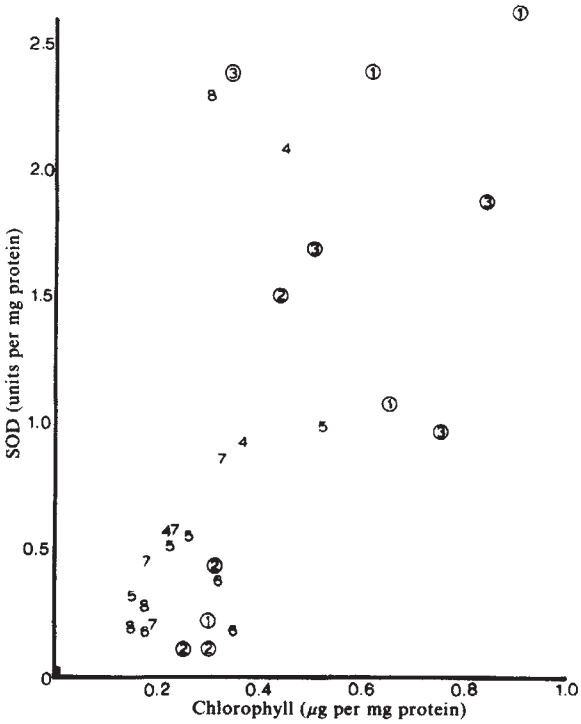

Fig. 2 Correlation between SOD activity and total chlorophyll in individuals from eight different clones of the sea anemone *A. elegantissima*. Clone-mates share numbers and symbiotic anemones collected from the upper areas of the intertidal zone are circled. The mean SOD activity of six aposymbiotic anemones totally lacking chlorophyll is represented by the single solid square (mean SOD activity = 1.14×10^{-2} U per mg animal protein). Regression: $y = 2.575x - 0.013$, $r = 0.738$, $P < 0.001$, $n = 35$. SOD activity, expressed in Units according to McCord and Fridovich[27], was determined using a Clark-type polarographic oxygen electrode and a procedure modified from Tyler[28], and from Marshall and Worsfold[29]. After homogenizing the anemone, the algal cells were removed by centrifugation (3,000*g*, 15 min) and the SOD activity reported is that of the resulting animal supernatant. Chlorophylls *a* and c_2 were calculated using the equations of Jeffrey and Humphrey[30], following two 10 h extractions in 100% acetone. No chlorophyll could be detected in the homogenization supernatant using this spectrophotometric procedure or by using the more sensitive fluorometric technique of Yentsch and Menzell[31], thus verifying that the algal cells had not been disrupted by the homogenization procedure and that the SOD activity is that of the animal tissue. Protein was determined according to Itzhaki and Gill[32], and, after tissue digestion in 5% KOH, by the technique of Bradford[33].

chemical inhibition or darkness decreased host SOD activity. It therefore appears that host SOD activity is altered in response to the amount of O_2 generated photosynthetically by its intracellular algal symbionts.

The net production of oxygen by alga–invertebrate associations has been interpreted almost exclusively as an indication of the nutritional contribution of the algae to the host[11,12,23], although intracellularly produced oxygen has been implicated as a buffer against environmental hypoxia and as a factor modifying behaviour in sea anemones[24–26]. The high levels of photosynthetically generated oxygen are a potentially deleterious factor in these symbioses. To exploit the benefits of the association while avoiding oxygen toxicity, the host sea anemones must maintain levels of SOD in direct proportion to their algal complement.

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Central connections of the retinal ON and OFF pathways

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Slaughter and Miller have recently demonstrated that in the isolated eye cup of the mudpuppy and the rabbit, DL-2-amino-4-phosphonobutyric acid (APB) reversibly blocks the ON responses in the retina: on infusion of APB, ON bipolar, ON amacrine and ON ganglion cells become unresponsive; receptors, horizontal cells, OFF bipolars, OFF amacrines and OFF ganglion cells are unaffected[1]. The centre-surround organization of OFF-centre ganglion cells appears unaltered. I have used these findings to determine how the ON-centre and OFF-centre retinal ganglion cells, whose characteristics have been extensively studied in mammals[2–7], exert their influence on the neurones of the central visual system. Recordings made in the lateral geniculate nucleus (LGN) and striate cortex of the rhesus monkey while the retinal ON responses were reversibly blocked with APB showed that in the LGN, retinal APB infusion blocked the responses of ON-centre cells and had little effect on OFF-centre cells. The centre-surround organization of OFF-centre cells was unaffected. APB infusion eliminated the light-edge responses of cortical cells, revealed a dark-edge response in some, but had no discernible effect on orientation and direction specificities. These results suggest that the ON and OFF systems do not interact significantly at the level of the LGN, but do so in the striate cortex.

Surgery was performed on rhesus monkeys under sodium pentobarbital anaesthesia. During recording, animals were paralysed with Flaxedil and were respirated with a 75–25% mixture of NO_2–O_2. The eye was perfused through a tube inserted behind the limbus into the vitreous chamber. The perfusate left the eye through a second tube. The control perfusate, which was similar to that reported by Cunningham and Miller[8], was dripped at the rate of 2–4 ml min^{-1}. A stopcock

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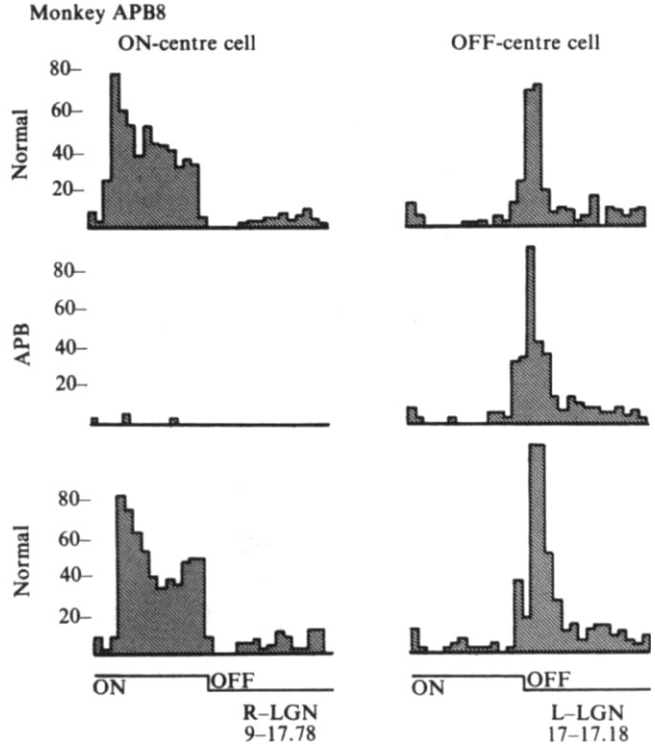

Fig. 1 The effect of retinal APB infusion into the left eye (200 μM APB for 1 min) on two simultaneously recorded single cells, one in layer 6 of the right LGN and the other in layer 2 of the left LGN. The receptive field centre of each cell was stimulated with a small spot of light, using two light beams, presented at the rate of 500 ms on and 500 ms off. Each histogram is based on responses elicited with 30 repeated stimulus presentations. The histograms on top were obtained before APB infusion, those in the middle after the infusion had blocked the retinal on responses, and those on the bottom after the ON-centre cell had recovered. APB abolished the responses of the ON-centre cell and had no significant effect on the OFF-centre cell.

allowed us selectively to infuse a solution which in addition to the perfusate contained 100–800 μM of APB. Electrodes were placed in the LGN and striate cortex of 10 animals. In most cases we recorded simultaneously with two electrodes, either with one in each LGN, or with one in the LGN and the other in the striate cortex. Stationary, flashing spots or moving light bars were presented on a tangent screen with a computer-driven optic bench[9]. The effects of APB infusion were studied at 124 LGN (78 single cells) and 55 cortex sites (41 single cells); their receptive fields were within 12° of the fovea. While recording in the LGN it was often also possible to monitor the activity of retinal ganglion cell axons[10].

In the LGN, brief 1–3-min infusion of 100–200 μM APB effectively blocked the activity of ON-centre cells within 1–3 min. In successful preparations, parvocellular ON-centre cells, most of which have colour-opponent receptive field properties[11–13], were blocked the whole time. Magnocellular ON-centre cells, which have broad-band receptive field properties[11–13], were more resistant to APB infusion; their responses were generally blocked several seconds later than those of parvocellular cells, and in some animals the response was reduced but not entirely eliminated. Therefore, to be certain about the extent to which ON responses were blocked while we studied ON–OFF interactions, we always assessed the responses of cells in the magnocellular lamina. The data reported here are based on those cases where we established that both the magnocellular and parvocellular responses were blocked by our infusions. For the most part, OFF-centre cells were not significantly affected by the APB. Figure 1 shows the effects of APB infusion on an ON-centre and an OFF-centre cell recorded simultaneously with two electrodes in the LGN. The infusion abolished the responses of the ON-centre cell and had little effect on the OFF-centre cell. Recordings from retinal ganglion cell axons showed similar effects.

To determine the extent to which the centre-surround antagonism observed in LGN cells is produced by the convergence of the ON and OFF channels, we selectively stimulated the centre and surround of LGN cells before and during APB infusion. Figure 2 shows one of the several methods we used. The receptive field centre was stimulated with a spot of light

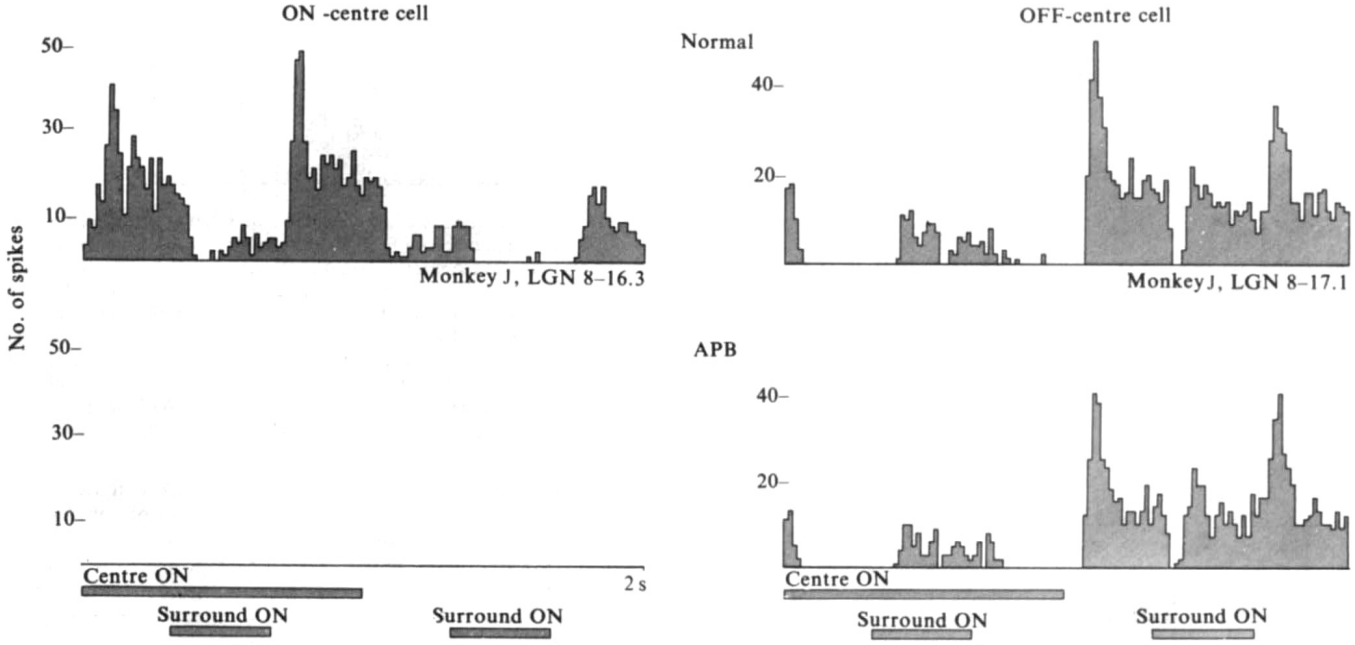

Fig. 2 The responses of an ON-centre and an OFF-centre colour-opponent cell to centre and surround stimulation before and after 200 μM infusion of APB into the eye. The centre stimulus (0.75° green spot for the ON-centre cell and 0.75° red spot for the OFF-centre cell) was flashed for 1 s every 2 s. The surround stimulus (9° red spot for the ON-centre cell and 9° green spot for the OFF-centre cell) was flashed on for 360 ms twice during each cycle, first 320 ms after the centre spot appeared and second 320 ms after the centre spot was extinguished. When the surround stimulus appeared alone, it yielded a response opposite in sign to that produced by the centre stimulus. Each histogram is based on 30 cycles. APB infusion blocked all the responses elicited from the ON-centre cell and had no significant effect on the responses of the OFF-centre cell.

flashed on for 1 s every 2 s. The surround was stimulated with a large spot of a different wavelength, which appeared once with the centre spot on and once with the spot extinguished. Figure 2 shows the responses of an ON- and an OFF-centre cell for such conditions of stimulation. Both were colour-opponent cells, and were stimulated accordingly. The centre of the first cell was activated with a small green spot, while the surround was stimulated with a large red spot. The opposite arrangement was used for the OFF-centre cell, which for its centre required a red stimulus and for its surround a large green spot.

In the ON-centre cell, the surround stimulus, when paired with the centre spot, caused inhibition; when it was presented alone, it yielded a response opposite in sign to that produced by the centre stimulus. APB infusion (200 μM for 1 min) eliminated all the responses of the ON-centre cell, including the OFF response produced by surround stimulation. Similar infusion did not have a differential effect on the centre and surround responses of the OFF-centre cell; the ON response produced by the surround stimulation while the centre spot was on persisted. The centre-surround organization of OFF-centre broad-band cells was also unaffected by APB infusion. These findings suggest that the enhanced centre-surround antagonism of LGN cells, evident in both cat and monkey[11,12,14], is not produced, in the monkey at least, by interactions between the ON and OFF channels, as some investigators have suggested for the cat[14,15].

In the striate cortex we examined the effects of APB infusion on the light- and dark-edge responses of cortical cells, their directionality and their orientation specificity[9,16]. When the ON responses were blocked by APB infusion in both the parvo-

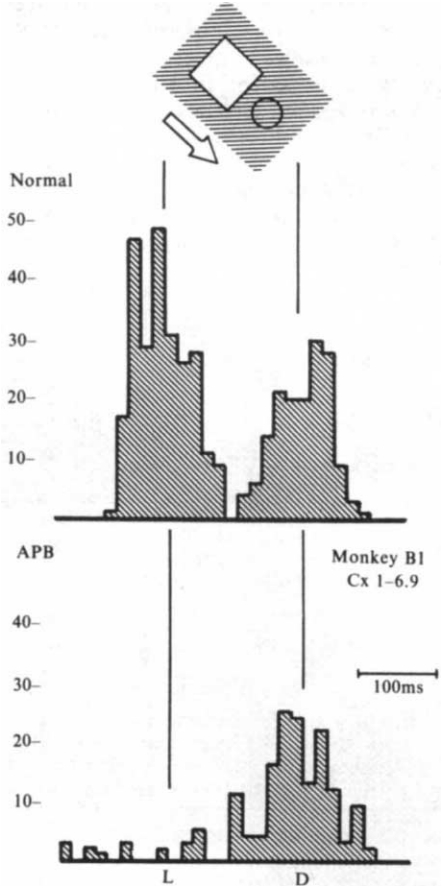

Fig. 3 The responses of a complex cell in striate cortex to a moving 1° wide light bar, as shown in the inset, before and after the infusion of 200 μM APB into the eye. The stimulus was moved across the receptive field at 4° per s. Data were collected for 30 repeated trials. The vertical lines show expected location of the light-edge (L) and dark-edge (D) responses. APB blocked the light-edge response and had little effect on the dark-edge response.

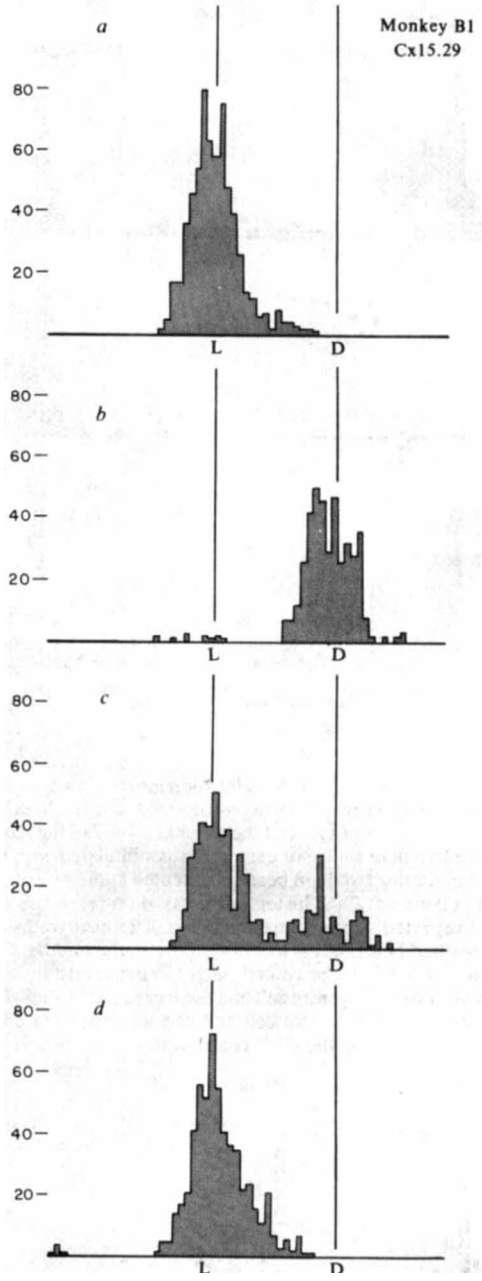

Fig. 4 The responses of a simple cell in the striate cortex before and after the infusion of APB. A 1° wide light bar was swept across the receptive field from left to right at 2° per s. Each histogram is the result of 30 repeated sweeps. The vertical lines show the expected location of the light-edge (L) and dark-edge (D) responses. *a*, Normal response during infusion of control perfusate; *b*, response after infusion of 100 μM APB had taken effect; *c*, partial recovery after termination of APB infusion; *d*, full recovery following termination of APB infusion. APB infusion uncovered a dark-edge response for this cell.

cellular and magnocellular layers of the LGN, the light-edge response of cortical cells was eliminated. An example obtained from a complex cell is shown in Fig. 3. The cell was stimulated with a moving light bar, which elicited a response to both the leading light edge and the trailing dark edge. Infusion of 200 μM APB eliminated the light-edge response, suggesting that it is produced by the input from the ON channel. In some cortical cells more complicated interactions were evident. Figure 4 shows one such example. This simple cell[9], when stimulated in the same fashion as the cell shown in Fig. 3, responded only to a light edge. Infusion of 100 μM of APB eliminated the light-edge response, and, unexpectedly, uncovered a dark-edge response. Part of the way to recovery, both responses were

evident. Recordings in magnocellular LGN have shown that APB infusion fully blocked the ON responses. These observations suggest that in addition to convergent excitation, significant inhibitory interactions can also occur between the ON and OFF channels in the striate cortex.

The orientation and direction specificities of cortical cells were assessed by examining the responses of cells to light and dark edges which were moved across receptive fields in various orientations in random order before, during and after blocking the retinal ON system. The directionality and orientation selectivity of the cortical cells we studied were unaltered by APB infusion, suggesting that these attributes are not produced by interactions between the ON and OFF channels[17]. Work carried out concurrently by J. C. Horton and H. Sherk using a different procedure has yielded similar results in the cat[18].

Thus, my results suggest that in the rhesus monkey ON-centre and OFF-centre retinal ganglion cells drive predominantly ON-centre and OFF-centre LGN cells respectively. The surround inhibition observed in LGN cells does not appear to be a product of the interaction between the ON and OFF channels. In the striate cortex the ON and OFF channels converge, with the light-edge response often produced by the input from ON-centre cells and the dark-edge response produced by OFF-centre cells. Inhibitory interactions between these channels are evident in the cortex. The orientation and direction specificity of cortical cells were unaffected by blocking the ON system, indicating that each channel has access to the mechanisms responsible for these attributes.

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Electrophysiological action of kainic acid and folates in the *in vitro* olfactory cortex slice

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Evidence has mounted that kainic acid (KA), a powerful neuroexcitatory and neurotoxic agent, acts at a specific class of receptors distinct from those mediating the excitatory actions of glutamate[1–3]. This has prompted a search for the endogenous ligand for the KA-specific receptors. Recently, Ruck *et al.*[4] reported that the naturally occurring folic acid derivative methyltetrahydrofolate (MTHF) is a potent and specific competitor for KA-binding sites in rat cerebellar membranes, having one-tenth the binding activity of KA at these sites. They suggested that MTHF may be the endogenous KA receptor ligand. Olney *et al.*[5] found that injections of various folates—MTHF, folic acid (pteroyl-L-glutamic acid, PGA), or folinic acid (formyltetrahydrofolate, FTHF)—into rat amygdala and striatum produced KA-like seizures and associated brain damage at sites distant from the site of injection. However, these folates did not mimic the direct neurotoxic effects of KA at the injection site. Furthermore, Roberts *et al.*[6] reported that MTHF injected into rat cerebellum produced slower and less complete neuronal degeneration than did KA and, unlike KA and glutamate, did not increase cerebellar cyclic GMP levels. In light of the contradictory results from these binding, histological and biochemical studies, we have tested the electrophysiological equivalency of KA and the folates in rat olfactory cortex slices. KA is a potent agonist at the receptors of the terminal synapses of the lateral olfactory tract (LOT); in fact, the natural transmitter at this synapse appears to act at a KA-preferring receptor[7]. Also, olfactory cortex is extremely sensitive to the neurotoxic action of KA[8]. We report here that MTHF, FTHF and PGA had little or no effect on LOT-stimulated field potentials even at millimolar concentrations, whereas KA exhibited excitatory effects at concentrations as low as 5×10^{-7} M. It therefore seems unlikely that MTHF or the other folates are endogenous ligands for the KA receptor in olfactory cortex.

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Thin slices (300–450 μm) of rat olfactory cortex were cut and incubated as previously described[7]. Each slice was transferred to a recording chamber that was constantly perfused with oxygenated normal Ringer's solution. The LOT was stimulated with double shocks of 20–50 V intensity and 50 μs duration via a tungsten bipolar electrode placed across the tract. Paired

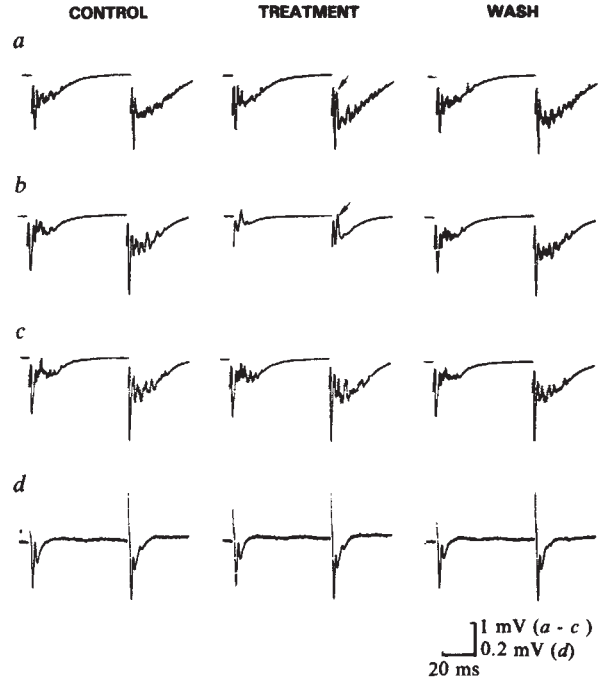

Fig. 1 Effects of KA and folates on LOT-stimulated field potentials in rat olfactory cortex slices. Each trace shows two field potentials in response to double shock stimulation of LOT. As previously reported, the second field potential of each pair is larger than the first[7,10]. Initial downward deflection in each trace is the beginning of the e.p.s.p.[11]. Rapid upward deflection is due to the superimposition of the population spike onto the slow wave component of the field potential[10,11]. The arrows in *a* and *b* indicate the population spike in the second field potential. Spiking in the late wave of the field potential indicates asynchronous neural firing. Slices were superfused with: *a*, 5×10^{-7} M KA for 15 min; *b*, 10^{-5} M KA for 5 min; *c*, 10^{-4} M FTHF for 15 min; and *d*, PGA for 20 min. The duration of wash was equal to or greater than duration of treatment. FTHF was prepared in oxygenated Ringer's just before use and was protected from light during experiment.