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Oscillations in the insect brain: Do they correspond to the cortical γ-waves of vertebrates?

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ABSTRACT **γ-waves, relatively high-frequency oscillations (30–80 Hz) that can be recorded in the olfactory system and the visual cortex of vertebrates, have recently attracted much attention. A role as an information carrier is under discussion, a possible involvement in "feature linking" has been suggested, and they have also been implicated functionally in phenomena such as mind consciousness or awareness. It has long been known that stimulus-dependent high-frequency oscillations (hf waves) can also be recorded from the optic lobes of arthropods. These oscillations in flies have been examined and found to be analogous to the γ-waves in many respects. Based on knowledge of the anatomy and physiology of the visual system in flies, the most plausible interpretation of the function of these oscillations differs from the interpretations of the vertebrate γ-waves currently under consideration.**

One of the greatest challenges in neurobiology is to explain the functioning of complex neural networks such as the vertebrate cortex. The problem lies not so much in acquiring experimental data as in providing a convincing functional interpretation of the data. A particular form of activity in the mammalian cortex, the γ-wave phenomenon, has recently been under study in various laboratories. These relatively high-frequency oscillations have certain characteristics that have suggested an involvement in complex functions (1–5). A proof for these functions, however, is still lacking.

Whenever the complexity of a system makes the solution to a problem inaccessible, a useful approach is to study analogous phenomena in a simpler system, where they may be easier to interpret. The application of this approach to brain oscillations is not new, as documented by the following remark by Lord Adrian in 1937: "The tendency to synchronization is now recognized to play a considerable part in the reactions of the central cortex, and it has become important to know more about the conditions which promote it. The optic ganglion of *Dytiscus* is in some ways an ideal preparation for a study of this kind" (6). For our experiments we chose not *Dytiscus* (a water beetle) but the blowfly *Calliphora*, which should facilitate functional interpretation because much more is known about the optic ganglia of flies than those of beetles.

MATERIALS AND METHODS

Female *Calliphora* were obtained from the Institute's colony. Several times (e.g., Figs. 1*c* and 2) instead of wild-type flies the chalky mutant was used. In this mutant, because of the lacking screening pigment, it is possible to stimulate many ommatidia even with a small photodiode, the intensity of which can be easily controlled. In experiments for which the recording site was irrelevant, summed potentials of the eye and brain were recorded noninvasively, by thin silver/silver chloride electrodes placed on the cornea and the back of the head capsule. Each electrode was kept in contact with the body fluids by means of a small drop of electrode gel. Local extracellular activity was recorded with 5-MΩ metal electrodes and, for intracellular recording, high-resistance (100 MΩ) capillary electrodes filled with 3 M potassium acetate were prepared by a standard technique (see, e.g., ref. 7).

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RESULTS

When one of the compound eyes of the blowfly is illuminated, extracellular oscillating potentials with an amplitude of up to 2 mV can be recorded from the region of the optic lobes. The frequency of oscillations is usually ≈150 Hz, although it may be lower (100 Hz) or higher (200 Hz). The oscillation can continue for many seconds, but it stops immediately as soon as the light is turned off. In general, the oscillation amplitude increases as the light intensity and/or the stimulated area of the eye are increased. Sometimes there are rhythmic fluctuations in amplitude, such as would be expected from superposition of the outputs of two or more oscillators at similar frequencies. The oscillations occur even in a completely intact animal and hence are not an artefact of dissection (8).

This much has been known for some time. In my experiments, the following observations have been made:

(*i*) The responses of the receptor cells in the eye do not include any frequency components in the range 100–200 Hz of the frequency spectrum prominent enough to give rise to the observed oscillations (Fig. 1); that is, the oscillations first arise in the central nervous system in the region of the optic ganglia. The situation in vertebrates is similar; under experimental conditions in which γ-waves are generated in the visual cortex of the cat, no discernible oscillatory components in the signals sent from the thalamus to the cortex can be seen (5).

(*ii*) In the frequency spectrum of the oscillations, the largest-amplitude component is essentially independent of the stimulus configuration. Neither the stimulus intensity nor the region of the eye that is stimulated affects the principal frequency component. This finding is also consistent with what is known of γ-waves. There is, however, a slight decrease in the principal frequency component in *Calliphora* as the stimulus duration is lengthened, probably because of light adaptation.

(*iii*) In the responses to a series of identical stimuli, the phase of oscillations is synchronized for a short time after stimulus onset, but later the oscillations in the consecutive responses are no longer in the same phase with respect to the onsets. The light need not be turned on suddenly in order to elicit oscillations. A slow increase in light intensity can also produce marked oscillations (Fig. 2). Furthermore, the oscillations do not always appear immediately after the beginning of the stimulus, nor do they always last until its end. Often they are limited to "spindles" 100–200 ms long. In this respect, too, the oscillations resemble γ-waves.

(*iv*) The amplitude of oscillations, and whether they appear at all, depends very much on the stimulus configuration—as in the case of the γ-waves of the visual cortex. For instance,

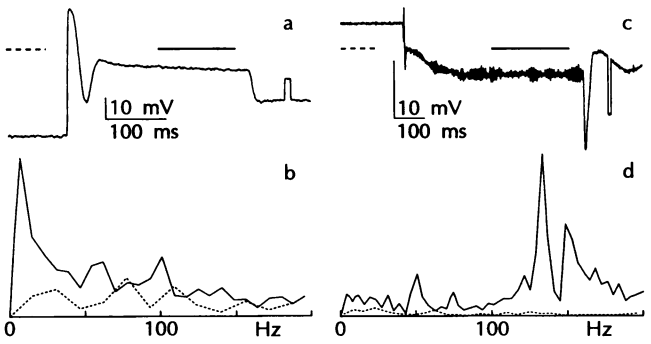

FIG. 1. (*a*) Intracellularly recorded response (receptor potential) of a photoreceptor in the retina of *Calliphora* to a 300-ms light flash (white light). (*b*) Frequency analysis of receptor potential (ordinate: amplitude, in relative units) shows that in the range 50–200 Hz no particular frequency band is emphasized. The peak at ≈10 Hz is the consequence of the slow decline of the receptor potential. For comparison, the dashed line shows the spectrum of the signal before stimulus onset. (*c*) Summed potential recorded from the intact animal consisting of the electroretinogram with superimposed oscillations. The stimulus was a light flash of 550 ms. (*d*) Frequency analysis of the summed potential exhibits a double peak in the region of 150 Hz. Often there is only one peak. In the spectrum of the signal before stimulus onset (dashed line) there is no such maximum. Horizontal bars above the potential curve (dashed line before stimulus on; solid line during stimulus) in *a* and *c* show the time span over which the spectra were analyzed.

three light sources in a row may elicit distinct oscillations when they are turned on simultaneously, whereas either the middle light or the two outer lights alone are much less effective (Fig. 3). This is consistent with the finding of Burkhardt (8) that the stimulated area of the eye is relevant.

(*v*) Oscillations recorded at different sites in the optic lobe, even widely separated sites, are to a great extent synchronized with one another (Fig. 4). Similarly, γ-waves can be synchronized at different sites in the cortex.

(*vi*) In the fly eye, the first relay station proximal to the retina is the lamina ganglionaris; in this first optic ganglion, most of the photoreceptor axons terminate, making synaptic contact with the second-order neurons, called L-neurons (Fig. 5). The latter respond to a light stimulus, given to the associated photoreceptor, with a graded, hyperpolarizing

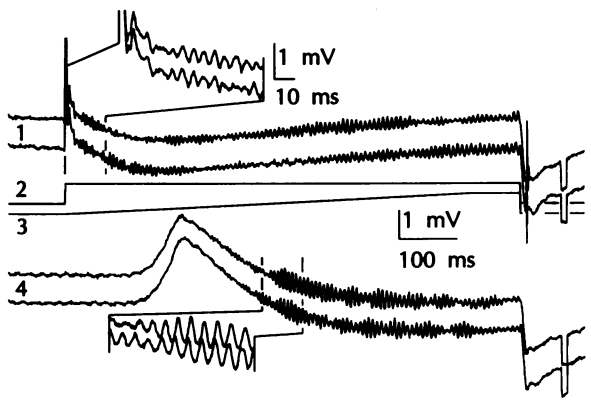

FIG. 2. Summed potentials, including hf-waves, recorded from the intact animal during different light stimuli, each presented twice. The light source was a green light-emitting diode (HS BG-5501; Stanley, Tokyo). Traces 1, responses to stepwise stimuli (time course of light intensity in trace 2). Oscillations during the two consecutive stimuli are both in the same phase with the step shortly after stimulus onset (see *Inset* with expanded time axis). Later, this phase relationship is lost. Traces 4, when the light intensity is slowly increased (trace 3), hf-waves can also occur, but their phase is independent of the stimulus onset (see *Inset* with expanded time axis).

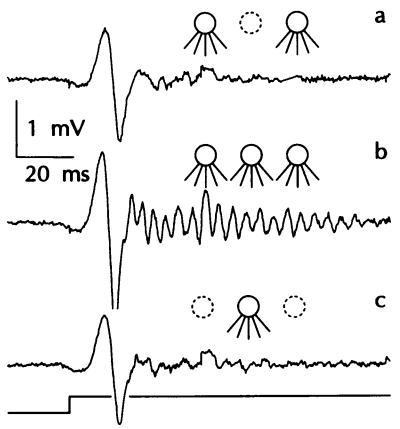

FIG. 3. hf-waves measured in the intact animal under various stimulation conditions: three light sources (light-emitting diodes as specified in Fig. 2) in a row elicit large hf-waves when they are turned on all at once (*b*), but when other parameters are kept the same and only the two outer sources are turned on (*a*), or the middle one alone is turned on (*c*), no oscillations appear. Signals have been high-pass filtered (50 Hz).

potential (7). The time course of the L-neuron potential is also affected by other factors. If the light source is not punctate but illuminates a large area, the initial hyperpolarization of the L-neuron is followed, after a certain latency, by marked depolarization. That is, lateral inhibition has occurred, for which various ionic mechanisms are responsible (7). Inhibition of this sort could easily result in oscillations, given the appropriate connectivity. For example, oscillations could be produced if the output of the L-neurons or neurons postsynaptic to them were fed back to the L-neuron synapse, as long as the gain is large enough and the delay is appropriate.

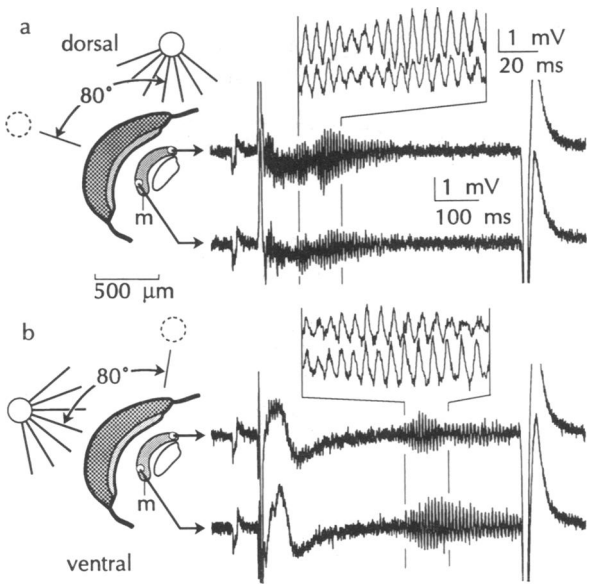

FIG. 4. hf-waves recorded by two extracellular electrodes at widely separated sites in the second optic ganglion [medulla (m) in diagram]. Even when the illumination is mainly limited to the dorsal part of the eye (*a*), hf-waves appear in both the dorsal and ventral medulla, and the two are strictly synchronized (*Inset*). The same applies when the light source is in a position 80° ventral to the first position (*b*). Position of the light source has some influence, however, in that the hf-waves recorded in the illuminated region are of larger amplitude (cf. *a* and *b*). In the diagram of the experimental arrangement (*Left*) the retina is indicated by coarse stippling and the optic ganglia—lamina and medulla (m)—are shaded. The light source (angular extension, ≈90°; white light) that is turned on in each case is represented by a circle with rays.

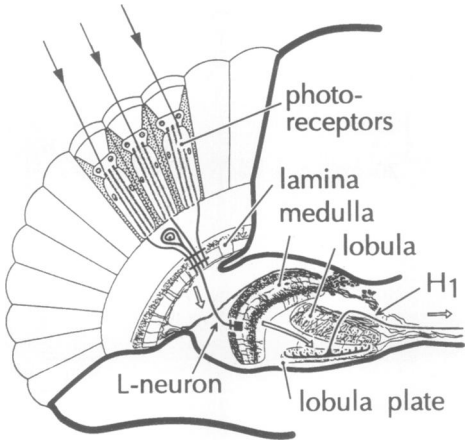

FIG. 5. Diagram showing position of the retina, with the photoreceptors, and of the three optic ganglia: lamina, medulla, and lobula/lobula plate (horizontal section). Diagram also includes an L-neuron in the lamina and a giant neuron (H_1) in the lobula plate. Also, several serotonergic giant neurons (9) are shown (not labeled): their arborizations cover the entire lamina, medulla, lobula, and lobula plate.

Indeed, intracellular recordings show that the L-neuron membranes often oscillate in synchrony with the high-frequency oscillations (hf-waves) (Fig. 6). Since the hf-waves are synchronized over large regions of the lamina, it follows that L-neurons considerable distances apart are active in synchrony with one another. Synchronous activity of neurons over relatively great distances is one of the chief characteristics reported for the γ-waves.

The purpose of the experiments described so far was to test whether the hf-waves in the optic lobes of *Calliphora* are

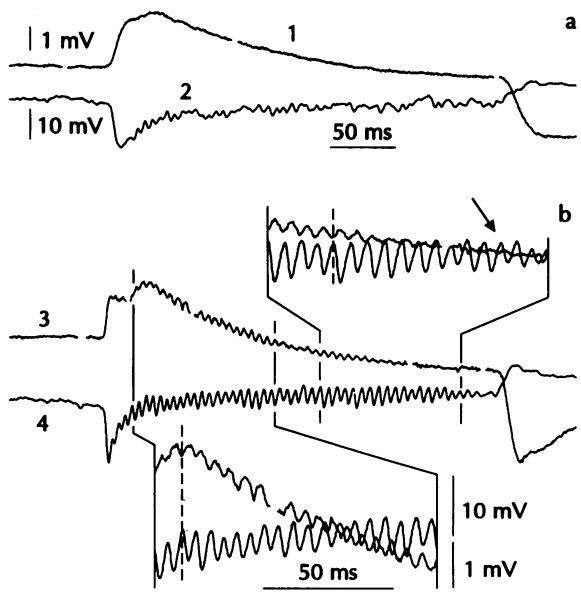

FIG. 6. Summed potentials (traces 1 and 3) and simultaneous intracellular L-neuron recordings (traces 2 and 4) for relative stimulus intensity $I = 1$ (*a*) and 10 (*b*). Whereas at $I = 1$ no hf-waves are triggered, at $I = 10$ hf-waves occur intra- and extracellularly. Oscillations in traces 3 and 4 remain over a relatively long time in counterphase (dashed vertical lines in insets with expanded time scale). The coupling, however, is not strict: in the extracellular recording, hf-waves disappear, whereas the membrane of the L-neuron still oscillates (arrow in *Inset b*). Stimulating light had an angular extension of ≈130° and was by short-pass filtering restricted to wavelengths <540 nm. By this means, scattering of the light over large areas of the retina is prevented.

formally analogous to the γ-waves in the visual cortex. To the extent revealed above, they are indeed analogous. Further experiments, exploiting special characteristics of the fly eye, were carried out to provide more detailed information about the manner of origin and possible functional significance of the oscillations in the fly. The results are as follows:

(*i*) The oscillations in different regions, while remaining primarily synchronous, can differ in relative amplitude depending on the nature of the stimulus (Fig. 4).

(*ii*) The oscillations cannot be extinguished by, for instance, turning on two lights that stimulate different parts of the eye with a delay equal to half an oscillation cycle. The implication is that only one or a few oscillators are being set into operation.

(*iii*) The oscillations do not always occur. The crucial parameters that determine whether they will appear are the size of the eye area stimulated, the distribution of the stimulus over the eye, the stimulus intensity, and the state of adaptation.

DISCUSSION

Is There a Binding Problem in the Fly's Optic Lobes? Given the anatomical and physiological properties of the visual system in flies, quite different from those in vertebrates, it is likely that oscillations have nothing to do with a complex function as discussed for γ-waves. That is, the array of L-neurons, the membrane potentials of which are oscillating, provides a retinotopic representation of the surroundings. There is no separate representation of features of objects, such as motion or color, in distinct groups of L-neurons in different parts of the lamina. Therefore, there is no feature linking or binding problem. Nor is there any indication that these oscillations could define "neuronal assemblies," as discussed in the context with γ-waves, or have anything to do with "awareness" or "mind."

Neuronal Assemblies vs. Gain Control. The functional interpretation examined in this section is speculative in that there is as little evidence for it as there is for the hypotheses proposed for the vertebrate γ-waves. However, it is easier to test experimentally.

Whenever enormous amounts of data are to be processed, it is likely that some of the numerical quantities will be small and others will be very large. With modern digital computers, this is generally not a problem, because each stored number is ordinarily represented by 64 bits, which gives precision to >15 significant figures and an additional 2000 powers of 10. This immense dynamic range usually means that no rescaling is required, even in fairly complex calculations. For neurons, it is a problem. In the time window relevant to perception, ≈100 ms, a neuron cannot assume even 100 different functional values (5 or 6 bits); the number depends to some extent on whether graded signals or only nerve impulses are counted. Indeed, according to an estimate of Barlow and Földiák (10), a cortical neuron has only four distinguishable levels of activity. Every nervous system, then, would be expected to need—and to possess—special mechanisms to solve this problem. Two particular requirements arise here: (*i*) the operating range of the neuron must be adjusted to the current input signals to ensure that the neuron can respond to changes in the input and is not overdriven, and (*ii*) the gain must be set suitably for each change in the input signal, so that inputs of different magnitude produce different degrees of neuronal excitation.

The problem is especially evident in photoreception at the level of the receptors themselves and of information processing in the higher-level neurons. To cope with the huge changes in mean brightness to which most organisms are exposed, photoreceptors incorporate not only the direct enzyme cascade for transduction (that is, the conversion of

absorbed light quanta into an electrical signal) but also molecular feedback loops to guarantee that the membrane potential of the cells never reaches an extreme value, even in very different light intensities. The membrane potential is kept at a level that allows a further response to a change in intensity. The neural networks that receive input from the photoreceptors—in the retina of vertebrates and the lamina of flies—serve a corresponding function; by way of self or lateral inhibition a (low-information) dc value is subtracted from the signal of the second-order neurons, so that departures from this mean can elicit a response with high gain (7).

Every pyramidal cell in the vertebrate cortex receives ≈10,000 synaptic inputs (11). In analogy with a photoreceptor, which can respond to single light quantum with discrete electrical events ("bumps") of the order of a few millivolts but is by no means "overdriven" in absorbing 10^8 quanta per s, it is conceivable that a pyramidal cell might give a suprathreshold response to activation of only a few synapses and yet not be driven to saturation even when thousands of synapses are active. For this situation to be achieved, the mean activity level and the gain of this neuron would have to be adjusted continually in accordance with the expected input. One mechanism to compensate for changing input magnitude is feedforward inhibition, in which massive input signals generate strong inhibition of higher-order neurons, which protects them from being overdriven. Although feedforward inhibition avoids the disadvantage of instability, it presents another problem: the point is to maintain a suitable output level of a neuron, but this output is not directly involved in the feedforward mechanism. By contrast, in negative feedback the output of a neuron (or neurons) has an inhibitory action on the elements that provide input to that neuron and hence is itself modified. But because of unavoidable delays, this arrangement can become unstable, producing oscillations.

hf-Waves in the Fly Brain Convey no Image Information. The evidence for this proposition is as follows:

(*i*) Because the oscillations are synchronous in regions of the brain corresponding to large parts of the visual field, they have essentially no angular selectivity and therefore cannot convey information about the image on the retina.

(*ii*) The probability that oscillations will occur and their amplitudes, when they do occur, are not unequivocally dependent on parameters of the visual stimulus. In addition, it is by no means the case that every stimulus elicits oscillations, not even a stimulus known to be effective in eliciting behavior.

(*iii*) The oscillations often appear relatively late after a stimulus (i.e., several hundred milliseconds; see Fig. 4*b*), later than many visually elicited behavioral responses.

A Possible Anatomical Substrate for Feedback Inhibition: Tangential Giant Neurons. Tangential cells in the optic lobes of flies have been described by several authors. These are cells that form links between different retinotopic units within one of the optic ganglia; they are oriented more or less perpendicular to the signal flow from the retina to the central brain. The category includes, for example, the serotonergic giant neurons in the optic lobes of flies (9), illustrated in the diagram of the eye in Fig. 5. They are distinguished by (*i*) their small number and (*ii*) their extensive arborizations, by which a single neuron typically connects different neuropil regions. Because such neurons are evidently unsuitable for the transmission of image information, they have been interpreted to be "modulating" neurons.

Whereas the phenomena of lateral inhibition and gain control in the fly's optic ganglia are well documented, the mechanisms are not yet clear (7). It is conceivable that tangential cells, creating extensive links in the brain, generate the long-range oscillations and ensure that the information-carrying neurons orthogonal to them are kept within a suitable working range.

In response to a light stimulus, the L-neurons of the lamina become hyperpolarized, at first with no oscillations. As the intensity increases, from a certain intensity on, the hf-waves appear (Fig. 6). They need not be a necessary "evil" of the delayed feedback, comparable to, e.g., epileptic convulsions. Their significance could be that they allow an inhibitory transmitter to be released more efficiently, perhaps by increasing the frequency with which rapidly inactivated channels are successively opened. In this case, the oscillations would indicate a particularly strong central nervous damping because of large overall input activity. The fact that the hf-waves are not detrimental to the function of higher-order neurons can be easily shown: spike activity was measured in a "motion-sensitive" neuron of the lobula plate, called H_1 (Fig. 5). The response of this neuron to a moving pattern was found to be not significantly modified irrespective of whether or not the light intensity was selected, for example, to induce hf-waves.

Could the Cortical γ-Waves Be the Manifestation of a Gain Control Mechanism? Broadly arborizing neurons (serotonergic, dopaminergic, noradrenergic, etc.) to which modulating functions have been ascribed are also present in the vertebrate cortex. According to the gain-control hypothesis, they could be organized as diagramed in Fig. 7. Region A represents a retinotopic cortical area containing motion-sensitive elements, and region B represents an area, perhaps in a distant part of the cortex, containing elements of a different kind—for instance, line detectors. The information-carrying part of the nervous system is indicated by solid lines. Dashed lines show the modulating part, which is thought to be responsible for keeping the information-carrying pathways in a suitable state of activity.

The modulating channels in Fig. 7 are laid out in such a way that they feed back not to a single retinotopic region but to several such centers at once—regions A, B, and C and perhaps still other regions of different modalities. This seems a useful arrangement; when an object appears at a particular place in the visual field, its representation is generated

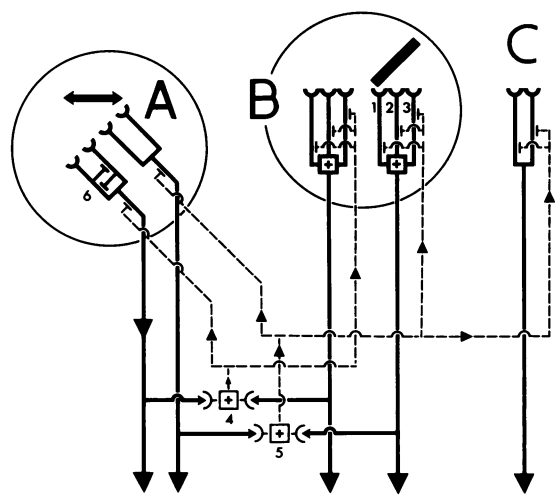

FIG. 7. Hypothetical structure to explain the origin of vertebrate cortical oscillations. Information-carrying channels are shown by thick lines, with inputs symbolized by semicircles. Gain-controlling channels are shown by dashed lines. The gain is controlled by a common feedback to corresponding sites in different centers; ensemble averaging in elements 4 and 5 prevents major fluctuations in the feedback signal. It is conceivable that the gain control also affects cortical regions that do not contribute to the ensemble average (region C). Where signal-carrying paths converge, signals are superimposed (in some cases indicated by +).

simultaneously at corresponding sites in different retinotopic centers (regions A, B, and C), so that it would be desirable to adjust the gain at all these sites by way of a common ensemble average, which is created by summation of signals converging from several different centers (Fig. 7; see nos. 4 and 5).

The diagram in Fig. 7 is meant merely to show the basic topography of the neural interactions with no specifics regarding gain-control mechanisms. Such mechanisms could take various forms; for example, the gain could be increased in the feedback loops by specific mechanisms when the inputs are small, and it could be reduced when they are large.

In principle, oscillations can develop even in a simple negative feedback system if it includes dead times or phase-shifting elements—as biological systems always do—and the gain is sufficiently large. As in the γ- and hf-waves, the frequency of the oscillation in such a system would depend on system parameters (duration of the delay, phase angle) and not on parameters of the input. Given a particular delay duration, whether the system oscillates or not would depend on the gain. In a situation such that oscillations may or may not occur, depending on the stimulus parameters, the implication would be that the gain is affected by these parameters. This dependence (for example, on stimulus intensity or configuration) is observed in the γ- and hf-waves. The influence of stimulus on gain could be exerted, for instance, by excitatory interactions, active outside the feedback loop, such as those indicated in Fig. 7 (no. 6 in region A). In fact, such excitatory interactions are included in the models used to interpret γ-waves as a mechanism for the formation of neuronal assemblies (4).

An interesting property of the network shown in Fig. 7 is that the phase difference between oscillations in regions A, B, and C depends not on the distance separating these regions, but rather on the conduction times from the input sites of the modulating systems (Fig. 7, nos. 4 and 5) to the sites of inhibition in the cortical regions. If these times are all approximately the same, the oscillations will be synchronous even in widely separated brain regions—which is often found experimentally in the cortex (1–5, 12).

Apart from the properties described above, which can be derived from the structure of the network in Fig. 7 and are observed in measurements of the γ-waves, there are additional γ-wave properties that fit better with the gain-control hypothesis than with the notion that γ-waves are the basis of perceptual functions. These are as follows:

(*i*) The latency of the γ-waves is long, often 150 ms or more, and variable (4). Pattern discrimination in the visual system occurs with only slightly varying latency and, at least for easy tasks, is possible in 100–150 ms (13). Clearly, the γ-waves are not an ideal precursor of such phenomena. In contrast, a gain-control mechanism might well function with a somewhat later onset.

(*ii*) The fact that γ-waves do not always appear, even in cases likely to involve detection and perception, tends to weigh against the neuronal-assembly hypothesis. In the gain-control hypothesis, on the other hand, oscillations are seen as the manifestation of especially powerful feedback.

(*iii*) γ-waves are also present in anesthetized animals. This would be unsurprising in the case of a gain-control system but unexpected for a phenomenon constituting the basis of consciousness.

A conspicuous difference between γ-waves and hf-waves is that the frequency of the hf-waves of flies is ≈3 times as high. One reason, presumably, is that conduction times are smaller in the fly brain, but the higher frequency probably also reflects the fact that the temporal resolution capacity of the dipteran visual system is ≈3 times as great as that of humans (14). As we know, flicker fusion frequency in the human visual system is at ≈30 Hz. Thus far, the frequency of the γ-waves is beyond the temporal resolution of our visual system. The hf-waves associated with the fly's responses to visual stimuli are equally unlikely to be temporally resolved and hence should not have an effect in the channel through which information about the visual surroundings is conducted to the center of the nervous system.

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