompanying blinking (Evinger et al. 1984; Collewijn et al. 1985) in the other monkey (monkey 591). At the beginning of each session, the lid coil was taped to the center of the lower margin of the upper eyelid. We calibrated the lid coil by rotating the coil through known angles of rotation in a separate session. In monkey 591, initial examination of the eye movements during blinks identified with a lid coil revealed that the vertical signal for each eye moved unequally during blinks (see Fig. 1C,D). The magnitude of the vertical eye movements increased concomitantly with the amplitude of lid closure. We did not determine whether this unequal binocular eye rotation was an accurate reflection of the eye movements accompanying blinks or perhaps due to an unusual movement of the wire leads within the orbit during blinks. The retraction of the globe caused by cocontraction of extraocular muscle pairs (Evinger et al. 1984; Collewijn et al. 1985; Evinger and Manning 1993) could have generated atypical movements of the eye coil leads. Whatever its cause, we routinely used the difference between the vertical position of the left and right eyes as an index of blink magnitude for this monkey. During voluntary eye movements, the two eyes moved conjugately. Thus, the difference between the vertical position of the left and right eyes provided a reliable and unique signal of reflex blinks. We refer to this index of blinks as the blink trace and to the signal from the lid coil as the lid trace.

Trigeminal reflex blinks were evoked by an air puff directed at one eye while the monkeys actively fixated a visual target. Compressed air was channeled through rubber tubing to the narrow opening in a plastic pipette (tip diameter less than 1 mm), positioned 30 mm from the eye. Air pressure at the source was 12 psi. A solenoid (General Valve) provided a 30-ms (monkey 530) or 50-ms (monkey 591) stimulus. The signal to the solenoid was monitored on a fifth analog-digital (A/D) channel of the computer. By directing the plastic pipette toward a microphone at the same distance as the monkey's eye, we determined the transport time for the air puff to reach the eye to be 23.7 ms (±2.1 SD). To maintain a constant stimulus magnitude, care was taken to insure that air puff stimuli occurred when the monkey fixated the target. To reduce habituation of the blink reflex, we presented air puff stimuli no more often than once every 20 s. We randomly interleaved eye movement trials with no air puff stimuli, air puff stimuli alone, collicular stimulation alone, and combined air puff and collicular stimulation. The effect of superior colliculus stimulation on reflex blinks was quantified by computing a normalized relative blink index, the ratio of blink magnitude evoked during tectal stimulation to blink magnitude evoked without tectal stimulation. The range of values between 0 and 1 corresponded to the range of effects from complete suppression of the reflex blink to no effect of tectal stimulation.

To investigate the latency of the collicular stimulation effect, during four recording sessions in monkey 530 we presented one to ten pulses of relatively high current (80–125 μA at 300 Hz) at various times during the blink reflex. The latency of the effect was determined by comparing each lid trace following the stimulus pulse(s) to a nominal blink profile created from superposition of 20 normal reflex blinks. The nominal response envelope was determined as the 99% confidence interval around the mean of the 20 normal blinks at each 2-ms time sample. We found that the most sensitive measure of midflight interruption was to compare the velocity profile of the lid. For each blink and for the composite nominal response envelope, we differentiated the lid position trace to obtain the lid velocity at each 2-ms interval (cutoff frequency 35 Hz at –20 dB). Individual traces were aligned at the peak velocity. On a given sample trial, the latency of a midflight interruption was determined as the time from the first stimulus pulse to the earliest point in the velocity trace that deviated outside the 99% confidence interval for four consecutive time points.

Histology

At the completion of the study, we mapped the collicular motor map using microstimulation (Robinson 1972) and made tracer injections at known locations in the motor map for use in another study. This allowed us to compare stimulation locations for the blink manipulations to histologically recovered positions within the superior colliculus. Then the animals were deeply anesthetized using pentobarbital sodium and terminated by transcardial perfusion of saline with heparin and sodium nitrate, followed by appropriate fixatives. The brain was removed and blocked in the coronal plane. Frozen sections were cut at 40 μm on a sliding microtome. Serial sections were prepared for identification of the injected tracers, Nissl and myelin.

Results

The air puff stimuli reliably evoked reflex blinks with a mean latency of 46.7 ms±8.5 SD (monkey 591) and 40.2 ms±5.3 SD(monkey 530) from the start of the solenoid pulse (Fig. 1A,C). Subtracting the transport time for the air puff to reach the eye (23.7 ms; see Materials and methods) yields a mean latency of approximately 20 ms from stimulus to response. While the animal fixated straight ahead, the air puff initiated a transient down and rightward rotation of the eyes that accompanied each reflex blink. As expected with co-contraction of agonist and antagonist extraocular muscles with reflex blinks (Evinger and Manning 1993), the direction and size of the eye movement exhibited some dependence upon initial eye position (not illustrated). For both monkeys, microstimulation of the superior colliculus could reduce the magnitude and peak velocity of the reflex blinks evoked by the air puff (Fig. 1B,D). In some cases, the blinks could be completely suppressed (e.g., Fig. 7C). Stimulation outside the "fixation zone" described by Munoz and Wurtz (1992) produced the classically described "staircase" of saccades (Robinson 1972). We did not find any systematic relationship between the cyclical occurrence of saccades during the stimulation and the blink suppression.

Throughout these experiments, we interleaved trials of combined presentation of collicular stimulation and the air puff stimulus, collicular stimulation alone, air puff alone, and fixation only. As long as air puffs were presented no more frequently than each 20 s, we did not observe systematic habituation of the reflex blinks. Nor did we observe any classical conditioning to the collicular stimulation. The collicular stimulation presented without the air puff elicited saccades but not blinks.

To identify the most effective locations within the superior colliculus for suppressing reflex blinks, we determined the position of the stimulating electrode within the collicular motor map using the saccades evoked by microstimulation. At each position within the motor map, we calculated the relative blink index at several stimulus currents and at several depths along each electrode tract. If the metrics of stimulus-evoked saccades changed slightly at different electrode depths (presumably due to curvature of the tectal layers relative to the electrode path), the saccades produced at the lowest threshold site served to establish the map position for

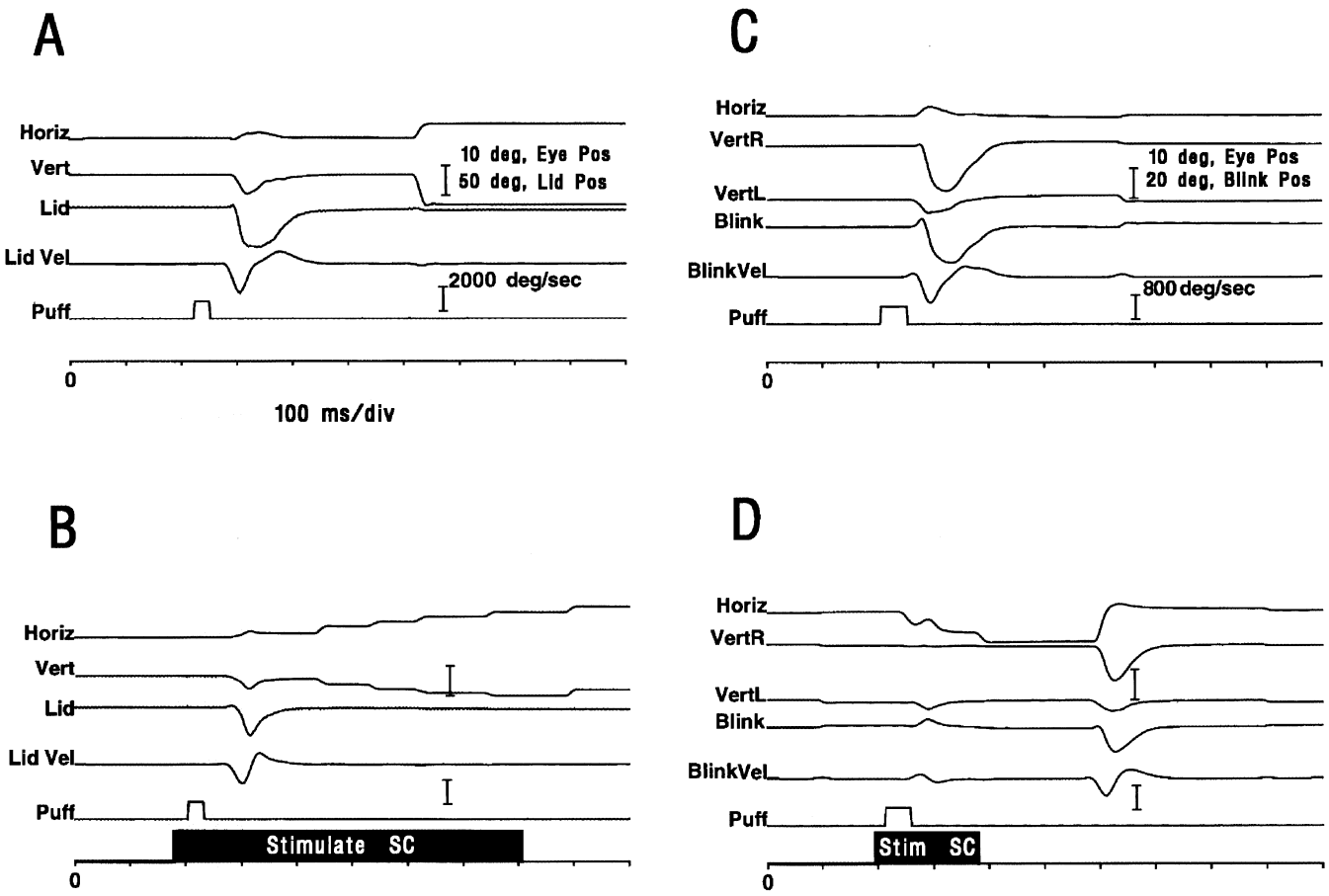

Fig. 1A–D Reflex blinks and suppression by stimulation in the superior colliculus. Examples of reflex blinks for an air puff to the eye for monkey 530 (**A, B**) and monkey 591 (**C, D**). **A, C** Normal reflex blinks; **B, D** blinks partially suppressed by microstimulation in the superior colliculus [*Horiz, Vert, VertL, VertR* horizontal and vertical eye position of the left eye and vertical position of the right eye, respectively; *Lid, Blink* position traces of the reflex blinks (see Materials and methods); *Lid Vel, Blink Vel* velocity of the lid and blink traces, respectively; *Puff* solenoid signal that delivers the air puff; *Stimulate SC* trains of biphasic microstimulation pulses delivered to the superior colliculus (300 Hz)]

that electrode penetration. For the six representative penetrations illustrated in Fig. 2, collicular microstimulation produced more suppression of reflex blinks as stimulation current increased. Also, the effectiveness of the stimulation systematically increased, then diminished at depths below that of the deep layers of the colliculus (greater than 3.5 mm).

For Fig. 3, the data from a total of 22 sites (14 from monkey 530 and 8 from monkey 591) were grouped into six categories of relative depths (0–6 mm) and five categories of currents (0–50 μA). The interaction of current and relative depth on blink amplitude was significant, ($F_{20,393}$=90.5), which indicated that the efficacy of the blink suppression by current intensity changed as a function of stimulation depth. For all but four stimulation sites, the stimulation reached 50% suppression at current values between 20 and 30 μA. The two stimulation sites that required the highest values of current to reach 50% suppression (45–50 μA) were located at positions within the tectum where stimulation tended to inhibit voluntary saccades (see Microstimulation in the fixation zone).

The depth for the greatest blink suppression occurred between 2 and 3.5 mm below the tectal surface for 84% of the penetrations. This relative depth corresponds to the intermediate and deep layers of the superior colliculus.

Position in the motor map

To test the prediction that the blink suppression should be greatest near the fixation zone and least in caudal colliculus, we grouped the data into two categories: (1) those sites in far rostral superior colliculus (amplitude of stimulated saccades of less than 5°), and (2) those sites in caudal colliculus (amplitude of stimulated saccades of more than 5°). Contrary to the prediction based on hypotheses about the importance of the fixation zone, the suppression was not greater for the rostral sites (Fig. 4). In fact, the caudal sites had a statistically reliable greater suppression than rostral sites ($F_{4,93}$=7.82).

Microstimulation in the fixation zone

Given our working hypothesis that the blink suppression effects might be due to recruitment of the brainstem

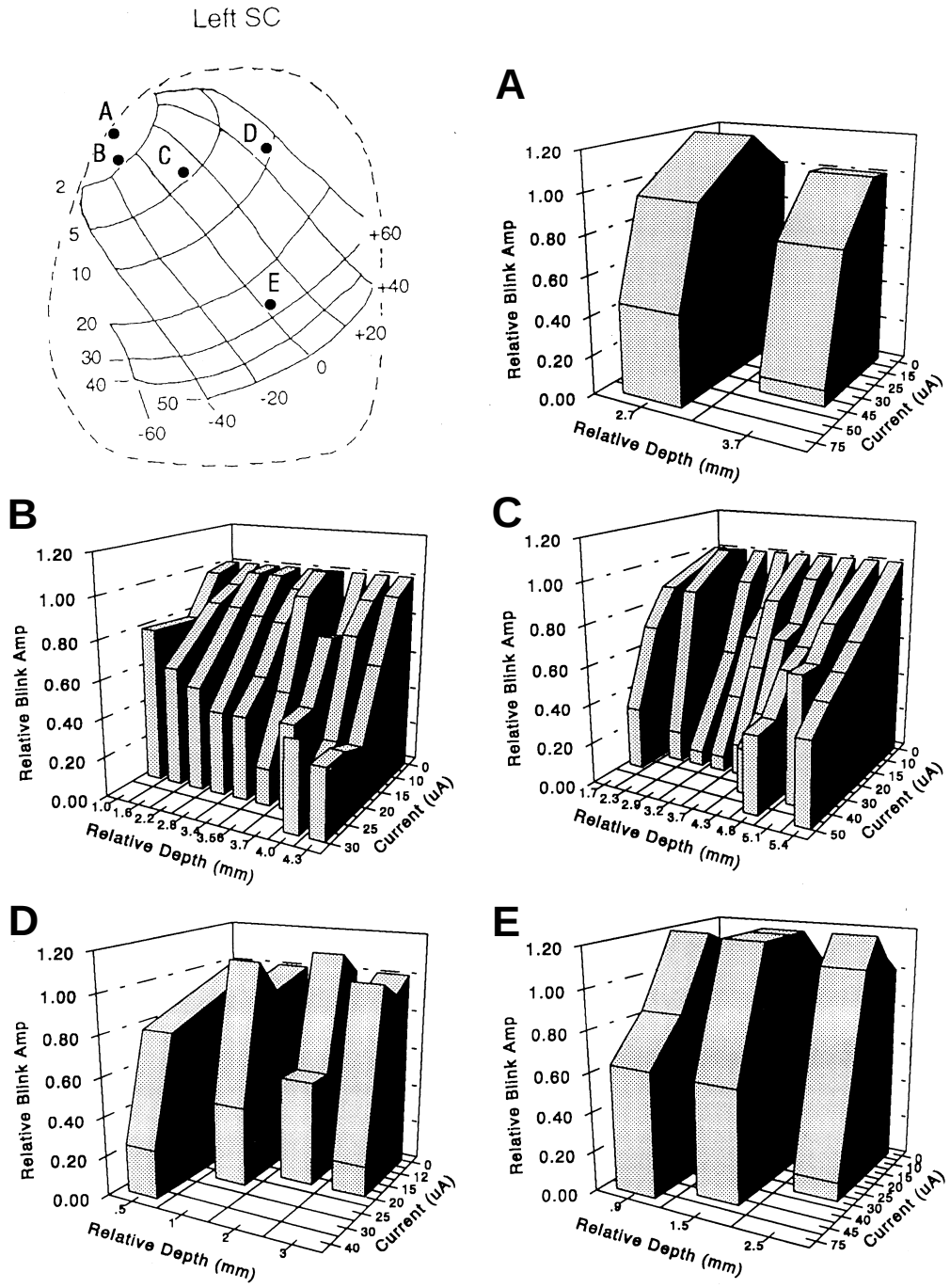

Fig. 2A–E Examples of blink suppression as a function of relative depth from the top of the superior colliculus for stimulus currents at several sites within the collicular motor map for saccades. The five families of curves (**A, D, E** from monkey 530; **B, C** from monkey 591) were collected from five different perpendicular tracts through the superior colliculus, as represented in the *top left inset*. The *inset* is a schematic of the collicular motor map for saccades as viewed from above (adapted from Robinson 1972). Each family of curves shows the decreasing relative blink amplitude (increasing blink suppression) for a mean of several trials at each of the values of stimulating current

OPNs via a rostral fixation zone, we gave special consideration to four sites within rostral colliculus where we could inhibit voluntary saccades by tectal stimulation. An example of inhibition of a voluntary saccade during stimulation at one of these sites is illustrated in Fig. 5. Stimulation at this site could reliably delay the movements beyond the normal response latency of approximately 200 ms for voluntary saccades. These physiological effects and evaluation of the microdrive coordinates suggested that these four penetrations passed through, or very near, the rostral fixation zone as defined by Munoz and Wurtz, (1992). The blink suppression at these sites either was nonexistent or required more than 30 μA of current to reach a 50% diminution. For two of the sites, the fixation effects occurred only at relatively shallow depths within the colliculus (0.8 mm and 1.8 mm below the surface), where blink suppression effects were minimal anyway. However, the other two sites were within the optimal depth for suppressing blinks, at 3.2 mm and 3.7 mm below the surface. These two sites required stimulation currents of 45–50 μA to reach 50% suppression. This was more than 10 μA greater than the site with the next highest value of 50% diminution at similar depths.

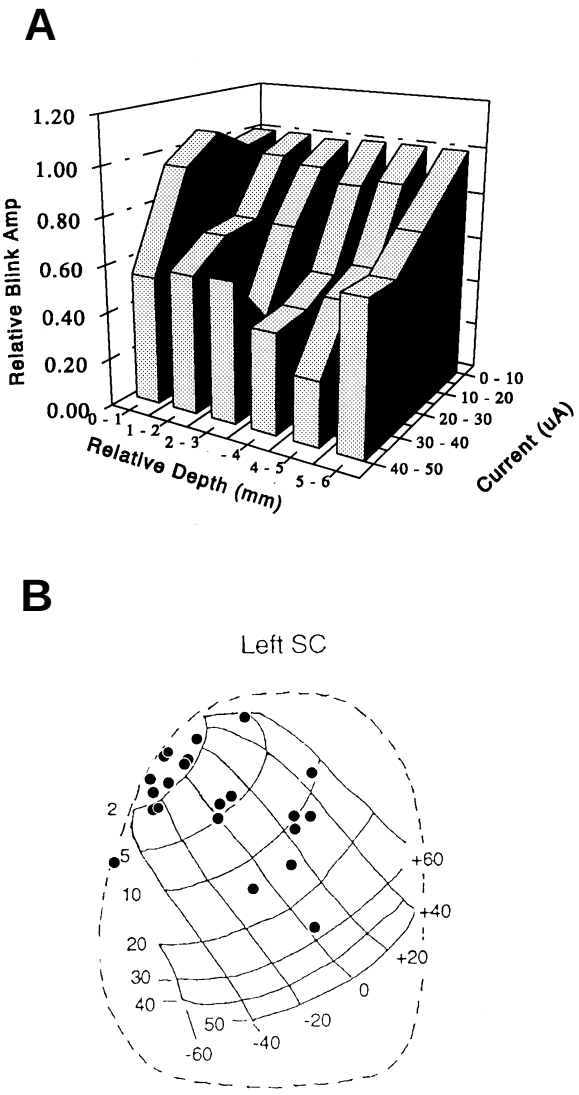

Fig. 3A, B Composite of the blink suppression for a total of 22 sites across the motor map from both monkeys. **A** Relative blink amplitude as a function of depth within the tectum and for the amount of stimulating current. **B** Location of all 22 electrode tracts within the motor map for saccades

Reflex blink suppression from brief trains of superior colliculus stimulation

To find the latency of the blink suppression from the stimulus, we delivered between one and ten pulses of stimulation to the tectum at various times during reflex blinks and at relatively high current (80–125 μA, 300 Hz). Pulse trains of four or less did not evoke saccades. There was a complex interaction on the latency, magnitude, and reliability of the blink perturbation from the number of pulses delivered and the relative timing between the stimulus and blink. The magnitude of the individual blink interruptions was grossly related to the number of stimulus pulses – seven or ten pulses usually produced larger, more reliable, and more persistent effects on the blink trajectories than did trains of four pulses or less. The incidence of producing measurable interruptions rose from 45% for one pulse to 100% for ten pulses (Fig. 6). However, the magnitude and latency of the perturbations were complexly affected by the relative timing between the stimulus and the blink. Specifically, the size, direction, and duration of the perturbations varied according to relative timing within the blink and

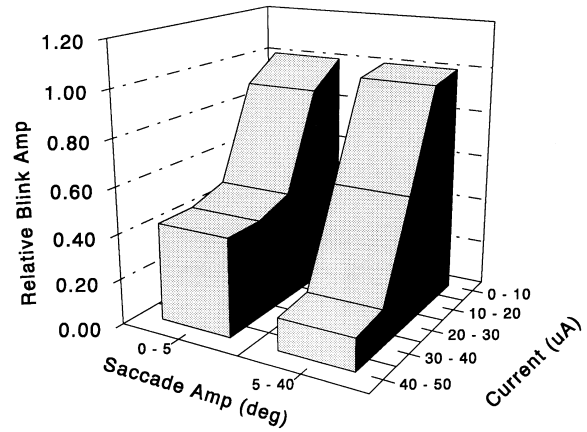

Fig. 4 Blink suppression in rostral compared with caudal colliculus. Composite stimulus/response curves for sites in rostral (saccade amplitude less than 5°) vs caudal (saccade amplitude more than 5°) colliculus

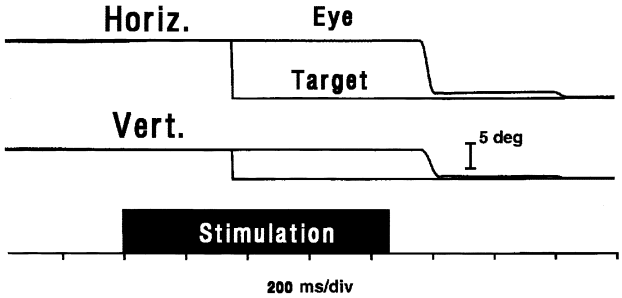

Fig. 5 Example of suppressed voluntary saccade by stimulation in the "fixation zone." Stimulation in rostral superior colliculus at this site consistently delayed impending voluntary saccades to the lower left quadrant. Microstimulation in the superior colliculus, 40 μA at 300 Hz (*Horiz.* horizontal position of the target and the left eye as a function of time, *Vert.* vertical position of the target and the left eye)

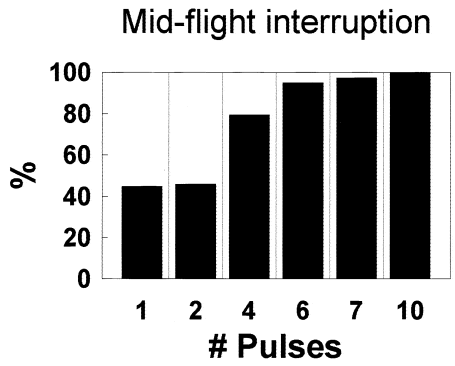

Fig. 6 Incidence of midflight interruption of reflex blinks as a function of number of pulses for brief stimulus trains

were too complex to be described by a single metric (see Fig. 7). Since we were primarily interested in determining the latency of the blink effects from the collicular stimulus, we did not attempt a quantitative assessment of the magnitude of the blink interruptions for these brief trains of collicular stimulation.

As described in Materials and methods, we found the latency of the stimulation effect by determining the first of four consecutive points in the velocity trace that deviated outside the 99% confidence envelope for normal reflex blinks. Figure 7 illustrates three examples of midflight blink interruptions or suppression produced at different times during the blinks and for different numbers of pulses. Each example compares the lid position and velocity for a single, tectal-stimulated trial to a nominal response envelope (mean and 99% confidence interval) of normal reflex blinks. The midflight interruptions can be seen where the lid velocity and position traces of the stimulated trial deviate outside the normal response envelope. Figure 7A shows slowing of the initial downphase of a blink by a single stimulation pulse. The earliest inflection of the blink velocity outside the 99% confidence interval of normal blinks occurred at 24 ms after the stimulus pulse. Figure 7B shows a mid-flight inflection from a train of four pulses delivered later in the blink when the lid-closing orbicularis activity was nearly complete. The latency to this upward-directed inflection during the slow-velocity return phase was 32 ms after the first pulse. Since the lid-closing orbicularis oculi muscle is quiescent during this period of the blink, we speculate that this effect may result from activation of the antagonist levator palpebrae muscle. With seven or ten pulses, we often got nearly complete suppression of the blinks, if the stimulation was delivered well before the blink started (Fig. 7C). Figure 7C also shows an example of a "rebound blink" at a 64-ms delay from the last stimulus pulse.

From data compiled from all the trials that exhibited measurable blink interruption, we found that the latencies ranged from 12 to 56 ms (Fig. 8). The mean of this distribution was 30.8 ms (±9.8 SD). However, we note that this distribution included data subsets that had confounding factors. First, since we initially did not know what the minimum latency would be, we delivered some trains of stimuli at times before the blinks much longer than the shortest possible latencies – up to 54 ms before the blink compared with perturbation latencies that eventually were measured at approximately 25 ms. Obviously, inhibition that would have arrived before the blink cannot be expressed until the time at which the reflex blink was triggered by the air puff. The persistence of the inhibition until the start of the blink was due to the fact that many of these trials included longer trains of pulses (Fig. 7C). Additionally, there was a persistence of the inhibitory effect well beyond the 0.15-ms time course of each pulse. This effect of "premature" delivery of the stimuli can account for 18 of the samples in Fig. 8 ("Early").

A second factor that contributed to the skewed distribution toward longer latencies was that the blinks were

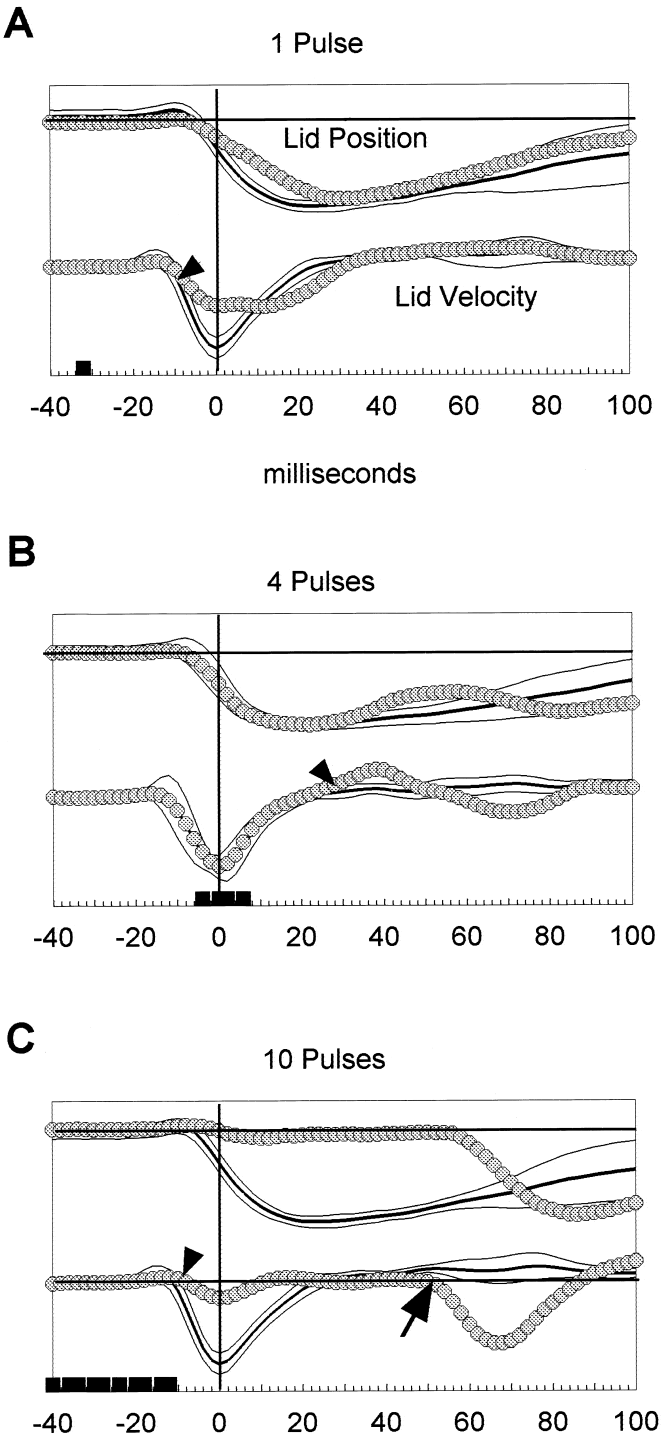

Fig. 7A–C Examples of midflight interruption of reflex blinks. Each panel shows the lid position and lid velocity for a blink perturbed by collicular stimulation superimposed on the mean nominal blink and the 99% confidence envelope for unperturbed blinks collected in interleaved fashion. Stimulus pulses delivered to the superior colliculus are shown as *event markers on the abscissa*. The *small arrows* indicate the first point at which the stimulation-induced perturbation deviated outside the 99% confidence envelope for normal reflex blinks. The *large arrow* in **C** indicates the start of a delayed "rebound blink.". In each case, the air puff stimulus began at 60 ms or more before the peak downward velocity, the alignment point for the lid traces. —— Mean, —— 99% c.i., ■ pulses

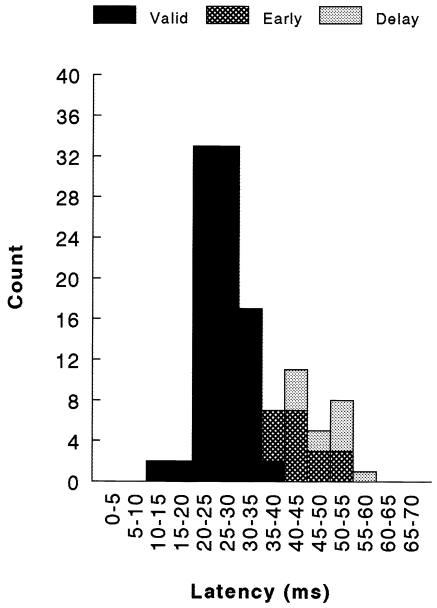

Fig. 8 Latency from the first pulse to the start of the mid-flight blink inflection for 119 trials that exhibited measurable perturbation. Data are compiled from stimulus trains with 1, 3, 4, 6, 7, and 10 pulses. (*Early* = samples from stimuli delivered so early that presumably the blink occurred after the earliest inhibition had already arrived, *Delay* samples with delayed latencies because the stimuli were delivered when the blinks were relatively refractory to inhibition, *Valid* samples not confounded by the early stimulus delivery or the relative refractory period)

relatively refractory to the perturbations at the time of peak downward velocity. Stimulus trains that were timed such that the inhibition arrived after the peak downward velocity, near the end of the orbicularis oculi activity, had lid perturbations delayed by approximately an additional 15 ms. Since these lid deviations resulted from activation of the levator palpebrae, rather than a reduction of orbicularis oculi activity, we labeled these 12 samples "Delay" (Fig. 8). Thus, excluding these 30 confounded samples from the distribution in Fig. 8 leaves the data subset labeled as "Valid." Using this sample as an estimate of the mean minimum latency gives a mean of 26.0 ms±4.6 SD.

As a measure of the persistence of the blink inhibition, we analyzed 23 trials for which we delivered either seven or ten pulses. For these trials, where there was near complete suppression of the blinks, we observed a consistent rebound blink at a relatively long latency (Fig. 7C). We interpret this effect as the release of a suppressed blink following eventual withdrawal of the blink inhibition. The mean latency to these rebound blinks from the last stimulus pulse was 63 ms±26.7 SD.

Discussion

In summary, we have shown that electrical microstimulation in the superior colliculus of primates can inhibit the blink reflex. A similar conclusion has been reached in rats by Basso et al. (1996). We can speculate that suppressing trigeminal reflex blinks might be useful during head and eye orienting movements to insure establishing unimpaired visual contact with the orienting target. Rotating the head causes wind to rush through the eyelashes, which could initiate a trigeminal reflex blink. Suppressing trigeminal reflex blinks as a component of the gaze shift would help insure unobscured vision upon reaching the target. While there are several possible roles for collicular modulation of trigeminal reflex blinks, it is remarkable that evolution retains this pathway in species as diverse as rats and monkeys.

In monkeys, blink suppression could be elicited from all sites within collicular motor map for saccades. However, the least effective sites for suppression were in the rostral superior colliculus near the fixation zone (Munoz and Wurtz 1992), where stimulation could inhibit voluntary saccades. The most effective depth at all positions within the motor map was ~3 mm below the top of the tectum in the intermediate to deep layers of the colliculus.

We also note that the inhibition appeared to have a persistent time course. The greater effectiveness of multiple pulses (Fig. 6) suggests a temporal summation mechanism. Further, from trains of seven or ten pulses there appeared to be an inhibition lasting approximately 60 ms (Fig. 7C). Collicular stimulation in the rodent also produced a 60 ms period of blink suppression (Basso and Evinger 1996).

Do the OPNs link the superior colliculus to trigeminal blink reflex circuits?

As reviewed in the introduction, Basso and Evinger (1996) have concluded that a class of tonically active inhibitory neurons is responsible for the link between superior colliculus and the inhibition of trigeminal reflex blinks. There are two types of inhibitory neurons known to be activated from the superior colliculus, both of which are involved in gaze and oculomotor control (for review, see Sparks and Mays 1990; Galiana and Guitton 1992): the OPNs and inhibitory burst neurons (Hikosaka and Kawakami 1977). Of these, only the OPNs are tonically active. The OPNs receive an excitatory input from the superior colliculus (Raybourn and Keller 1977; King et al. 1980; Kaneko and Fuchs 1982), and microstimulation of the OPNs suppresses reflex blinks (Cohen and Henn 1972; Fuchs et al. 1991; Mays and Morrisse 1995). Thus, it is reasonable that collicular microstimulation recruits OPNs, which in turn inhibits trigeminal reflex blinks. Despite the feasibility of this line of reasoning, several lines of evidence indicate that the OPNs do not fulfill this role.

First, despite the current evidence that the strongest excitatory drive from the superior colliculus onto OPNs arises from the rostral colliculus (Paré and Guitton 1994), our data show that microstimulation in the rostral colliculus produced the least amount of reflex blink sup-

pression (Fig. 4). This result could occur if OPNs do not participate in collicular modulation of reflex blinks or if fixation zone stimulation activated a mixed population of neurons that canceled the OPN excitation. In the latter condition, the stimulation in caudal colliculus might have produced stronger reflex blink suppression, because it activated caudally projecting collaterals of fixation zone neurons without also activating neurons that cancel fixation zone activation of OPNs. The behavior of OPNs during caudal collicular stimulation, however, argues against this explanation.

If OPNs suppress reflex blinks with collicular stimulation, then OPNs should be activated by microstimulation in caudal colliculus and in the fixation zone. In contradiction to this, Raybourn and Keller (1977) have shown that, following an initial excitation, the OPNs exhibit a prominent suppression to stimulation in the caudal colliculus. Even if one would argue that our brief trains of tectal stimulation could have had a net excitatory drive on OPNs (e.g., Raybourn and Keller 1977), our prolonged trains of suprathreshold collicular stimulation often induced a "staircase" of repeated saccades (e.g., Fig. 1). We have argued that these staircase movements result from oscillations of the saccadic feedback circuit in an unlatched state for which the OPNs are silent throughout the stimulation (Breznen et al. 1996; Breznen and Gnadt 1997). Data from Reusser et al. (1996) support this prediction. Furthermore, our data do not show any evidence for cyclic periods of blink suppression associated with the periodic staircase of movements. Thus, several lines of evidence show that the prominent effect of microstimulation of the caudal superior colliculus is to *inhibit* OPN activity. Yet despite this inhibition, blinks are suppressed during the collicular stimulation.

Our data using electrical stimulation suggest that tectal efferent fibers that recruit the blink inhibition either originate or pass through the intermediate and deep layers of the superior colliculus, throughout its extent. The fact that Basso et al. (1996) could manipulate blink amplitude with chemical manipulations in the tectum argues against the possibility that our electrical stimulation is activating collaterals of fibers afferent to the superior colliculus.

Thus, as with the rat (Basso et al. 1996), stimulation of the superior colliculus in monkeys suppresses trigeminal reflex blinks. These data from the monkey clearly do not support a role for the OPNs in mediating this collicular effect on reflex blinks. Further, the present data suggest that this suppression does not involve saccadic eye movement circuits. Basso and Evinger (1996) have reached a similar conclusion in a rodent preparation, showing that the superior colliculus modulates trigeminal reflex blinks by activating the nucleus raphe magnus, which inhibits spinal trigeminal neurons that are an element of the reflex blink circuit.

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