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Horizontal Propagation of Excitation in Rat Visual Cortical Slices Revealed by Optical Imaging

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Optical imaging with high spatial and temporal resolution of neural activity in rat cortical slices was used to investigate the dynamics of signal transmission through neural connections in the visual cortex. When inhibition due to γ-aminobutyric acid was slightly suppressed, horizontal propagation of excitation in both the supra- and infragranular layers became prominent. This propagation was not affected by vertical cuts in either the supra- or infragranular layer, which suggests that excitation is at least partially conveyed horizontally by reciprocal vertical connections between neurons in these layers.

The integration of information from different parts of the visual field is an essential aspect of information processing. In the primary visual cortex (VC), horizontal connections extending along cortical layers and forming clustered terminals on distant but similar functional columns have been proposed to represent such integrations (*1–5*). Besides those horizontal clustered connections, an analysis of dendritic and axonal arborizations of individual VC cells has revealed that vertical interlaminar connections also have some horizontal spread (*2*, *4*, *6*). Thus, horizontal interaction can be based on the vertical interlaminar connections as well as on the horizontal clustered connections, but their relative contributions in sending excitation horizontally have still not been clarified. In order to reveal pathways where excitation is conveyed horizontally, we tried to visualize the propagation of neural activity by using optical imaging techniques and voltage-sensitive dyes (*7–9*).

Neural activity evoked by stimulation of white matter (WM) in frontal sections of the rat VC was recorded as an absorption change in a voltage-sensitive dye by optical recording apparatus with high spatial (128 by 128 photodiodes) and temporal (0.6 ms) resolution (*10–14*). Stimulation first evoked vertical propagation toward the cortical surface (Fig. 1A); this response was separated spatially into three components: (i) early excitations in layer VI (latency, 2.4 ms) and (ii) in layer IV (4.8 ms), where geniculate axons are known to innervate cortical cells, and (iii) a later excitation in layers II-III (7.2 ms) (*8*). The vertical propagation was followed by a horizontal spread in supra- and infragranular layers (SGLs and IGLs), especially in layers II-III and V (Fig. 1A) (24 ms). The range of the spread was varied in different slices, but mostly was restricted to a short distance [0.886 ± 0.220 mm and 0.976 ± 0.271 mm in layers II-III and layer V, respectively (n = 5)] (*15*).

Cortical excitation is thought to be limited by the γ-aminobutyric acid (GABA)–mediated inhibitory mechanism, and the difference in the horizontal spread is probably due to the strength of the GABA-mediated inhibition. In fact, the horizontal spread increased after addition of 1 μM bicuculline methiodide (BMI), a $GABA_A$ receptor antagonist (Fig. 1B). The range of the horizontal spread was dose-dependent at 0.948 ± 0.224 mm, 1.218 ± 0.361 mm, 2.007 ± 0.379 mm, and 2.247 ± 0.501 mm at 0.5, 1.0, 2.0, and 5 μM BMI, respectively (layers II-III, n = 5). Further, the layers showing horizontal spread within this range of BMI concentration were the same as in the control solution (brackets in Fig. 1, A and B, at 24 ms), and no significant change in the vertical propagation was observed, except for an increase in the signal intensity (Fig. 1, A and B, at 7.2 ms). Thus, the excitatory connections underlying the horizontal propagation in the presence of BMI were probably the same as those in the control solution, at least within this lower range of BMI concentration.

One way to test whether the horizontal propagation is due to the horizontal clustered connections is to examine the effects of a vertical cut in parts of cortical layers. If this were the case, the cut in SGLs, for example, should disrupt propagation in SGLs but not in IGLs. Although the experiment is simple, the results may be doubtful, because a cut may have other effects on a slice. Thus, the effect of a cut on vertical propagation was examined by making a cut just above the stimulation electrode along a line of vertical propagation through layer I to layer IV (*16*). We found that vertical propagation was separated on the left and right sides of the cut but the overall pattern of propagation was the same as in the control slice (*17*) (n = 4). This result suggests that a cut can be used to disrupt certain parts of neural connections without affecting other properties of slices.

Figure 1C shows the effects of a vertical cut in SGLs on horizontal propagation. Contrary to expectation, in three out of four cases a vertical cut did not interrupt propagation in either SGLs or IGLs (Fig. 1C). In the remaining case, propagation was interrupted in both SGLs and IGLs at the cut. For the former cases, we analyzed the propagation on an expanded time scale around the time when it passed through the cut (Fig. 2). In all of these cases, the neural excitation in SGLs did not propagate directly through the cut in a horizontal direction, but reciprocal connections between SGLs and IGLs allowed horizontal propagation parallel to the lamina to bypass the cut (Fig. 2, 24 ms through 29.4 ms). These vertical propagations seemed to be essential to maintain horizontal propagation crossing over the cut in SGLs as well as in IGLs. As in the latter observation, when the upward vertical propagation from IGLs to SGLs was not evoked sufficiently, horizontal propagations in both layers were interrupted at the cut.

Similarly, when a vertical cut was made in IGLs, horizontal propagations in both IGLs and SGLs were not interrupted by the cut (Fig. 3) (n = 3). The stimulation of WM evoked horizontal propagations in both the SGLs and IGLs up to the cut. When the excitation reached the cut (Fig. 3, 36 ms), it propagated vertically from the SGLs down to the IGLs, skipped over the cut (Fig. 3, 48 ms through 84 ms), and then continued to propagate horizontally in both the SGLs and IGLs (Fig. 3, 96 ms).

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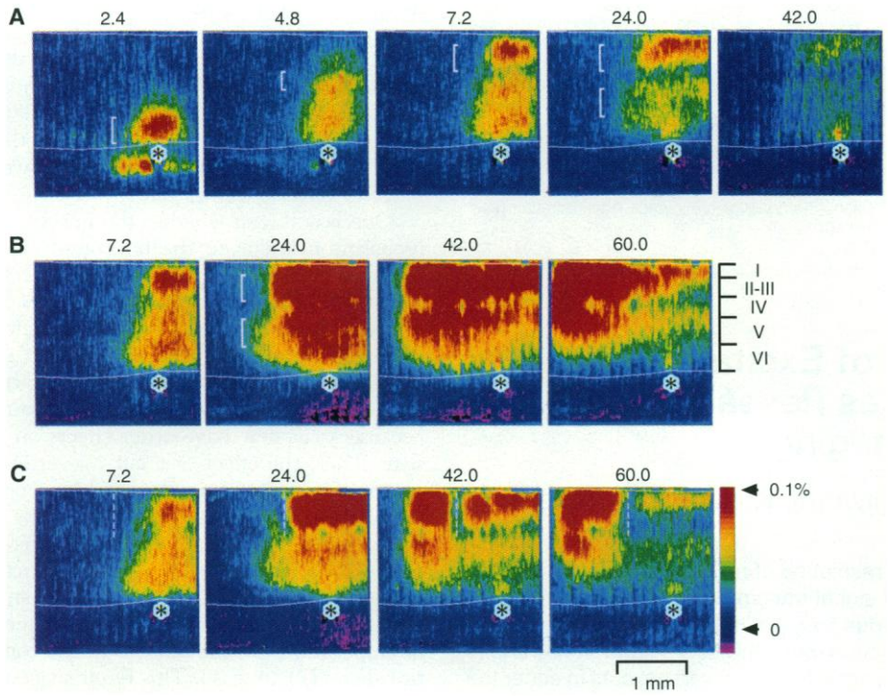


Fig. 1. Time-lapse pictures of excitation propagation in a VC slice in the control solution (**A**), in medium containing 1 μM BMI (**B**), and after a vertical cut was made from layer I to layer IV (**C**). The number on each frame indicates the time after stimulation of WM. Brackets in (A) at 2.4 ms, 4.8 ms, and 7.2 ms indicate layers VI, IV, and II-III, respectively, and those at 24 ms in (A) and (B) indicate layers II-III and V. Borders between layers are indicated on the right in (B). Colors indicate changes in light absorption (percent). Solid white lines represent the cortical surface (upper line) and the border between WM and layer VI (lower line). Asterisks indicate the location of the cathodes used for stimulation. The position of the vertical cut in (C) is indicated by dotted white lines.

Fig. 2. Vertical propagation from IGLs to SGLs in an expanded time scale of the same record as in Fig. 1C. Labels are the same as in Fig. 1.

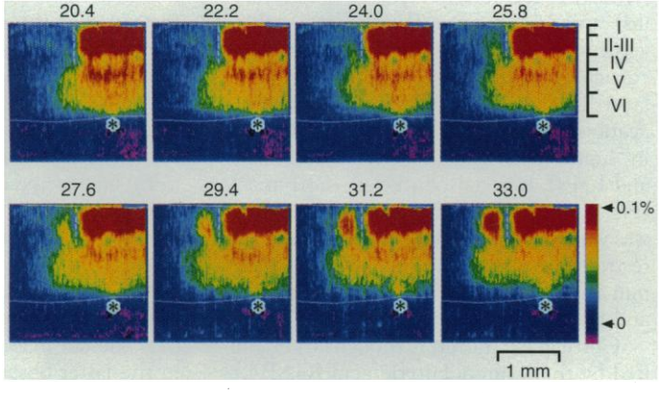
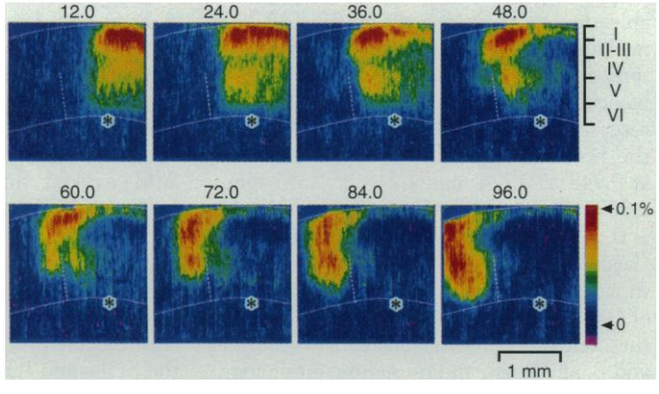


Fig. 3. The effect of a vertical cut in IGLs on horizontal propagation. The concentration of BMI was 1 μM. Labels are the same as in Fig. 1.



Other evidence supports the involvement of vertical connections in horizontal propagation. First, a horizontal cut along layer IV completely suppressed horizontal propagation in both SGLs and IGLs, although the effect of injury on the apical and basal dendrites of cortical cells could not be disregarded in these cases (*17*). Second, the conduction velocities in the horizontal direction were 0.060 ± 0.035 m/s and 0.067 ± 0.027 m/s in layers II-III and in layer V, respectively, but the velocity in the vertical direction was 0.204 ± 0.051 m/s (n = 5) (*18*). The fact that the velocity was three times smaller in the horizontal direction suggests that more synapses were involved in the horizontal propagation.

Morphological and electrophysiological investigations have shown that there are extensive neural connections between one layer and another, including connections between SGLs and IGLs, and that they can provide closed-loop circuits between SGLs and IGLs, but their role in excitation transfer has not been clarified (*4*, *6*, *19*). Although these connections seem to extend vertically and are mostly restricted in a narrow band toward the cortical surface (*20*), our results suggest that one possible role of the reciprocal connections between SGLs and IGLs would be to enhance lateral interaction through these connections.

Much attention has recently been paid to the horizontal clustered connections (*2*, *3*, *5*). Unfortunately, we did not observe any horizontal propagation reflecting such connections except in a single case, in which vertical and horizontal propagations were followed by a horizontal spread extending 3 mm along SGLs but not in IGLs (*17*). Perhaps fibers traveling a long distance are likely to be truncated in slice preparation, or it may be that the horizontal clustered connections could not evoke sufficient excitation to be detectable by the optical recording (*21*). Excitation propagation due to horizontal clustered connections and its relation to horizontal propagation that is based on reciprocal connections between SGLs and IGLs remain to be investigated.

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10. VC slices (400 μm thick) were dissected from rats 4 to 8 weeks old [T. Kurotani, N. Yamamoto, K. Toyama, *Dev. Brain Res.* **71**, 151 (1993)] and were continuously superfused with Krebs-Ringer solution (control) consisting of 124 mM NaCl, 5.0 mM KCl, 1.24 mM KH_2PO_4, 2.0 mM $MgSO_4$, 2.0 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose. The temperature was kept at 30°C throughout the experiment. Each slice was stained with a voltage-sensitive dye, RH482 (catalog no. NK3630, Nippon Kankoh-Shikiso Kenkyusho, Okayama, Japan) (at 0.1 mg/ml for 20 min), which is known to represent neural activity faithfully (*8*) [A. Konnerth, A. L. Obaid, B. M. Salzberg, *J. Physiol.* **393**, 681 (1987); M. Tanifuji, A. Yamanaka, K. Toyama, *Soc. Neurosci. Abstr.* **17**, 114 (1991)].
11. Stained slices were mounted on an inverted microscope (IMT-2, Olympus, Japan) that was equipped with a tungsten-halogen lamp (150 W), heat absorption (HA30, Kenko, Japan) and interference filters (700 ± 30 nm) (BPF-4, Vacuum Optics, Japan), a mechanical shutter, and an objective (PlanApo ×4, 0.2 numerical aperture, Nikon, Japan). The tungsten bipolar stimulation electrode (interpolar distance, 150 μm) was placed perpendicularly in the WM of a slice, where the cathode of the electrode was positioned on the border between WM and the gray matter. A glass electrode filled with 2% Pontamine sky blue and 0.85% NaCl (5 megohm) was placed in layer II-III to record field potentials. The length of illumination for the optical recording was minimized by the shutter (857 ms) to avoid dye bleaching and photodynamic damage. Stimulation was applied every 15 s. Stimulus intensity was adjusted to the strength that produced supramaximum responses of field potentials in layer II-III, which was in the range of 0.5 to 2.0 mA. Stimulus duration was 80 μs.
12. The field potential was continuously monitored throughout the experiment (peak amplitude, 1.57 ± 0.78 mV; latency, 10.01 ± 1.39 ms; $n = 27$), and no photodynamic effect on its amplitude and latency was observed by the optical recording.
13. The optical response evoked by stimulation of WM was recorded by a Fujix SD1001 (Fujifilm Microdevices, Japan). The recording system, consisting of a camera head with a 128 by 128 photodiode array and a processing unit that included digital frame memories, was based on a system designed by M. Ichikawa and colleagues [M. Ichikawa, T. Iijima, G. Matsumoto, in *Brain Mechanisms of Perception and Memory*, T. Ono, L. R. Squire, M. E. Raichle, D. I. Perrett, M. Fukuda, Eds. (Oxford Univ. Press, New York, 1993), pp. 638–648]. In each trial, a real-time image was taken about 500 ms before the stimulation and stored in one frame memory (reference image). Subsequent frames taken every 0.6 ms were subtracted from the reference image, amplified 400 times, and then stored in another frame memory (difference image). By this subtraction process, an accuracy (gray-level resolution) equivalent to 16-bit digitization was achieved. The difference image was usually averaged for 16 trials, and each frame was divided by a real-time image of the slice to express the optical responses as a percent of change in light absorption. Because of limited illumination by the shutter, this division process made no difference in the resulting image whether the real-time image used was taken before or after the optical recording ($n = 5$). Conventionally, the reference image taken at the last of 16 trials was used for division. The noise that accompanied the optical responses was due mainly to shot noise arising from the statistical noise of light. This noise was about 10 times smaller than the peak amplitude of the optical signals after averaging for 16 trials.
14. The time courses of the optical responses in each layer were reconstructed from serial frames. They consisted of rapidly rising and gradually recovering phases, which were similar to postsynaptic potentials in VC cells. The transient spiking responses were observed only around the cathode of the electrode and probably represented impulses evoked in the stimulated axons.
15. The horizontal spread was evaluated as being half the maximum width in a time-averaged response image constructed from serial frames of optical responses.
16. The cut was made with a fragment of a sharp razor blade held on a micromanipulator. The efficiency and exact position of the cut were examined by histology.
17. M. Tanifuji, T. Sugiyama, K. Murase, unpublished data.
18. The conduction velocity was calculated from the onsets of the optical responses along layers II-III and V (horizontal direction) and along a vertical line just above the stimulation electrode (vertical direction).
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A Molecular Determinant for Submillisecond Desensitization in Glutamate Receptors

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The decay of excitatory postsynaptic currents in central neurons mediated by α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors is likely to be shaped either by receptor desensitization or by offset after removal of glutamate from the synaptic cleft. Native AMPA receptors show desensitization time constants of 1 to about 10 milliseconds, but the underlying molecular determinants of these large differences are unknown. Cloned AMPA receptors carrying the "flop" splice variants of glutamate receptor subtype C (GluR-C) and GluR-D are shown to have desensitization time constants of around 1 millisecond, whereas those with the "flip" variants are about four times slower. Cerebellar granule cells switch their expression of GluR-D splice variants from mostly flip forms in early stages to predominantly flop forms in the adult rat brain. These findings suggest that rapid desensitization of AMPA receptors can be regulated by the expression and alternative splicing of GluR-D gene transcripts.

Native AMPA receptors have been studied in outside-out patches with rapid solution exchange techniques that allow agonist application times of less than 0.3 ms (*1*, *2*). These studies have allowed an estimate of the correlation between channel kinetics and the time course of synaptic events. Because data for the four cloned AMPA receptor subunits were obtained from whole-cell recordings (*3*–*6*) where the speed of agonist application is not faster than about 3 ms, current kinetics in the range between 1 and 3 ms will be underestimated. We therefore measured desensitization of different homo- and heterooligomeric AMPA receptors recombinantly expressed in *Xenopus laevis* oocytes in outside-out patches with a piezo-driven application technique (*1*, *7*, *8*).

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Homomeric AMPA receptor channels formed by the glutamate receptor D_{flop} (GluR-D_o) subunit desensitize four times faster than GluR-D_{flip} (GluR-D_i) channels (Fig. 1A and Table 1). The decay time constant of currents mediated by homomeric GluR-D_o channels in response to a 60-ms pulse of 1 mM glutamate (τ_{des}) was 0.9 ± 0.1 ms (mean ± SD). In contrast, for the splice variant GluR-D_i, which differs from the flop variant in only 11 amino acid residues (*3*), the corresponding value was 3.6 ± 0.6 ms. A similar difference in desensitization was found for GluR-C_o and GluR-C_i channels (Table 1). The occurrence of differences in desensitization time course is, however, not a general property of the splice variants for all four subunits. For GluR-A channels, there was no statistically significant difference between the flip-flop splice variants (Fig. 1B) (τ_{des} = 3.7 ± 0.7 ms for GluR-A_o and 3.4 ± 0.6 ms for GluR-A_i). GluR-B channels could not be measured in outside-out patches because of the small currents mediated by homooligomeric GluR-B channels.

We also investigated the current response to shorter glutamate pulses, where desensitization was incomplete and the offset after rapid removal of glutamate