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The brain-stem parabrachial region controls mode of response to visual stimulation of neurons in the cat's lateral geniculate nucleus

SHAO-MING LU, WILLIAM GUIDO, AND S. MURRAY SHERMAN
Department of Neurobiology, State University of New York, Stony Brook

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Abstract

We recorded the responses of neurons from the cat's lateral geniculate nucleus to drifting sine-wave grating stimuli both before and during electrical stimulation of the parabrachial region of the midbrain. The parabrachial region provides a mostly cholinergic input to the lateral geniculate nucleus, and our goal was to study its effect on responses of geniculate cells to visual stimulation. Geniculate neurons respond to visual stimuli in one of two modes. At relatively hyperpolarized membrane potentials, low threshold (LT) Ca^{2+} spikes are activated, leading to high-frequency burst discharges (*burst* mode). At more depolarized levels, the low threshold Ca^{2+} spike is inactivated, permitting a more tonic response (*relay* or *tonic* mode). During our intracellular recordings of geniculate cells, we found that, at initially hyperpolarized membrane potentials, LT spiking in response to visual stimulation was pronounced, but that parabrachial activation abolished this LT spiking and associated burst discharges. Coupled with the elimination of LT spiking, parabrachial activation also led to a progressive increase in tonic responsiveness. Parabrachial activation thus effectively switched the responses to visual stimulation of geniculate neurons from the burst to relay mode. Accompanying this switch was a gradual depolarization of resting membrane potential by about 5-10 mV and a reduction in the hyperpolarization that normally occurs in response to the inhibitory phase of the visual stimulus. Presumably, the membrane depolarization was sufficient to inactivate the LT spikes. We were able to extend and confirm our intracellular observations on the effects of parabrachial activation to a sample of cells recorded extracellularly. This was made possible by adopting empirically determined criteria to distinguish LT bursts from tonic responses solely on the basis of the temporal pattern of action potentials. During parabrachial activation, every cell responded only in the relay mode, an effect that corresponds to our intracellular observations. We quantified the effects of parabrachial activation on various response measures. The fundamental Fourier response amplitude (F1) was calculated separately for the total response, the tonic response component, and the LT burst component. Parabrachial activation resulted in an increased F1 amplitude for the total response. This increase was due to an increase in the tonic response component. For a subset of cells showing epochs of LT bursting, parabrachial activation concurrently reduced LT bursting and increased the amplitude of the tonic response. Parabrachial activation, by eliminating LT bursting, also caused cells to respond with more linearity. By keeping geniculate cells in the relay mode, the parabrachial region serves to maintain a more linear retinogeniculate transfer of information to cortex, and this may be important for detailed analysis of visual targets. However, when a geniculate neuron becomes hyperpolarized, as may occur during states of visual inattention, it would not respond well to visual stimuli without the sort of nonlinear amplification provided by the LT spike. Thus, the LT spike may permit hyperpolarized cells to relay to cortex the presence of a potentially salient or dangerous stimulus, but this is done at the expense of linearity. This may serve as a sort of "wake-up call" that redirects attention to a particular stimulus and eventually enhances activity of appropriate parabrachial inputs to switch the critical geniculate neurons into the relay mode.

Keywords: Visual system, X cell, Y cell, Low threshold calcium conductance, Response mode, Acetylcholine

Introduction

It is now quite clear that the lateral geniculate nucleus of the dorsal thalamus represents much more than just a simple relay station for signals from the retina *en route* to cortex (reviewed in Burke & Cole, 1978; Sherman & Koch, 1986, 1990; Singer, 1977; Steriade & Llinás, 1988). Instead, this nucleus serves as a variable gateway, determining what, when, and how much of the retinal information is passed on to cortex. One of several keys to this variable gating relates to the observation that thalamic relay cells, including those of the lateral geniculate nucleus, exhibit a number of voltage-dependent conductances.

Reprint requests to: S. Murray Sherman, Department of Neurobiology, State University of New York, Stony Brook, NY 11794-5230, USA.

These conductances can dramatically affect responses of geniculate relay cells to their retinal inputs and thus affect retinogeniculate transmissions. Because these conductances are voltage dependent, inputs that control membrane potential of the relay cells, effectively control retinogeniculate transmission. It is noteworthy that 80–90% of synaptic input to geniculate relay cells is nonretinal, deriving from local inhibitory neurons (GABAergic interneurons and cells of the nearby thalamic reticular nucleus, or perigeniculate nucleus), descending axons of the visual cortex, and ascending axons of the brain-stem reticular formation (Sherman & Koch, 1986, 1990). It is of obvious importance to understand how these nonretinal sources control relay cell responsiveness.

We tried to address this issue initially in cats by concentrating on one voltage-dependent conductance and its control *via* inputs to the lateral geniculate nucleus from the parabrachial region of the brain stem. This region provides a mostly cholinergic input (de Lima et al., 1985; de Lima & Singer, 1987; Fitzpatrick et al., 1989; Raczkowski & Fitzpatrick, 1989; Smith et al., 1988). The conductance of interest is the low threshold (LT) Ca^{2+} spike, which results from a voltage-dependent Ca^{2+} conductance seen in all thalamic relay cells, including those of the cat (e.g. Deschênes et al., 1984; Jahnsen & Llinás, 1984*a*,*b*; Lo et al., 1991; McCormick & Feeser, 1990; Steriade & Llinás, 1988). This conductance is inactivated at membrane potentials more depolarized than roughly −60 mV, but is de-inactivated at more hyperpolarized levels. At such hyperpolarized levels, a depolarization, such as a retinal excitatory postsynaptic potential (EPSP), can activate the Ca^{2+} conductance, leading to a large, triangular depolarization; this is the LT spike, and it is "low threshold," because its activation threshold lies at a hyperpolarized level with respect to that of conventional action potentials. Typically riding the crest of an LT spike is a burst of 2–7 conventional action potentials with brief interspike intervals (≤4 ms), and these action potentials represent the signal relayed to cortex. This pattern of responsiveness evoked by retinal or other excitatory inputs is known as the *burst mode*. When a relay cell depolarizes sufficiently to inactivate the LT spike, it responds with action potentials in a steady stream as long as the membrane remains adequately depolarized. Such a pattern of responses evoked by retinal or other excitatory inputs is known as the *relay* or *tonic mode*. A relay cell's membrane potential determines with which firing mode (i.e. burst or relay) it responds to retinal input, and this has implications for the nature of retinogeniculate transmission (Guido et al., 1992; Lo et al., 1991; Lu et al., 1992; McCormick & Feeser, 1990; Sherman & Koch, 1986, 1990; Steriade & Llinás, 1988).

We have previously shown with *in vivo* recording that virtually all relay cells of the cat's lateral geniculate nucleus exhibit LT spikes, and that these spikes and their subsequent LT bursts can be evoked by retinal activation, either through electrical stimulation of the optic chiasm or visual stimulation of the neuronal receptive fields (Guido et al., 1992; Lo et al., 1991; Lu et al., 1992). We have also shown that electrical stimulation of the parabrachial region dramatically reduces and usually abolishes LT spiking in geniculate neurons, perhaps by depolarizing relay cells (see Discussion), but this prior study was limited to LT spikes occurring spontaneously or evoked *via* stimulation of the optic chiasm (Lo et al., 1991).

In the present study, we sought to confirm this observation and extend it to LT spikes evoked by visual stimulation. The pattern of activation due to visually driven responses is quite different from that evoked by stimulation of the optic chiasm, and it is thus not obvious that parabrachial activation will have the same effects on LT spike reduction under the two stimulus conditions. More importantly, we hoped to describe effects of a nonretinal input on retinogeniculate transmission during more natural activation of retinal afferents *via* visual stimulation.

Methods

Animal preparation and geniculate recording

We performed experiments on adult cats (1.8–3.0 kg) using methods that have been described in detail elsewhere (Bloomfield et al., 1987; Bloomfield & Sherman, 1988; Lo et al., 1991; Lu et al., 1992) and are only briefly outlined here. For initial surgical preparation, we anesthetized the cats with 2–3% halothane in N_2O/O_2 mixed in a 1:1 ratio, and we maintained anesthesia with 0.5% halothane in a 7:3 mixture of N_2O/O_2 throughout the recording session. For paralysis, we administered 5.0 mg gallamine triethiodide followed by 3.6 mg/h of gallamine triethiodide plus 0.7 mg/h of d-tubocurarine in 5% lactated Ringers solution. Cats were artificially respired *via* a tracheal cannula. Rectal temperature, heart rate, and end-tidal CO_2 were monitored and kept within normal physiological limits.

In our anesthetized, paralyzed preparation, we observed EEG activity that was usually synchronized in a fashion that is characteristic of slow wave sleep, but occasionally this was interrupted by brief periods of spindle activity (Funke & Eysel, 1992; Ikeda & Wright, 1974). We made no attempt to correlate EEG activity with changes in response mode or with parabrachial activation.

We mounted the cat in a stereotoxic apparatus and opened the skull to allow recording from the lateral geniculate nucleus. A plastic well was built around the craniotomy, and the chamber was sealed with agar and wax to improve stability during recording. We inserted a pair of bipolar stimulating electrodes into the brain to straddle the optic chiasm and applied single pulses (0.1-ms duration, 100–500 μA, <1 Hz) across these electrodes to activate geniculate cells orthodromically from the optic tract. We also inserted a pair of stimulating electrodes into the brain stem at AP:+1,−1,L:3, and D:−1, an area representing the midbrain parabrachial region (Berman, 1968; Uhlrich et al., 1988). We stimulated this parabrachial region with square-wave pulse trains (100-μs pulses of 100–500 μA at 330 Hz for 50–80 ms) to activate its ascending input to the lateral geniculate nucleus (see Burke & Cole, 1978; Sherman & Koch, 1986, 1990; Singer, 1977; Steriade & Deschênes, 1988).

The pupils were dilated, accommodation was blocked pharmacologically with 1% atropine sulfate solution applied topically, and the corneas were protected with zero-power contact lenses that contained a 3-mm-diameter artificial pupil. We used a fiber-optic light source to plot and project retinal landmarks, including the *area centralis*, onto a tangent screen. Spectacle lenses focused the eyes onto the same tangent screen or onto an electronic display monitor placed in front of the cat, 28.5 cm from the nodal points of the eyes.

Single neurons in the A-laminae of the lateral geniculate nucleus were recorded intracellularly and extracellularly using fine-tipped micropipettes filled with 4 M KAc. We pulled the electrode to an initial impedance of 40–50 MΩ and then beveled the tip to a final impedance of 20–30 MΩ. Requirements

for acceptable intracellular impalement included a d.c. drop in resting potential to −50 mV or more negative, an action potential amplitude of ≥40 mV, and a neuronal input resistance ≥10 MΩ. We amplified neuronal activity through a high-impedance amplifier equipped with a bridge and current injection circuitry. We displayed all recordings on an oscilloscope, fed them to an audio monitor, and stored them on an 8-channel FM tape recorder interfaced with a computer for off-line analysis. Action potentials were led through a window discriminator and digitized for off-line computer analysis.

Visual stimulation and geniculate cell classification

For the initial evaluation of neuronal responses, visual stimuli were presented on a tangent screen using a hand-held projector. We used flashed spots of light to determine ocular dominance, receptive-field location, receptive-field size, and OFF- or ON-center type. We then replaced the tangent screen with a display monitor to present vertically oriented, sine-wave grating stimuli. The gratings were produced with an Innisfree Image Generator controlled by a computer. The gratings had a mean luminance of 30 cd/m^2 and could be drifted or counterphase modulated. Other stimulus parameters, such as spatial frequency, contrast, and temporal frequency, were varied independently. We classified all geniculate neurons as X or Y using a standard battery of tests. This included response linearity in response to grating stimuli, receptive-field center size, response latency to electrical stimulation of optic chiasm, and response to a large, fast moving stimulus of high contrast to activate the surround (i.e. dark for an ON-center cell).

Responses to drifting gratings

Typically, we presented drifting sine-wave gratings of various temporal and spatial frequencies at a contrast of 0.5. We stored the spike arrival times of the responses to visual stimuli with a resolution of 0.1 ms. Responses were evaluated by computing the Fourier components from the averaged response histogram after a stimulation period of 20–40 s or 30–80 stimulus cycles. We typically used the fundamental Fourier component (F1) as the response measure, although we also computed the d.c. (F0) and second harmonic (F2) components. Equivalent epochs in which a uniform luminance equal to the mean luminance of the gratings was also presented in order to obtain a measure of spontaneous activity. For many neurons, we constructed complete spatial- and temporal-frequency tuning functions. However, the data presented in this paper are limited to responses to gratings drifted at or near optimal values of spatial and temporal frequency (typically 0.1–1 cycle/deg at 4 Hz).

We were interested in the effects of two manipulations on responses to the drifting gratings: parabrachial stimulation and, for cells recorded intracellularly, effects of varying membrane potential. For the former, we electrically activated the parabrachial region as described above. To be certain that the effects of this activation were general and not limited to a particular portion of the stimulus cycle, we varied phase between the visual and parabrachial stimuli, and averaged response histograms were constructed in phase with the visual stimulus (Uhlrich et al., 1990). This ensured that parabrachial effects were not limited to one portion of an average response histogram, but rather affected the entire response period. For the latter, we simply varied membrane voltage during visual stimulation by direct current injection through the recording electrode.

Results

We recorded the responses of 21 geniculate cells (7 X and 14 Y) to sine-wave gratings drifted at or near optimal stimulus parameters (i.e. spatial and temporal frequency) both before and during electrical stimulation of the parabrachial region. All cells were recorded in the A-laminae. Among these, three were recorded intracellularly (1 X and 2 Y), and 18 were recorded extracellularly (6 X and 12 Y).

Intracellular recording

As has been shown previously, a geniculate neuron switches response modes depending on its membrane potential: at hyperpolarized levels, the low threshold (LT) Ca^{2+} spike can be activated, leading to LT bursts, and this pattern of firing is called the *burst mode*; at depolarized levels, the low threshold spike is inactivated, permitting a more tonic response and more faithful relay of retinal information to cortex, and this pattern is known as the *relay* (or *tonic*) *mode* (see Introduction). We could thus readily regulate response modes of the intracellularly recorded neurons by altering resting membrane potential *via* current injection through the recording electrode, and we could directly determine by the presence or absence of the large, triangular depolarization characteristic of the LT Ca^{2+} conductance which firing mode the neuron expressed during any response to a visual stimulus (Lu et al., 1992). If a cell is sufficiently hyperpolarized, the only response typically seen to visual stimuli is an LT burst. However, if less hyperpolarized, a cell can respond with a combination of LT bursts and tonic activity (see Figs. 1–4), but the LT burst *always* occurred first. This may simply reflect the fact that the stimulus-evoked depolarization early in the cycle can activate an LT spike, but later in the cycle, the cell becomes sufficiently depolarized to inactivate further LT spikes, leading to tonic firing.

The intracellular traces of Figs. 1–4 illustrate many of the basic results of our study. These show for an X (Figs. 1 and 3) and a Y cell (Figs. 2 and 4) the effects of parabrachial activation during visual stimulation. Initially, both neurons were held at relatively hyperpolarized membrane potentials at which the LT spike was de-inactivated and could be activated by appropriate levels of depolarization (Deschênes et al., 1984; Jahnsen & Llinás, 1984*a*,*b*; Lo et al., 1991; Lu et al., 1992). Before the start of parabrachial activation (the first arrowhead in the top trace of Figs. 1 and 2), both cells initially responded in the burst mode to every visual stimulus cycle. That is, each initial response began with an LT spike (marked by an asterisk), riding the crest of which was a burst of action potentials with interspike intervals ≤4 ms. This is the LT burst, and following each LT burst was a stream of tonic activity. This pattern of response is identical to that reported previously for cells responding to visual stimuli at more hyperpolarized membrane levels at which the LT spike was de-inactivated (Lu et al., 1992).

During parabrachial activation, several changes in the response were observed. First, parabrachial activation abolished LT spiking and the associated burst discharges. However this effect was not immediate, since LT bursting persisted in both cells for a number of stimulus cycles after the onset of parabrachial activation. This is shown more clearly by Figs. 3 and

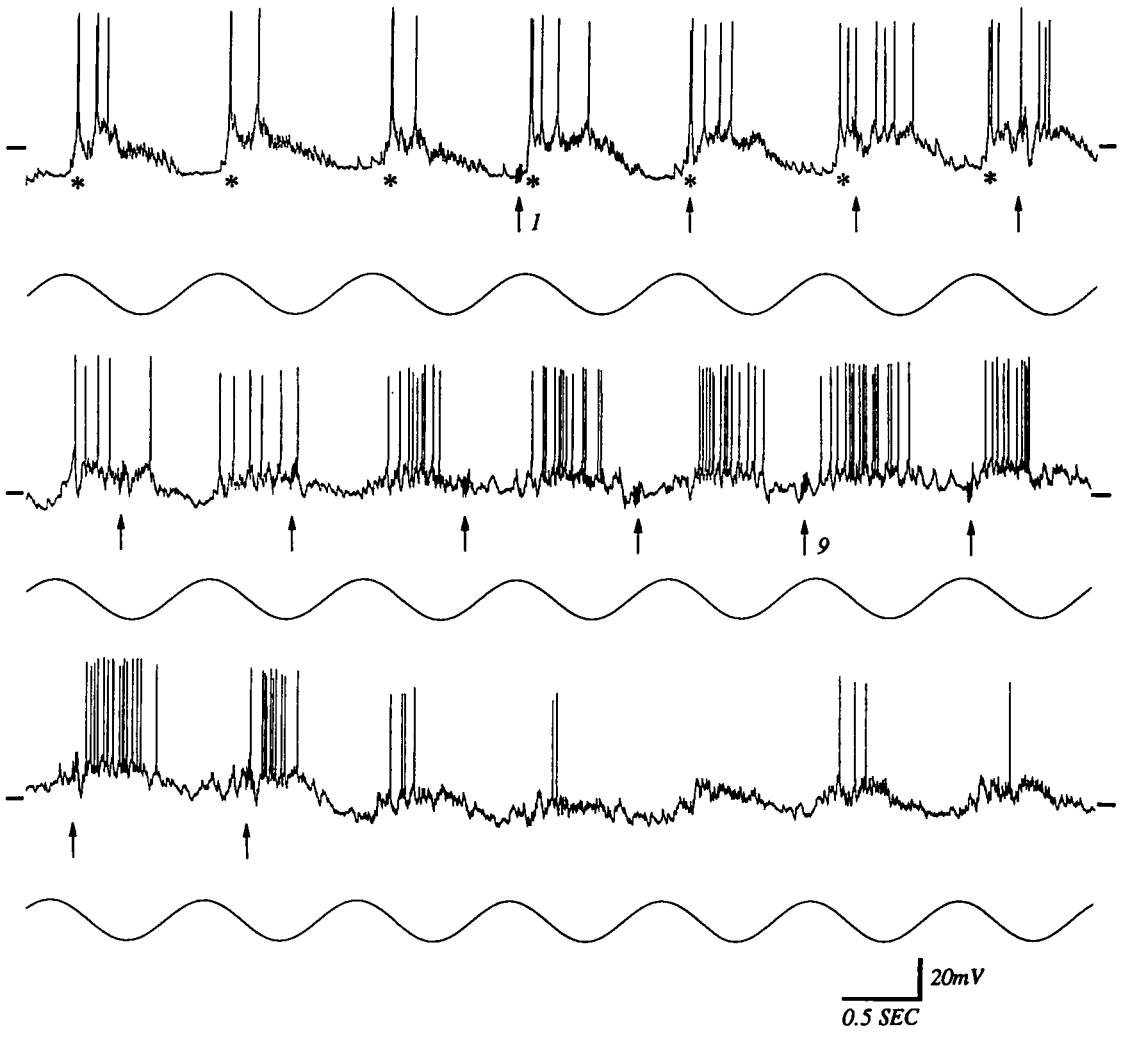

Fig. 1. Intracellular records showing responses of a geniculate X cell to a drifting grating before and during electrical activation of the parabrachial region of the brain-stem. Before this activation, the cell's resting membrane potential was about −75 mV, and the tick marks to both sides of each trace represent a voltage level of −75 mV. The response traces represent a single, unbroken sequence that is continuous from the right end of a trace to the left end of the trace immediately below. The sinusoidal waveform beneath each response trace represents the sinusoidal contrast changes presented by the drifting grating. Each LT spike is indicated by an asterisk below the trace, and each arrow indicates the stimulus artifact produced by parabrachial activation. The numbered arrows mark trace segments shown at an expanded time base in Fig. 3. Note that, before parabrachial activation, LT spikes were activated by the visual stimuli, and the cell responded in the burst mode. When LT spikes occurred they were always the first response, and tonic responses occurred later. Soon after parabrachial activation, LT spiking was abolished, and only tonic activity ensued. Note also the gradual depolarization of the resting membrane (refer to tick marks) and the eventual reduction in the amplitude of the hyperpolarization that occurs in response to the inhibitory phase of the drifting grating.

4, which show segments of the traces in Figs. 1 and 2 at an expanded time scale. Immediately after initiation of parabrachial activation, the grating still evoked an LT spike and burst discharge in each cell (top traces of Figs. 3 and 4), but after several hundred milliseconds of parabrachial activation, no more LT bursts were evoked (bottom traces of Figs. 3 and 4). Second, and associated with the elimination of LT spiking, parabrachial activation led to a progressive increase in tonic responses that eventually reached a plateau. Thus, parabrachial activation switched response modes of both neurons from burst to relay. Third, there was a gradual depolarization of the resting membrane voltage that reached a steady state of about 5–10 mV. In both cells, this steady state took roughly 3 s to achieve following initiation of parabrachial activation. Fourth and finally, there was an eventual reduction in the amplitude of the hyperpolarization that normally occurs in response to the inhibitory phase of the visual stimulation (i.e. when a dark cycle of the grating passes through an ON-center receptive field or *vice versa* for an OFF-center receptive field). For instance, in the top trace of Fig. 2, before parabrachial activation, the membrane potential of the cell clearly hyperpolarizes during inhibitory phases of the stimulus, but in the bottom two traces, after the parabrachial region had been activated for some time, the membrane potential no longer displays such hyperpolarization. A similar

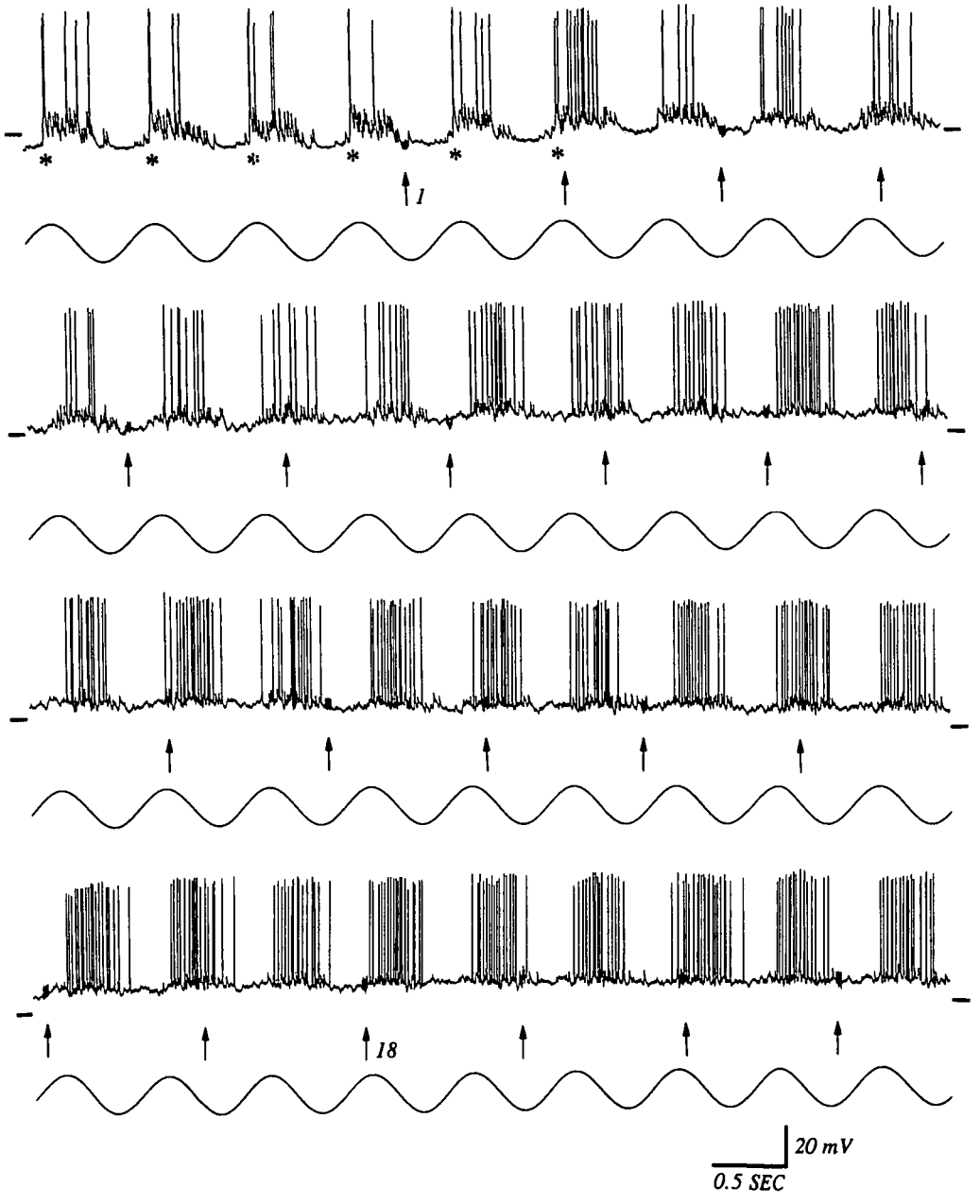

Fig. 2. *Intracellular records showing responses of a geniculate Y cell to a drifting grating before and during parabrachial activation*; conventions are as in Fig. 1. The tick marks are at −80 mV, and the effects of parabrachial activation are essentially the same as shown in Fig. 1.

phenomenon was seen for the cell illustrated in Fig. 1, although the spontaneous EPSPs seen during the inhibitory phases of the stimulus make this more difficult to discern from the illustration.

The simplest explanation for the switching from burst to relay mode due to parabrachial activation is that the gradual depolarization caused by this stimulation is sufficient to inactivate the LT Ca^{2+} spike. To establish this would require a careful determination for each cell precisely at what level this inactivation occurs and whether it is at this level that the effects of parabrachial activation cause the switch from burst to relay mode. However, from our limited intracellular recording, we were unable to test this hypothesis, and we cannot rule out the possibility that parabrachial activation could have some direct effect on the LT spike other than that caused purely by changes in membrane voltage. This issue requires further study.

This switching by parabrachial activation of a geniculate cell's response mode from burst to relay is the chief observation of this study. It also confirms and extends our earlier observation (Lo et al., 1991) that parabrachial activation produces a similar switch in response modes when the geniculate cell is either spontaneously active or responding to electrical activation of the optic chiasm. This switching of response mode is further illustrated by Fig. 5, in which we compare average response histograms to three consecutive stimulus cycles generated at a

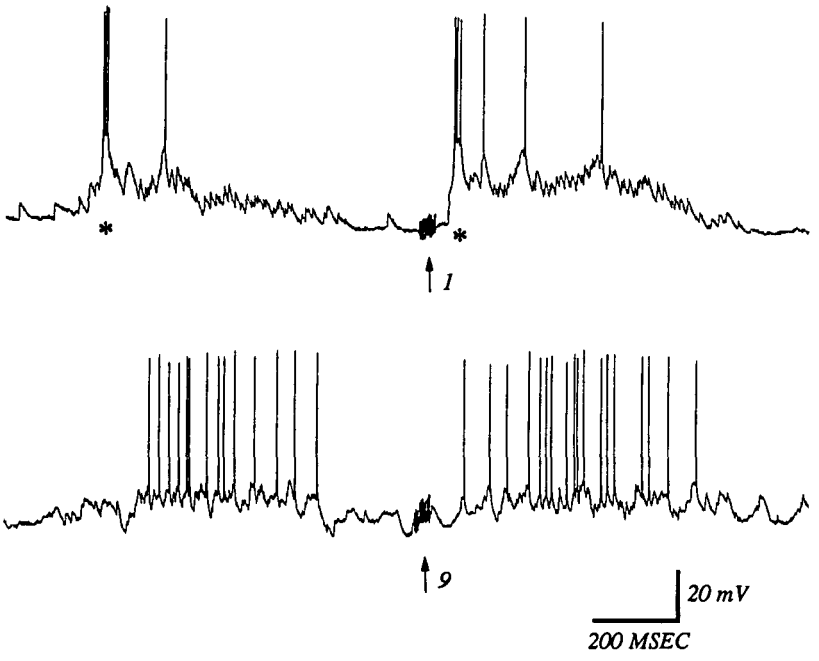

Fig. 3. Intracellular records showing responses before and during parabrachial activation. These traces are expanded sequences of the X-cell response shown in Fig. 1 (marked with numbered arrows); conventions are as in Fig. 1. Top trace: Response immediately before and after initial activation of the parabrachial region. Bottom trace: Response immediately before and after the ninth parabrachial activation. Immediately after the initiation of parabrachial activation, the grating still evoked LT bursts (top trace). However, after several hundred milliseconds of parabrachial activation, LT bursts were eliminated, and only tonic activity was evoked.

depolarized membrane potential (Fig. 5A) to those that were generated at a more hyperpolarized membrane potential both before and during parabrachial activation (Figs. 5B and 5C, respectively). At the depolarized membrane voltage (Fig. 5A), the cell responds in the relay mode. This response appears to exhibit considerable linearity, because the sinusoidal shape of the response reflects the sinusoidal shape of the contrast changes presented by the drifting grating. At the hyperpolarized membrane voltage (Fig. 5B), the cell responds in the burst mode. This response clearly exhibits less linearity than that of Fig. 5A. It has a prominent transient peak early in the response that reflects LT bursting, and this is followed by a weaker response that reflects a tonic response component following the LT bursting in each cycle (see Figs. 1 and 2). However, soon after we triggered the parabrachial activation from the same initial membrane potential (Fig. 5C), the cell began to respond in the tonic mode with no LT bursts and with much more linearity. Indeed, the response became very much like that seen in Fig. 5A. The only notable difference was a slight disparity in response amplitude, which is most likely due to the fact that the membrane voltage of the cell during the response illustrated in Fig. 5C eventually climbed to no higher than −70 mV and thus remained hyperpolarized with respect to the situation of Fig. 5A. Finally, Figs. 5B and 5C show that when parabrachial activation causes

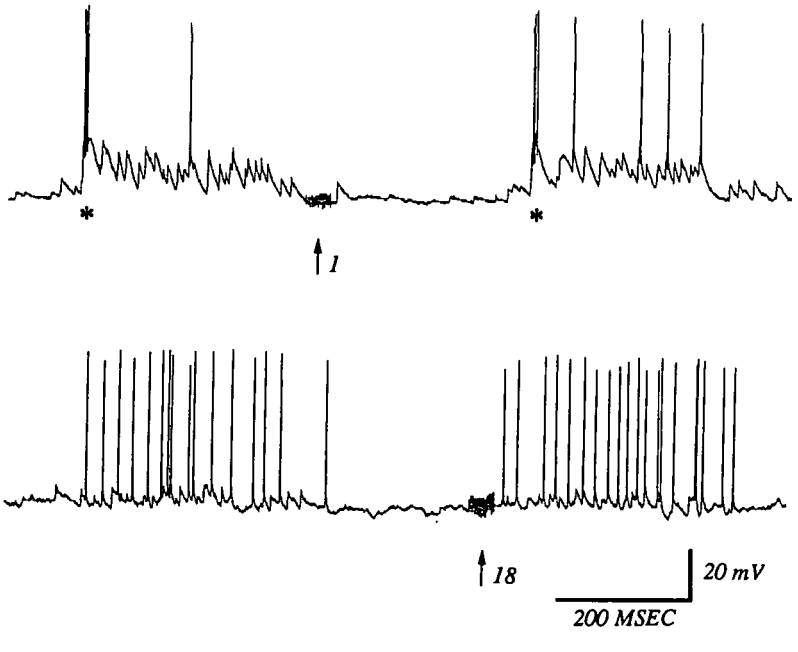

Fig. 4. Intracellular records showing responses in expanded time base before and during parabrachial activation for cell shown in Fig. 2; conventions and observations are as in Fig. 3.

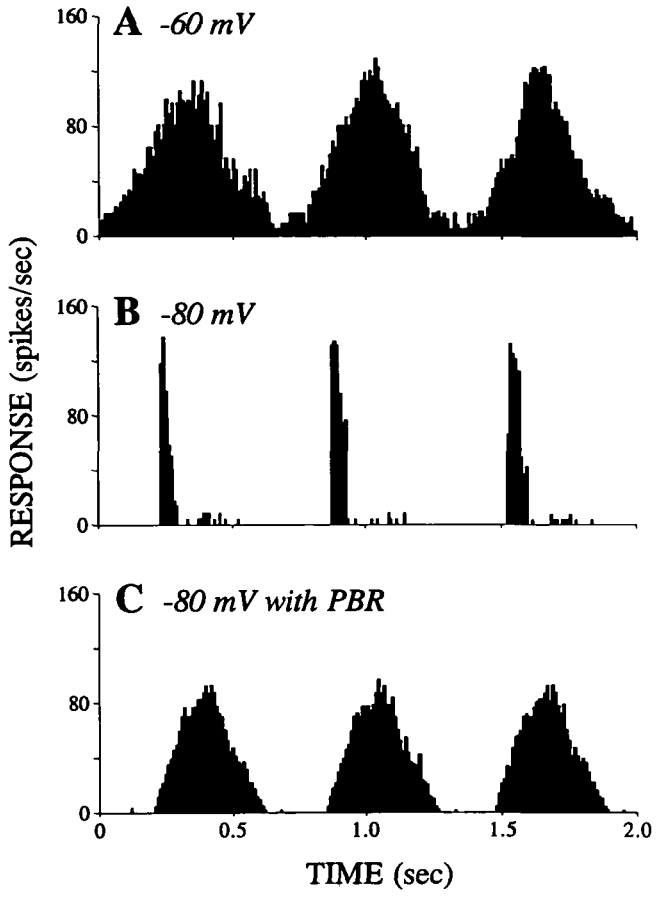

Fig. 5. Typical response histograms to three successive stimulus cycles of a drifting grating for the Y cell shown in Figs. 2 and 4. A: Responses at −60 mV without parabrachial activation. There is no evidence of LT spiking, and the cell thus responded in the relay mode. Note the fairly sinusoidal response profile, matching the contrast changes in the stimulus, thereby indicating considerable linear response summation. B: Responses at −80 mV before parabrachial activation. LT spiking was evident, and the cell thus responded in the burst mode. Note that compared to A, the response profile here is less sinusoidal, indicating more nonlinear distortion in the response. C: Responses during parabrachial activation beginning at the same membrane potential (−80 mV) as in B. Soon after parabrachial activation, the cell switched from the burst to the relay mode, and the membrane gradually depolarized by 5–10 mV. As the LT bursts were eliminated, the response shows much more linearity. Note the similarity between histograms in A and C.

the switch from burst to relay it also greatly enhances overall response. This is because the elimination of LT bursting is more than compensated by the great increase in the tonic response.

Extracellular recording

Although the above observations could be clearly made from our intracellular records, the sheer difficulty in sustaining good-quality intracellular recording *in vivo* long enough to make these observations means that such intracellular recording is an impractical means of validating these observations on a larger cell sample. One could readily obtain a larger cell sample through extracellular recording, but identifying the burst or relay response modes, which is central to the above observations, is not straightforward with extracellular recording. However, we solved this problem of response mode identification by adopting the empirical criteria set forth in our recent papers (Guido et al., 1992; Lu et al., 1992). Briefly, a response episode is considered to be an LT burst if its component action potentials exhibit interspike intervals ≤4 ms and if the first action potential in the burst episode occurs after a silent period of ≥100 ms for the rates of temporal stimulation (≤8 Hz) used in the present study. All other response episodes are considered to be part of the tonic response component.

We thus were able to extend and confirm our intracellular observations on the effects of parabrachial activation across a sample of 18 geniculate cells (6 X and 12 Y) recorded extracellularly. As in our previous study of extracellularly recorded responses to visual stimulation (Guido et al., 1992), we found that most cells exhibited epochs of LT bursting interspersed with tonic response components, while a minority responded exclusively in the relay mode. Overall, 13 of our cells in the study (2 X and 11 Y) responded with significant LT bursting, and five (4 X and 1 Y) responded solely in the relay mode. Thus more of our Y-cell sample (11 of 12) exhibited LT bursting than did our X-cell sample (2 of 6), a difference that is statistically significant ($P < 0.05$ on a χ^2 test). This difference between X and Y cells has been noted before (Guido et al., 1992; Lo et al., 1991).

As with intracellular recording, we found that every cell recorded extracellularly responded only in the tonic mode during parabrachial activation. Fig. 6 shows an example for an X cell recorded extracellularly. Prior to parabrachial activation (Fig. 6A), the cell responded with a mixture of LT bursting and tonic responses. By using our above mentioned criteria to distinguish these response components (see Lu et al., 1992), we could separate the total response (Fig. 6A, top histogram) into an LT burst component (Fig. 6A, middle histogram) and tonic response component (Fig. 6A, bottom histogram). Note that the total response is not especially sinusoidal in shape, suggesting limited linearity. Note also that the prominent LT burst precedes the tonic response component (Lu et al., 1992) and is largely responsible for the nonlinearity of the total response (Guido et al., 1992). During parabrachial activation (Fig. 6B), the LT bursting was *completely* eliminated, resulting in a purely tonic response. There has thus been a dramatic switch in response mode from mostly burst mode to completely relay mode. Compared to the response before parabrachial activation, the response during parabrachial activation is much more sinusoidal in shape and thus more linear, and it also is larger in amplitude because the elimination of LT bursts is more than compensated by the increase in the tonic response component. A comparison of Figs. 5 and 6 shows that these basic features seen during extracellular recording mimic those seen with intracellular recording.

By combining the extracellular and intracellular results, we were able to quantify the effects of parabrachial activation on various response measures. Fig. 7 shows the effects of this activation on responses to visual stimuli. The fundamental (F1) Fourier response amplitude was calculated for the total response (Fig. 7A), and then separately for the tonic response (Fig. 7B), and the LT burst components (Fig. 7C). Parabrachial activation resulted in an increase in the total response for all but one cell—an X cell (Fig. 7A). This happens to be one of the X cells recorded extracellularly that responded exclusively in the relay mode prior to parabrachial activation. Fig. 7B shows that the

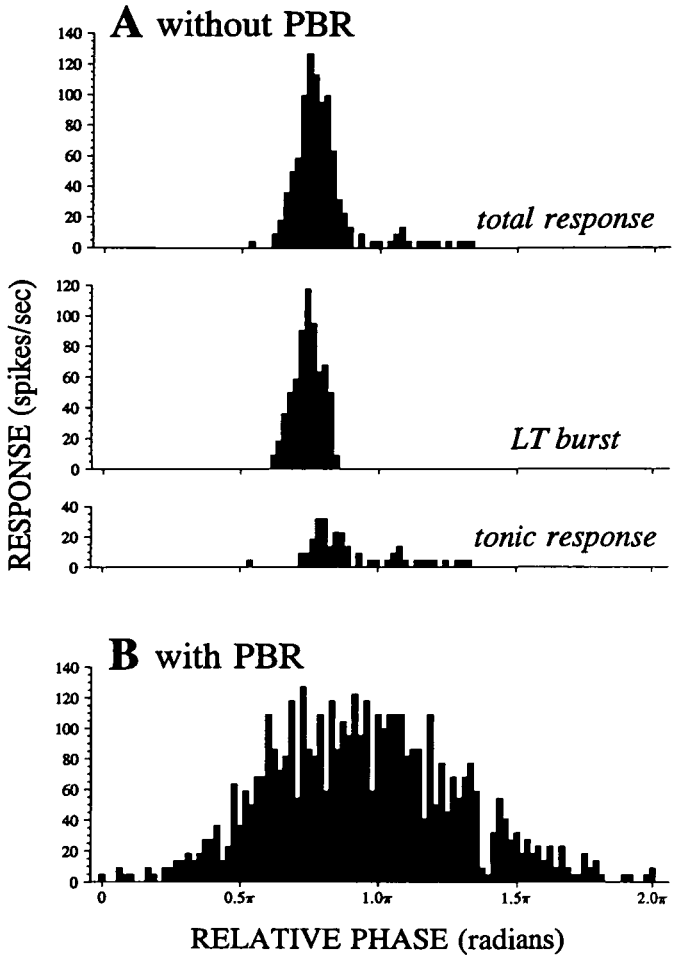

Fig. 6. Average histograms showing response to one cycle of a drifting grating for an X cell recorded extracellularly. A: Response without parabrachial activation. Shown is the total response (top histogram), which includes both the LT burst (middle histogram) and tonic response component (bottom histogram). B: Response during parabrachial activation. LT bursting was completely eliminated, and the response became purely tonic.

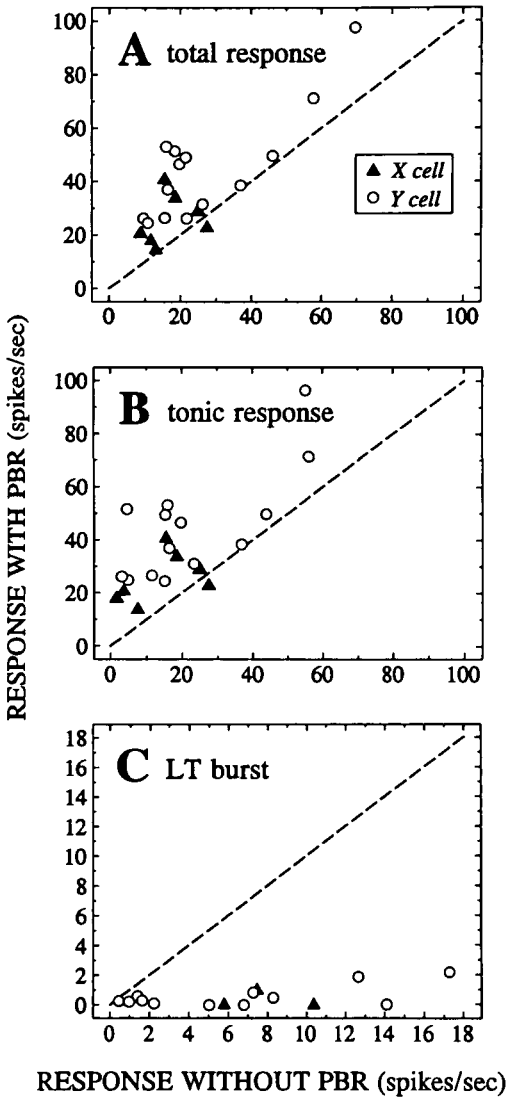

Fig. 7. Scatterplot showing the fundamental (F1) Fourier response amplitude before and during parabrachial activation for our sample of 21 geniculate cells; X and Y cells are separately indicated. In each plot, the line of slope 1 is shown to clearly indicate whether parabrachial activation increases or decreases the response amplitude. A: Total response. All but one cell (an X cell) showed an increase in F1 response amplitude with parabrachial activation. B: Tonic response component. Every cell showed an increased response with parabrachial activation except for the same X cell that failed to do so in A. Note that the increase in the total response (A) could be accounted for by an increase in the tonic response component. C: LT burst component for the subset of 13 cells displaying LT bursting before parabrachial activation. Note that parabrachial activation dramatically reduced LT bursting.

tonic response component of all but one cell (the same X cell as noted above) also increases as a result of parabrachial activation. This was equally true for cells initially responding purely in the relay mode as well as for cells responding with LT bursts. Finally, Fig. 7C shows that the subset of cells displaying LT bursting before parabrachial activation (3 X and 12 Y) show little or none during such activation. Note, however, the different scales of Figs. 7A and 7B vs. 7C, indicating that, in absolute terms, more of the fundamental Fourier response is contained in the tonic response component than in the LT bursts. Thus parabrachial activation, through its dramatic reduction in LT bursting (Fig. 7C), removes fewer action potentials from the overall response than it adds to the tonic response component (Fig. 7B), resulting in a larger overall response (Fig. 7A).

The above analysis indicates that parabrachial activation concurrently reduces LT bursting as it increases the amplitude of the tonic response component. Fig. 8 explores this further for the subset of cells displaying LT bursts before parabrachial activation, and it shows for each cell the effects of this activation on both components. This is expressed as a change in the F1 Fourier response amplitude, with negative values indicating a reduction. We found that these two effects of parabrachial activation were negatively correlated ($r = -0.57$; $P < 0.05$). The correlation, while significant, shows considerable variation, perhaps because of a "basement" effect: in each cell, LT bursting is reduced to nearly zero from varying levels, while the extent of increase of the tonic response component does not encroach upon an analogous nonlinear limit.

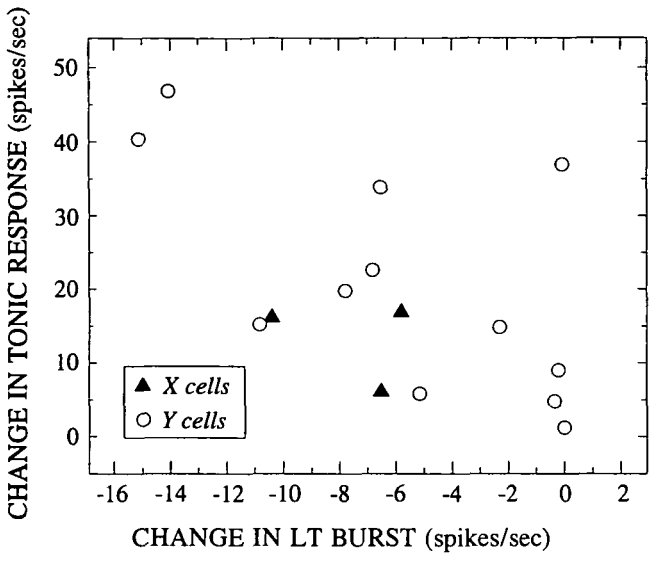

Fig. 8. Scatterplot showing the conjoint effects of parabrachial activation on the LT burst and tonic response components; X and Y cells are separately indicated. Each point represents the change induced by parabrachial activation in the F1 response amplitude for each component, and negative values indicate a reduction in response. Note the tendency for parabrachial activation to reduce LT bursting as it increases the tonic response ($r = -0.57$; $P < 0.05$).

Finally, parabrachial activation causes geniculate cells displaying LT bursting to respond more linearly to visual stimuli. This is because parabrachial activation reduces LT bursting, and LT bursts add a substantial nonlinearity to responses to visual stimuli (see Figs. 5 and 6; see also Guido et al., 1992). To illustrate this effect more quantitatively, we computed both the first and second harmonic Fourier amplitudes (F1 and F2, respectively) of the response, because the F1 value reflects the linear portion of the response, while the F2 value reflects some of the nonlinearities in the overall response. We then calculated an index of response-nonlinearity for each cell before and during parabrachial activation. This index is the F2/F1 ratio. Hochstein and Shapley (1976) also used the F2/F1 ratio as a nonlinearity index, but their use differs from ours. They stimulated cells with a counterphase modulated grating, and the resulting nonlinearities are most pronounced at even higher harmonics, being most prominent in the F2 response. Such pronounced doubling is not seen with the drifting gratings that we used. Rather, the F2 response generated by drifting gratings reflects an estimate of nonlinearity that is more evenly spread across even *and* odd higher harmonics, and we use the F2/F1 ratio here as a simple measure of this nonlinearity. Fig. 9 shows that every one of the cells displaying LT bursting before parabrachial activation showed a decreased F2/F1 ratio during this activation.

Discussion

We found that activation of the parabrachial input to the lateral geniculate nucleus has a dramatic effect on the responses to visual stimuli of geniculate neurons. That is, parabrachial activation led to a pronounced reduction in LT bursting. These results extend our earlier observations based on responses of geniculate neurons to electrical activation (Lo et al., 1991), and they also help to provide a clearer picture regarding effects of nonretinal inputs on retinogeniculate transmission. Furthermore, as parabrachial activation reduced LT bursting, it simultaneously enhanced the tonic response component, and since this latter component tended to be larger, this activation also served to enhance the overall response of most cells.

Mechanisms of the effects of parabrachial activation

Our results are most parsimoniously explained by the depolarization of geniculate relay cells *via* parabrachial activation. Such depolarization not only inactivates the LT Ca^{2+} spike but also will enhance the tonic response component. There seem to be two related mechanisms by which parabrachial activation achieves this: direct excitation of relay cells and disinhibition of these cells by direct inhibition of the local inhibitory GABAergic cells, the interneurons and perigeniculate cells. Our intracellular records are consistent with this view, since we noted that parabrachial activation both enhanced depolarization and reduced hyperpolarization in response to visual stimuli. Furthermore, several *in vivo* electrophysiological studies have shown that electrical activation of the parabrachial region causes excitation of relay cells and inhibition of perigeniculate cells (e.g. Ahlsén, 1984; Ahlsén et al., 1984; Deschênes & Hu, 1990; Fourment et al., 1988; Francesconi et al., 1988; Hu et al., 1989; Lo et al., 1991; Steriade & Deschênes, 1988). Unfortunately, with the techniques used in the present account and these prior studies, it is not possible to distinguish monosynaptic from multisynaptic effects.

However, a combination of morphological and *in vitro* pharmacological studies indicates that both of these effects, excitation of relay cells and inhibition of local inhibitory cells, result respectively from monosynaptic action of parabrachial axons

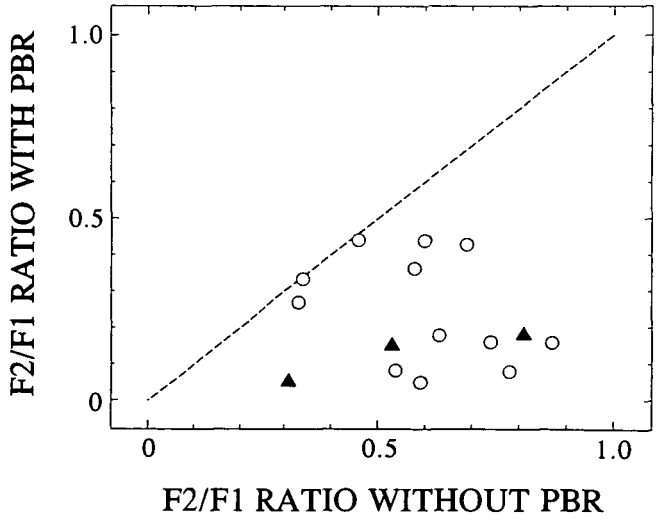

Fig. 9. Scatterplot showing the ratios F2/F1 Fourier response amplitudes of a cell's total response measured before and during parabrachial activation; X (solid triangles) and Y cells (open circles) are separately indicated. The dashed line has a slope of 1. All points are below this line, indicating that every cell exhibiting LT bursting before parabrachial activation showed a decreased F2/F1 ratio during such activation. Thus by eliminating LT bursts, parabrachial activation led to more linear-response summation.

on relay cells and local inhibitory cells. Immunocytochemical studies with the electron microscope have shown that cholinergic terminals within the A-laminae of the lateral geniculate nucleus form synaptic contacts onto both relay cells and interneurons, and the major source of cholinergic terminals to these laminae derives from the parabrachial region (de Lima et al., 1985; de Lima & Singer, 1987; Fitzpatrick et al., 1989; Raczkowski & Fitzpatrick, 1989; Smith et al., 1988). Complementary studies in which single parabrachial axons were labeled and reconstructed in the thalamus show that they innervate both the lateral geniculate and perigeniculate nuclei and that, within the A-laminae, single axons contact both relay cells and interneurons (Uhlrich et al., 1988; Cucchiaro et al., 1988). Pharmacological studies of geniculate cells recorded *in vitro* further support this conclusion (McCormick, 1992; McCormick & Pape, 1988; McCormick & Prince, 1987). Application of ACh depolarizes relay cells, chiefly through a nicotinic receptor that increases conductance to a variety of cations and also through an M1 muscarinic receptor that decreases a K^+ conductance. In contrast, ACh hyperpolarizes interneurons and perigeniculate cells through an M2 muscarinic receptor that increases a K^+ conductance. Therefore, a single cholinergic axon from the parabrachial region can simultaneously excite relay cells and inhibit local inhibitory neurons.

Both the direct excitation and disinhibition of relay cells will tend to depolarize them, and as noted above, this may be sufficient to explain our current observations. If so, then one should be able during intracellular recording to mimic the effects of parabrachial activation on responses to visual stimulation simply by appropriately adjusting membrane voltage. To date, our intracellular recordings have not been directed at such precise matching of membrane potentials. Until such a result is obtained, it is not possible to conclude that the only effects of parabrachial action on relay cell activity and LT spikes are due to changes in membrane voltage.

Effects of parabrachial activation on transmission of receptive-field properties

Effects on response mode

An active parabrachial input obviously causes geniculate cells to respond more in the relay that in the burst mode. As we have noted before (Guido et al., 1992; Lo et al., 1991; Lu et al., 1992), geniculate neurons, at least under the physiological conditions of our recording preparations, seem to operate very close to the threshold for LT spike activation and inactivation. While all of our cell sample recorded intracellularly responded in either mode depending on the imposed membrane potential, most cells recorded extracellularly alternated between visually evoked response modes in a seemingly random fashion. This may simply reflect variation in activity among any given cell's parabrachial afferents. Interestingly, we had previously noted that several cells recorded simultaneously could switch between modes out of phase with one another so that some cells respond in burst mode while others do so in relay mode (Guido et al., 1992). This suggests that variations in activity among parabrachial inputs need not reflect the entire pathway but may instead be rather specific for geniculate cells or subsets thereof. More importantly, because geniculate cells in our preparation seem to reside close to the inactivation threshold for LT spiking, there need be only minor changes in activity from the parabrachial region to effect a switch between the burst and relay response modes. Of course, other inputs, such as those from retina and visual cortex, may also change membrane potential and thus switch a geniculate cell between response modes.

Effects on response linearity

Our current results confirm our earlier ones: the tonic response component and relay mode represents a period during which the neuron responds to visual stimuli with a relatively high degree of linearity, while LT bursts add considerable harmonic distortion to the response (Guido et al., 1992; Lu et al., 1992). Thus, one of the main consequences of parabrachial activation is that it helps to preserve linearity in the responses of geniculate cells to visual targets. This is a direct consequence of the reduction in LT bursting induced by parabrachial activation.

In order for the cortex to decode completely the signals reaching it from the lateral geniculate nucleus and reconstruct the visual world, it is important to preserve as much linearity as possible in these signals. Once lost or distorted, this information cannot be retrieved. By enhancing linearity in retinogeniculate transmission, the parabrachial region helps to maximize the information relayed to the cortex *via* the lateral geniculate nucleus.

Hypothesis for role played by LT spikes and the parabrachial region

An obvious question that needs to be addressed is the larger functional role played by LT spikes and the need to control them *via* inputs from the parabrachial region. Why pay the price for LT spikes if they distort the visual signal? We can suggest a plausible hypothesis, but we emphasize that many features of it have yet to be tested.

In a general way, activity in the parabrachial region might relate to overall arousal and visual attention for the animal (reviewed in Steriade & Deschênes, 1988; Steriade & McCarley, 1990; see also Livingstone & Hubel, 1981; McCarley et al., 1983). When less attentive or aroused, a lower activity level in parabrachial axons would result in geniculate neurons becoming hyperpolarized. This would result from a removal of both excitation and disinhibition. It is important that potentially salient or threatening visual objects be detected reliably and analyzed efficiently by the cortex so that attention can be switched on, much like the "searchlight" suggested by Crick (1984).

A problem is that, without some boosting or amplification, a hyperpolarized geniculate relay cell may not be able to relay a potentially important signal from retina to cortex. A solution is to empower geniculate (and other thalamic) relay cells with a nonlinear amplification that permits a detection signal to get through at the expense of retaining all of the information about the stimulus originally encoded by the retina. This nonlinear amplification is the LT Ca^{2+} spike. Once the cortex is notified of a potentially interesting or threatening visual stimulus, its next task is to gain more detailed information about the visual stimulus. This can be achieved by depolarizing the appropriate relay cells, which serves both to eliminate LT bursts, removing the nonlinear distortions, and to make the cell more responsive to retinal inputs.

Precisely how the cortex achieves this depolarization is even more speculative, but two complementary mechanisms can be considered. First, the descending corticogeniculate pathway,

which contacts both relay cells and the local GABAergic cells (i.e. interneurons and perigeniculate cell or cells of the thalamic reticular nucleus), can depolarize certain relay cells in a very specific, highly topographic manner (for details, see Koch, 1987; Sherman & Koch, 1986, 1990). Second, the cortex might influence the parabrachial region by some unspecified route. For example, a potential pathway exists from visual cortex to striatum to substantia nigra to the parabrachial region (Hall et al., 1989). Also, projections from the visual cortex to the superior colliculus could perhaps serve as a link to the parabrachial region *via* local connections. If so, then visual cortex may influence parabrachial cells to become more active, and this could serve to maintain large ensembles of relay cells in the relay firing mode.

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References

AHLSÉN, G. (1984). Brainstem neurones with differential projection to functional subregions of the dorsal lateral geniculate complex in the cat. *Neuroscience* **12**, 817–838.

AHLSÉN, G., LINDSTRÖM, S. & LO, F.-S. (1984). Inhibition from the brainstem of inhibitory interneurones of the cat's dorsal lateral geniculate nucleus. *Journal of Physiology* (London) **347**, 593–609.

BERMAN, A.I. (1968). *The brainstem of the Cat. A Cytoarchitectonic Atlas with Stereotaxic Coordinates.* Madison, Wisconsin: University of Wisconsin Press.

BLOOMFIELD, S.A., HAMOS, J.E. & SHERMAN, S.M. (1987). Passive cable properties and morphological correlates of neurones in the lateral geniculate nucleus of the cat. *Journal of Physiology* (London) **383**, 653–692.

BLOOMFIELD, S.A. & SHERMAN, S.M. (1988). Postsynaptic potentials recorded in neurons of the cat's lateral geniculate nucleus following electrical stimulation of the optic chiasm. *Journal of Neurophysiology* **60**, 1924–1945.

BURKE, W. & COLE, A.M. (1978). Extraretinal influence on the lateral geniculate nucleus. *Reviews in Physiology, Biochemistry and Pharmacology* **80**, 105–166.

CRICK, F. (1984). Function of the thalamic reticular complex: The searchlight hypothesis. *Proceedings of the National Academy of Sciences of the U.S.A.* **81**, 4586–4590.

CUCCHIARO, J.B., UHLRICH, D.J. & SHERMAN, S.M. (1988). Parabrachial innervation of the cat's dorsal lateral geniculate nucleus: An electron microscopic study using the tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L). *Journal of Neuroscience* **8**, 4576–4588.

DE LIMA, A.D., MONTERO, V.M. & SINGER, W. (1985). The cholinergic innervation of the visual thalamus: An EM immunocytochemical study. *Experimental Brain Research* **59**, 206–212.

DE LIMA, A.D. & SINGER, W. (1987). The brainstem projection to the lateral geniculate nucleus in the cat: Identification of cholinergic and monoaminergic elements. *Journal of Comparative Neurology* **259**, 92–121.

DESCHÊNES, M. & HU, B. (1990). Membrane resistance increase induced in thalamic neurons by stimulation of brainstem cholinergic afferents. *Brain Research* **513**, 339–342.

DESCHÊNES, M., PARADIS, M., ROY, J.P. & STERIADE, M. (1984). Electrophysiology of neurons of lateral thalamic nuclei in cat: Resting properties and burst discharges. *Journal of Neurophysiology* **51**, 1196–1219.

FITZPATRICK, D., DIAMOND, I.T. & RACZKOWSKI, D. (1989). Cholinergic and monoaminergic innervation of the cat's thalamus: Comparison of the lateral geniculate nucleus with other principal sensory nuclei. *Journal of Comparative Neurology* **288**, 647–675.

FOURMENT, A., HIRSCH, J.C. & MARC, M.E. (1988). Reticular control of thalamic transmission during behavioral states: A study in dorsal lateral geniculate nucleus relay neurons of the cat. *Experimental Neurology* **100**, 305–321.

FRANCESCONI, W., MÜLLER, C.M. & SINGER, W. (1988). Cholinergic mechanisms in the reticular control of transmission in the cat lateral geniculate nucleus. *Journal of Neurophysiology* **59**, 1690–1718.

FUNKE, K. & EYSEL, U.T. (1992). EEG-dependent modulation of response dynamics of cat dLGN relay cells and the contribution of corticogeniculate feedback. *Brain Research* **573**, 217–227.

GUIDO, W., LU, S.-M. & SHERMAN, S.M. (1992). Relative contributions of burst and tonic responses to the receptive-field properties of lateral geniculate neurons in the cat. *Journal of Neurophysiology* **68**, 2199–2211.

HALL, W.C., FITZPATRICK, D., KLATT, L.L. & RACZKOWSKI, D. (1989). Cholinergic innervation of the superior colliculus in the cat. *Journal of Comparative Neurology* **287**, 495–514.

HOCKSTEIN, S. & SHAPLEY, R.M. (1976). Quantitative analysis of retinal ganglion cell classifications. *Journal of Physiology* (London) **262**, 237–264.

HU, B., STERIADE, M. & DESCHÊNES, M. (1989). The effects of brainstem peribrachial stimulation on neurons of the lateral geniculate nucleus. *Neuroscience* **31**, 13–24.

IKEDA, H. & WRIGHT, M.J. (1974). Sensitivity of neurons in the visual cortex (area 17) under different levels of anesthesia. *Experimental Brain Research* **20**, 471–484.

JAHNSEN, H. & LLINÁS, R. (1984*a*). Electrophysiological properties of guinea-pig thalamic neurones: An *in vitro* study. *Journal of Physiology* (London) **349**, 205–226.

JAHNSEN, H. & LLINÁS, R. (1984*b*). Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurones *in vitro*. *Journal of Physiology* (London) **349**, 227–247.

KOCH, C. (1987). The action of the corticofugal pathway on sensory thalamic nuclei: A hypothesis. *Neuroscience* **23**, 399–406.

LIVINGSTONE, M.S. & HUBEL, D.H. (1981). Effects of sleep and arousal on the processing of visual information in the cat. *Nature* **291**, 554–561.

LO, F.-S., LU, S.-M. & SHERMAN, S.M. (1991). Intracellular and extracellular *in vivo* recording of different response modes for relay cells of the cat's lateral geniculate nucleus. *Experimental Brain Research* **83**, 317–328.

LU, S.-M., GUIDO, W. & SHERMAN, S.M. (1992). Effects of membrane voltage on receptive-field properties of lateral geniculate neurons in the cat: Contributions of the low threshold Ca^{2+} conductance. *Journal of Neuroscience* **68**, 2185–2198.

MCCARLEY, R.W., BENOIT, O. & BARRIONUEVO, G. (1983). Lateral geniculate nucleus unitary discharge in sleep and waking: State- and rate-specific aspects. *Journal of Neurophysiology* **50**, 798–818.

MCCORMICK, D.A. (1992). Cellular mechanisms underlying cholinergic and noradrenergic modulation of neuronal firing mode in the cat and guinea pig dorsal lateral geniculate nucleus. *Journal of Neuroscience* **12**, 278–289.

MCCORMICK, D.A. & FEESER, H.R. (1990). Functional implications of burst firing and single spike activity in lateral geniculate relay neurons. *Neuroscience* **39**, 103–113.

MCCORMICK, D.A. & PAPE, H.-C. (1988). Acetylcholine inhibits identified interneurons in the cat lateral geniculate nucleus. *Nature* **334**, 246–248.

MCCORMICK, D.A. & PRINCE, D.A. (1987). Actions of acetylcholine in the guinea-pig and cat medial and lateral geniculate nuclei, *in vitro*. *Journal of Physiology* (London) **392**, 147–165.

RACZKOWSKI, D. & FITZPATRICK, D. (1989). Organization of cholinergic synapses in the cat's dorsal lateral geniculate and perigeniculate nuclei. *Journal of Comparative Neurology* **288**, 676–690.

SHERMAN, S.M. & KOCH, C. (1986). The control of retinogeniculate transmission in the mammalian lateral geniculate nucleus. *Experimental Brain Research* **63**, 1–20.

SHERMAN, S.M. & KOCH, C. (1990). Thalamus. In *Synaptic Organization of the Brain*, 3rd edition, ed. SHEPHERD, G.M., pp. 246–278. New York: Oxford University Press.

SINGER, W. (1977). Control of thalamic transmission by corticofugal and ascending reticular pathways in the visual system. *Physiological Reviews* **57**, 386–420.

SMITH, Y., PARÉ, D., DESCHÊNES, M., PARENT, A. & STERIADE, M.

(1988). Cholinergic and non-cholinergic projections from the upper brainstem core to the visual thalamus in the cat. *Experimental Brain Research* **70**, 166–180.

STERIADE, M. & DESCHÊNES, M. (1988). Intrathalamic and brainstem-thalamic networks involved in resting and alert states. In *Cellular Thalamic Mechanisms*, ed. BENTIVOGLIO, M. & SPREAFICO, R., pp. 37–62. Amsterdam: Elsevier.

STERIADE, M. & LLINÁS, R. (1988). The functional states of the thalamus and the associated neuronal interplay. *Physiological Reviews* **68**, 649–742.

STERIADE, M. & MCCARLEY, R.W. (1990). *Brainstem Control of Wakefulness and Sleep*. New York: Plenum Press.

UHLRICH, D.J., CUCCHIARO, J.B. & SHERMAN, S.M. (1988). The projection of individual axons from the parabrachial region of the brainstem to the dorsal lateral geniculate nucleus in the cat. *Journal of Neuroscience* **8**, 4565–4575.

UHLRICH, D.J., TAMAMAKI, N. & SHERMAN, S.M. (1990). Brainstem control of response modes in neurons of the cat's lateral geniculate nucleus. *Proceedings of the National Academy of Sciences of the U.S.A.* **87**, 2560–2563.