Role of Reticular Activation in the Modulation of Intracortical Synchronization

Matthias H. J. Munk,* Pieter R. Roelfsema, Peter König,† Andreas K. Engel, Wolf Singer

During aroused states of the brain, electroencephalographic activity is characterized by fast, irregular fluctuations of low amplitude, which are thought to reflect desynchronization of neuronal activity. This phenomenon seems at odds with the proposal that synchronization of cortical responses may play an important role in the processing of sensory signals. Here, activation of the mesencephalic reticular formation (MRF), an effective way to "desynchronize the electroencephalogram," was shown to facilitate oscillatory activity in the gamma frequency range and to enhance the stimulus-specific synchronization of neuronal spike responses in the visual cortex of cats.

During drowsiness, deep sleep, and anesthesia, electroencephalographic activity is characterized by low-frequency oscillations (<10 Hz) of high amplitude (*1*), which are thought to reflect the synchronous and periodic activation of large cell populations (*2*). With arousal, fast, irregular fluctuations of small amplitude replace the low-frequency activity. Arousal and its corresponding electroencephalogram (EEG) patterns can be caused by electrical stimulation of the MRF (*3*). Although surface recordings appear flat and irregular in aroused states, regular periodic activity patterns do occur in the cortical EEG and field potentials, albeit at higher frequencies (*4*, *5*) and exhibiting less coherence compared with slow oscillations (*6*).

Intracerebral recording techniques have recently uncovered synchronization between spike responses of cortical neurons in the awake or lightly anesthetized animal that occurred in response to appropriate sensory activation (*7*) or in association with solving sensorimotor tasks (*5*). This synchronization occurs with a precision in the millisecond range, is found over large cortical distances (*8–10*), and is often associated with oscillatory activity in the gamma frequency band (>30 Hz) (*5*, *8*, *9*, *11–14*). There is evidence that gamma-range oscillations can be enhanced by activation of the MRF in the sensory (*15*), association, and motor areas of the cortex (*6*, *16*). In the visual cortex, synchronization of evoked activity depends on the configuration of the activating stimuli (*8*, *10*, *12–14*) and is particularly strong between cells responding to features of a single coherent object. The hypothesis that neuronal synchronization could play a role in response selection and perceptual grouping (*7*) seems at odds with the evidence that wakefulness is associated with "EEG desynchronization."

To test the extent to which the occurrence and feature selectivity of stimulus-induced response synchronization in the visual cortex are influenced by activation of the MRF and its desynchronizing effect on the EEG, we recorded multiunit activity (MUA) and local field potentials (LFPs) from 48 pairs of spatially separate sites in the visual cortex of four lightly anesthetized cats (*9*, *10*) while increasing the degree of EEG desynchronization by electrical stimulation of the MRF (*17*). The signal power in the gamma frequency band of LFPs and the correlation strength of spike responses were compared for distinct periods before and after MRF stimulation. A typical result from an experiment in which neuronal responses were recorded with two electrodes from corresponding sites in area 17 of the two hemispheres is shown in Fig. 1. Stimulation of the MRF applied shortly before the onset of each light-evoked response facilitated the strength of synchronization (N = 12, average increase 35%), or, as in this case, made responses synchronize that lacked any signs of synchrony without MRF stimulation (N = 9, average correlation strength 17%). This facilitation of response synchronization occurred without a change in the strength of the visual responses (compare Fig. 1, C and E), which indicated that the occurrence of correlated discharges was not simply a result of enhanced discharge rates.

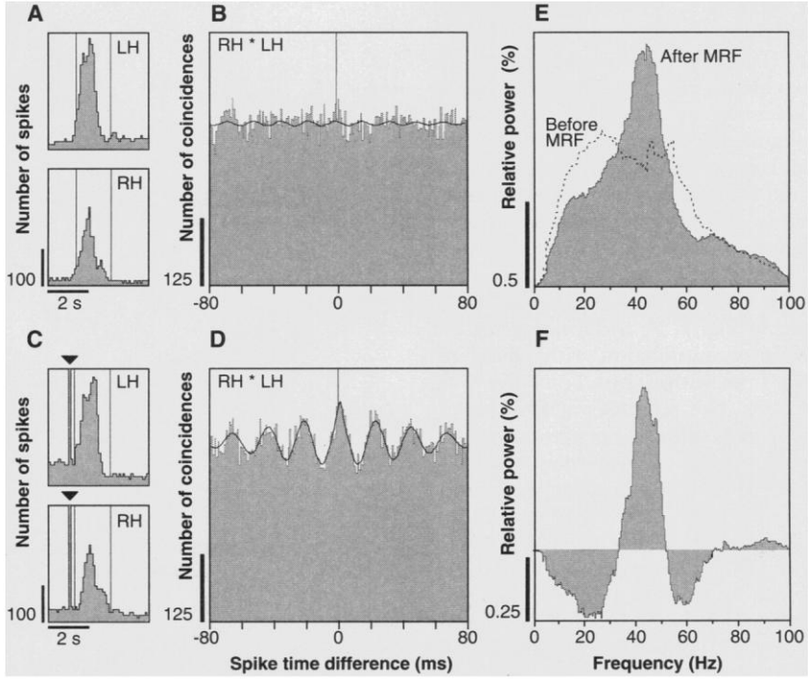

Fig. 1. MRF stimulation facilitates synchronization of visual responses. MUA was recorded from neurons in area 17 close to the representation of the vertical meridian in the left (LH) and right (RH) hemisphere, and simultaneous responses were evoked with a moving light bar. (**A** and **C**) PSTHs of responses (summed over 10 successive trials) recorded from the left and right hemispheres, without (A) and with (C) preceding reticular stimulation [artifact indicated by arrowhead in (C)]. Vertical lines delineate the response periods included in cross-correlation analysis. (**B** and **D**) Cross-correlograms between responses shown in (A) and (C), respectively. The thin line corresponds to the fitted Gabor function (*16*). In (D), it accounts for 71% of the variance and exhibits a periodic modulation with a frequency of 45 Hz and a center peak with a relative modulation amplitude of 22%. (**E** and **F**) Power spectra of LFP responses before and after MRF stimulation (E) and their difference (F) were recorded simultaneously with the unit responses in (A) and (C). Spectra were computed from the same signal periods as unit correlograms.

Abteilung Neurophysiologie, Max-Planck-Institut für Himforschung, Deutschordenstrasse 46, 60528 Frankfurt, Germany.

*To whom correspondence should be addressed. E-mail: munk@mpih-frankfurt.mpg.d400.de

†Present address: Neurosciences Institute, 10640 John Jay Hopkins Drive, San Diego, CA 92121, USA.

To assess the desynchronizing effect of MRF stimulation, we analyzed frequency spectra of the LFP during spontaneous and visual activity before and after MRF stimulation (*18*). In the entire sample of LFP recordings, MRF stimulation enhanced the relative signal power in frequency bands above 14 Hz during periods of spontaneous and light-evoked activity and decreased the power in the low-frequency bands (*19*). The example shown in Fig. 1E demonstrates the reduced contribution of lower frequencies to the power spectrum, here between 3 and 32 Hz, and a strong increase of the frequency components in the lower gamma band (32 to 53 Hz). In this case there was also a decrease of frequencies between 53 and 70 Hz (Fig. 1F), but this decrease was not a general feature and therefore did not reach significance in the entire sample (see Fig. 2, third row, for another example that lacks this reduction of high frequencies). The dominant frequency of the periodic modulation in the cross-correlograms (Figs. 1D and 2C, second row) was often close to the peak frequency of the power spectrum of the LFP (Figs. 1F and 2C, third row).

To separate the effect of reticular activation from spontaneous fluctuations of synchronization, we divided each of 85 response sequences, derived from 48 recording site pairs, into four blocks of five responses (Fig. 2) (*20*). The responses in the first two blocks (Fig. 2, A and B) and those in the second two blocks (Fig. 2, C and D) were obtained without and with MRF stimulation, respectively. Spontaneous fluctuations and MRF effects were determined by comparing changes in correlation strength between the first two blocks (Fig. 2, A and B) with those occurring between the second and third block (Fig. 2, B and C). The example in Fig. 2, A to D, illustrates our method of quantification with paired recordings from within area 17 of the same hemisphere. The responses at the two recording sites exhibited no significant synchronization without MRF stimulation (Fig. 2, A and B) but showed strong synchronization immediately after MRF stimulation (Fig. 2C, third block). This synchronizing effect of MRF stimulation had a tendency to decline, as indicated by the less pronounced synchronization in the fourth block (Fig. 2D). In individual recordings, the strength of synchronization exhibited considerable spontaneous fluctuations, as assessed from comparison of the first two blocks, but for the population these fluctuations did not deviate from zero (Fig. 2E, $P > 0.4$, one-sample t test). Because we had multiple measurement series for most of the recording sites, population data are provided as distributions of the average change in correlation strength per recording site pair. For further analysis, only recording sequences whose spontaneous fluctuations remained within 1 SD ($N = 64$) were selected. For these recordings, stimulation of the MRF resulted in a significant increase of spike synchronization (Fig. 2E, $P < 0.02$).

For quantification of the changes in the LFP, data were subdivided into the same four consecutive blocks as for the evaluation of correlation in the MUA responses (Fig. 2). Comparison of the respective power spectra in the example presented in Fig. 2 (third row) revealed that the pronounced shift from low to high frequencies occurred between the second and third blocks and hence was the result of MRF stimulation (compare Fig. 2, B and C). However, this example also showed a trend toward higher frequencies from the first to the second block (compare Fig. 2, A and B), which probably indicates the influence of repeated light stimulation. For the sample of recordings with coherent visual stimulation ($N = 85$), computed as average value per recording site pair ($N = 48$), the cumulative distributions of the difference in relative gamma power (Fig. 2F) showed no significant difference in gamma power between the first and second block (B – A), but a significant increase from the second to the third block (C – B) ($P < 0.02$, t test; $P < 0.02$, sign test).

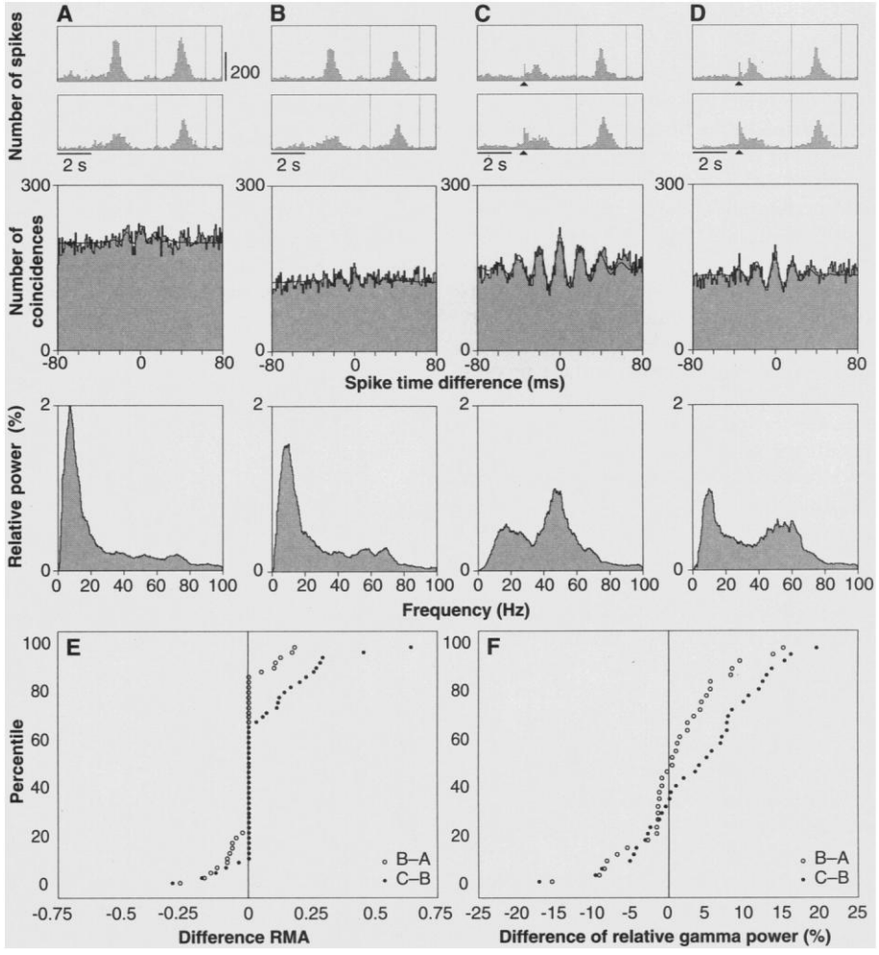

Fig. 2. Demonstration of quantification methods and population data of the reticular effect on spike correlation and gamma power. (**A** to **D**) An example of the effect of reticular stimulation on synchronization between visual responses at two area 17 recording sites in the same hemisphere. Without reticular stimulation [(A) and (B), sum over five responses each] there was no significant correlation, whereas the visual responses to the backward stimulus movement were strongly synchronized (C and D) when, during each cycle of visual stimulation, a short train of electrical pulses (see legend to Fig. 1) was delivered to the MRF (artifacts marked by triangles). Correlograms were computed for the periods indicated by the vertical lines in the PSTHs. The effect of reticular activation on the strength of the synchronization was quantified by subtracting the relative modulation amplitude of the correlograms before reticular stimulation from that during reticular stimulation (C – B). (**E**) The cumulative distributions of these differences (●) indicate an increase in the strength of synchrony during reticular stimulation. In contrast, the spontaneous fluctuations in synchronization strength (B – A, ○) were not significantly different from zero. (**F**) Cumulative distributions of differences in gamma power between blocks A and B (○) and between blocks B and C (●) from 35 recording site pairs (*18*). The abscissa is the difference of power in the gamma band (30 to 47.5 + 52.5 to 100 Hz) in percent.

To test whether MRF stimulation alters the dependence of synchronization on the global configuration of the visual stimulus, we presented different configurations of visual stimuli with and without MRF stimulation in an interleaved protocol. If neurons at two recording sites are activated with a single light bar, they tend to synchronize their responses, but this does not occur if the neurons are activated with two light bars moving in counterphase (*12, 13*). MRF stimulation substantially enhanced synchronization of responses evoked by a single light bar (compare Fig. 3, A and C), but did not synchronize responses elicited by two bars moving in counterphase (Fig. 3, B and D). For the whole sample of cell pairs tested (N = 48, Fig. 3E), MRF stimulation significantly enhanced the synchronization of responses evoked by coherent stimuli ($P < 0.02$, same data as in Fig. 2E), but did not affect the rather sparse occurrence of synchronization during responses to noncoherent visual stimulation ($P > 0.45$), which was as weak and rare as during noncoherent visual stimulation without MRF activation ($P > 0.75$) (*21*). This result indicates that MRF stimulation facilitates response synchronization but does not impair the selectivity with which it reflects global stimulus properties.

The probability and strength of synchronization are constrained by the architecture of intracortical connections (*7, 9, 10, 22*). If the synchronization among responses of cortical neurons actually reflects the way in which the visual system segments visual images, then the criteria for perceptual grouping should reside in the functional architecture of these connections (*7*). Our results show that the strength of stimulus-dependent synchrony is also controlled dynamically by modulatory systems. This offers the possibility that the system could actively adjust synchronization probability and strength and thereby could tune the sensitivity of the grouping mechanism and, presumably, the spatial extent over which it acts. The fact that MRF stimulation favors the occurrence of stimulus-specific response synchronization among spatially distributed neurons while shifting the power of LFP oscillations into the gamma frequency range is further support for the hypothesis that gamma activity serves as a carrier for synchronization phenomena characterized by high temporal precision (*23*).

Synchronization of neuronal responses selectively raises their saliency because coincident synaptic inputs generate responses at later processing stages with higher probability and shorter latency than do noncoincident inputs. It has been proposed, therefore, that response synchronization serves to select and group together subsets of distributed neuronal responses for further joint processing (*7*). Such flexible grouping of responses can be exploited to solve binding problems that are common in processing architectures that rely on population coding (*24*). Reticular activation induces states whose EEG signature closely resembles that of an aroused, performing brain, shifting the power of the EEG toward higher frequencies and favoring the occurrence of oscillatory responses in the gamma frequency range (*6*). The finding that reticular activation enhances response synchronization without decreasing the specificity of its dependence on stimulus configuration suggests that central core systems can dynamically influence the way in which responses are selected for integration at the respective higher processing stages. Such dynamic adjustment could play an important role in attentional processes and sensorimotor integration.

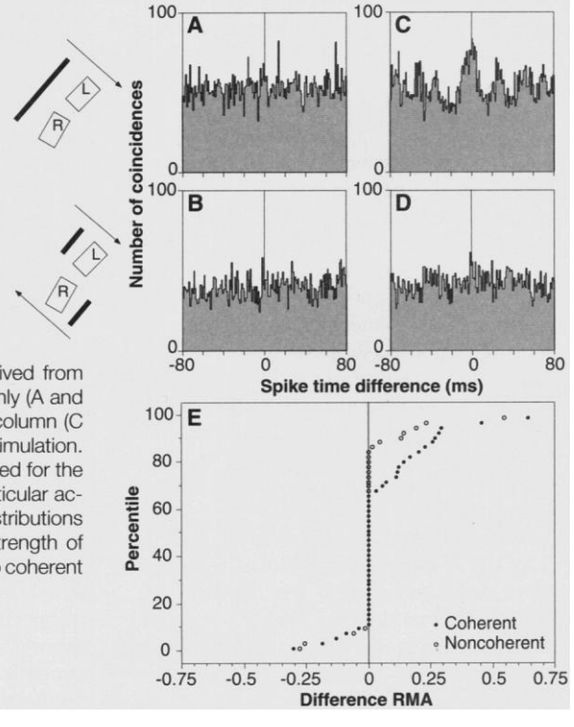

Fig. 3. Stimulus specificity of synchronization probability is not reduced by MRF stimulation, as shown by comparison of the influence of different visual stimuli and reticular activation on the synchronization behavior of cortical neurons. The upper two histograms (**A** and **C**) are computed from responses to a single moving stimulus (coherent), whereas for the lower two histograms (**B** and **D**) the stimulus consisted of two bars moving in counterphase (noncoherent; see diagram at the left). Data presented in the left column are derived from recordings during visual stimulation only (A and B), whereas for the data in the right column (C and D) we additionally applied MRF stimulation. Significant correlation was only obtained for the coherent stimulus condition during reticular activation (RMA, 46%). (**E**) Cumulative distributions of MRF-induced differences in the strength of synchronization between responses to coherent (●) and noncoherent (○) visual stimuli.

REFERENCES AND NOTES

1. M. Steriade, D. A. McCormick, T. J. Sejnowski, *Science* **262**, 679 (1993).
2. M. Abeles, *Local Cortical Circuits, An Electrophysiological Study* (Springer, Heidelberg, 1982).
3. G. Moruzzi and H. W. Magoun, *Electroencephalogr. Clin. Neurophysiol.* **1**, 455 (1949).
4. J. J. Bouyer, M. F. Montaron, A. Rougeul, *ibid.* **51**, 244 (1981).
5. V. N. Murthy and E. E. Fetz, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5670 (1992).
6. M. Steriade, F. Amzica, D. Contreras, *J. Neurosci.* **16**, 392 (1996).
7. W. Singer, *Annu. Rev. Physiol.* **55**, 349 (1993); W. Singer and C. M. Gray, *Annu. Rev. Neurosci.* **18**, 555 (1995).
8. A. K. Engel, A. K. Kreiter, P. König, W. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6048 (1991).
9. A. K. Engel, P. König, A. K. Kreiter, W. Singer, *Science* **252**, 1177 (1991).
10. L. G. Nowak, M. H. J. Munk, J. I. Nelson, A. C. James, J. Bullier, *J. Neurophysiol.* **74**, 2379 (1995).
11. C. M. Gray and W. Singer, *Neuroscience* **22**, S434 (1987); R. Eckhorn *et al.*, *Biol. Cybern.* **60**, 121 (1988); A. K. Kreiter and W. Singer, *Eur. J. Neurosci.* **4**, 369 (1992); R. Eckhorn, A. Frien, R. Bauer, T. Woelbern, H. Kehr, *Neuroreport* **4**, 243 (1993).
12. C. M. Gray and W. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1698 (1989).
13. C. M. Gray, P. König, A. K. Engel, W. Singer, *Nature* **338**, 334 (1989); A. K. Engel, P. König, W. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9136 (1991).
14. A. K. Kreiter and W. Singer, *J. Neurosci*, in press.
15. W. Singer, in *Brain Cholinergic Systems*, M. Steriade and D. Biesold, Eds. (Oxford Univ. Press, Oxford, 1990), pp. 314–336.
16. M. Steriade, R. C. Dossi, D. Pare, G. Oakson, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4396 (1991).
17. This manipulation has been shown to produce a transient desynchronization of the EEG, generating activation patterns that closely resemble those observed during wakefulness as well as during paradoxical sleep [characterized by rapid eye movements and dreaming (*1*)]. Simultaneous MUA and LFP recordings from four to eight electrodes were obtained either within area 17 of the same hemisphere or at the area 17/18 border region of opposite hemispheres in four lightly anesthetized (N_2O/O_2 = 70/30%, supplemented with 0.1 to 0.8% halothane) and paralyzed (pancuronium, 0.08 mg/kg · hour) cats. The animal experiments were performed according to the German Law for the Protection of Experimental Animals and conformed with NIH and American Society for Neuroscience regulations. Recording sites were selected whose receptive fields permitted simultaneous activation with a single moving light bar. In addition, the MRF was stimulated with concentric electrodes at Horsley Clark coordinates (A2-4/L2/H8) with short trains (60 to 100 ms) of constant-current pulses (0.25 to 2 mA) delivered at 75 to 200 Hz, 300 to 500 ms before the onset of the visual responses. The location of the stimulation electrodes was adjusted to produce maximal facilitation of cortical LFP responses evoked by stimulation of the optic chiasm. Histological reconstruction of electrolytic lesions placed along the track of the stimulation electrodes revealed locations within or close to the parabrachial nucleus. Data analysis is based on the computation and quantitative analysis of poststimulus time histograms (PSTHs) and auto- and cross-correlograms [see P. König, *J. Neurosci. Methods* **54**, 31 (1994)]. In short, correlations

were rated as significant if the χ^2 reduction of the fitting procedure exceeded 15% and a z score of >3 was reached, corresponding to a probability of <0.0027 of detection of false positive peaks.

18. LFPs were recorded from the same microelectrodes as MUA by differential filtering (1 to 100 Hz, 3 dB per octave) and digitized at a sampling rate of 1 kHz. Power spectra were computed with a resolution of 0.5 Hz and normalized to the total power between 0.1 and 100 Hz. Frequencies between 47.5 and 52.5 Hz were routinely excluded from analysis, and in the figures they are displayed as linear interpolation between flanking values. The sample of sufficiently noise-free data was recruited from $N = 35$ recording site pairs (see Fig. 2F). To assess the desynchronizing effect of MRF stimulation, we computed the power spectra of the LFPs before and after MRF stimulation from the same periods of light responses used for cross-correlation analysis and from periods of spontaneous activity that had the same duration.
19. In the entire sample, MRF stimulation enhanced the relative (normalized) power of the LFPs in frequency bands above 14 Hz during periods of both spontaneous and light-evoked activity [beta (14 to 30 Hz) and gamma (>30 Hz), $P < 0.05$ in a one-sample t test] and decreased the power in the low-frequency bands for spontaneous activity [alpha (8 to 13 Hz, $P < 0.001$), theta (4 to 7 Hz, $P < 0.01$), and delta (1 to 3 Hz, $P < 0.05$)]. For periods of light-evoked activity, the relative decrease of power in the low-frequency range (<14 Hz) reached significance [alpha ($P < 0.05$), theta ($P < 0.01$), and delta ($P < 0.01$)] only for responses to coherent visual stimuli that also induced response synchronization, but not when compared across all stimulation conditions.
20. For our sample of 760 recording sequences, in which visual coactivation yielded at least 1.5 times as many spikes during responses as during spontaneous activity in five subsequent stimulus presentations, averaged PSTHs and cross-correlograms were computed for the responses to these five stimulus presentations (example in Fig. 2, A to D). Of these 760 sequences, 134 sequences originating from 48 pairs of recording sites showed significant correlation in at least one of the four blocks; 85 sequences were recorded with coherent and 49 with noncoherent visual stimulation (see Fig. 3). A robust measure for correlation strength, which is close to the mean percentage increase in firing probability [see T. C. Cope, E. E. Fetz, M. Matsumura, *J. Physiol. (London)* **390**, 161 (1987)], is the relative modulation amplitude (RMA) of the center peak in the correlogram, defined as the ratio of its height to the mean of the correlation function, expressed either as a real number between 0 and 1 or as a percentage. Computing the differences of RMA preserves the identity of individual measurement series before pooling. For the same reason, power changes in the gamma frequency band of the LFP are also expressed as differences. Because we had multiple measurement sequences (2.8 on average) for most of the recording site pairs, data are presented (Figs. 2, E and F, and 3E) and used in statistical evaluation as average values per pair. We did not normalize the correlograms for the number of stimulus presentations because this would provide no additional information and because the number of trials for each recording sequence (4 times 5) was the same.
21. The analysis was restricted to those cases where spontaneous fluctuations in correlation strength (RMA) remained within 1 SD of the entire distribution, which corresponded to a value of 0.251 RMA. This requirement was met by 64 of 85 recording sequences during coherent visual stimulation and by 38 of 49 recording sequences during noncoherent visual stimulation. Data for correlation changes during noncoherent visual stimulation without MRF activation are not shown.
22. C. Schwarz and J. Bolz, *J. Neurosci.* **11**, 2995 (1991); J. I. Nelson, P. A. Salin, M. H. J. Munk, M. Arzi, J. Bullier, *Visual Neurosci.* **9**, 21 (1992); P. König, A. K. Engel, S. Löwel, W. Singer, *Eur. J. Neurosci.* **5**, 501 (1993); M. H. J. Munk, L. G. Nowak, J. I. Nelson, J. Bullier, *J. Neurophysiol.* **74**, 2401 (1995).
23. P. König, A. K. Engel, W. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 290 (1995); A. K. Engel, P. König, T. B. Schillen, *Curr. Biol.* **2**, 332 (1992).
24. W. Singer, *Science* **270**, 758 (1995).
25. We thank S. Herzog and H. Klon-Lipok for help with the experiments, M. Sum and E. Raulf for expert histology, and R. Ruhl for support with the graphics.

4 October 1995; accepted 6 February 1996

Short-Term Plasticity of a Thalamocortical Pathway Dynamically Modulated by Behavioral State

Manuel A. Castro-Alamancos* and Barry W. Connors

The neocortex receives information about the environment and the rest of the brain through pathways from the thalamus. These pathways have frequency-dependent properties that can strongly influence their effect on the neocortex. In 1943 Morison and Dempsey described "augmenting responses," a form of short-term plasticity in some thalamocortical pathways that is triggered by 8- to 15-hertz activation. Results from anesthetized rats showed that the augmenting response is initiated by pyramidal cells in layer V. The augmenting response was also observed in awake, unrestrained animals and was found to be dynamically modulated by their behavioral state.

Synaptic pathways originating in the thalamus provide sensory and motor information to the neocortex (*1*, *2*). The response characteristics of these pathways are not static but display short-term plasticity (that is, frequency-dependent properties). Thalamocortical pathways are known to be modulated during sleep-wake cycles (*1*, *2*), but the regulation of their plasticity during different waking states has not been studied previously. If this short-term plasticity varied dynamically with behavioral state, the capacity for information processing could be increased. In 1943 Morison and Dempsey showed that low-frequency (8- to 15-Hz) activation of certain thalamic pathways causes a progressively "augmenting response" in the neocortex (*3*). This robust form of short-term plasticity has been demonstrated repeatedly in both motor (*4*) and sensory (*5*) regions of the neocortex. But despite extensive study, there is no consensus regarding the mechanisms of the augmenting response (*6*) or its relation to behavior. We have investigated the augmenting response in a synaptic pathway from the ventrolateral nucleus (VL) of the thalamus to the sensorimotor neocortex and explored its mechanisms and modulation by behavioral state.

Single electrical stimuli delivered to the VL of the anesthetized rat evoked a characteristic field potential in the depth of the parietofrontal cortex (Fig. 1A) (*7*). A short-latency primary response was followed 175 to 200 ms later by a long-latency potential. Paired stimuli, separated by 100 ms, generated an augmenting response (Fig. 1B); the second response at this interval was several times larger than the first and was also followed by the long-latency potential. The narrow effective time window for generating an augmenting response, illustrated in Fig. 1C, was between about 50 ms and the peak of the long-latency potential (200 ms), after which the second response was not augmented. Current-source density (CSD) analysis revealed that the primary VL response, the onset of the augmenting response, and the long-latency potential were all generated by neurons of layer V (Fig. 1D). After the onset of the augmenting response, strong current sinks spread quickly into upper cortical layers and horizontally into adjacent cortical regions. The area of horizontal spread of the augmenting response in the frontoparietal neocortex is shown in Fig. 1E (*8*).

The relevance of the augmenting response to behavior has not been demonstrated, although the response has been shown to vary between sleep and waking (*9*). We found that the VL-generated augmenting response was strong and reliable in awake, unrestrained rats, with characteristics virtually identical to those observed in anesthetized animals (Fig. 2A, "resting"). However, the augmenting response, but not the primary response, was strongly influenced by the behavioral state of the animal. Three states were distinguished in awake rats that were allowed to move freely about an open field (*10*): resting, exploration, and immobility. The augmenting response was strong when the animal was resting (but not sleeping), but strongly suppressed when the animal was moving about and actively exploring the environment (Fig. 2A, "explo-

Department of Neuroscience, Brown University, Providence, RI 02912, USA.

* To whom correspondence should be addressed. E-mail: manuel_castro@ brown.edu