Spatiotemporal Properties of Neural Activity Propagation from the Subicular Complex to the Posterior Cingulate Cortex in Rat Brain Slices Detected by the Optical Recording Technique

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Abstract: Using the optical technique, we investigated the functional neural circuits from the subicular complex to the posterior cingulate cortex in rat brain slices. In 11 out of 98 slices, an electrical stimulation to the subicular complex induced an excitation wave that spread into the posterior cingulate cortex (PCC) and propagated along its superficial layer. The process of this propagation was clearly divided into three steps. The first step was a fast conduction process in the superficial layers of the subiculum, which might arise from propagation of action potentials directly evoked by the stimulation. The second one was a slow process around the boundary between the subiculum and PCC, during which significant signal enhancement was observed via a pathway in the middle to deep layers. The third

step was a slow propagating process along the superficial layers of PCC. Application of the non-NMDA receptor antagonist, CNQX, restricted propagation in the first step, suggesting that a synaptic relay exists between the first and second steps. In the rest of the slices (87 out of 98), signal propagation showed only the first step in response to electrical stimulation. However, when bicuculline, a GABA_A receptor antagonist, was applied to these slices, the signal propagation spread into PCC in a manner indistinguishable from the one characterized above. It is therefore plausible that, under the conditions we adopted for the slices, the propagation pathway to PCC usually remains suppressed by GABAergic synaptic mechanisms. [*Japanese Journal of Physiology*, 49, 445–455, 1999]

Key words: voltage-sensitive dyes, optical recordings of neural activity, posterior cingulate cortex, subicular complex, signal enhancement.

Anatomical and neurophysiological studies have shown that the subicular complex is a major efferent structure of hippocampal formation [1]. The subicular complex, which could be divided into four subregions (presubiculum, parasubiculum, postsubicular and subiculum) by their input and output patterns, is situated between the hippocampus and the posterior cingulate cortex (PCC) [2–5]. The PCC is one of the principal intermediate cortical areas between the hippocampus and sensory association cortex, which has an important role in the process of learning and memory [6–8]. The projections from the hippocampal formation to the subcortical regions and entorhinal cor-

tex have been investigated extensively, however, few studies have conducted on the connections between the hippocampal formation and PCC.

Neuroanatomical and electrophysiological studies have characterized several major afferents to PCC. Pyramidal neurons in the subicular complex relay outputs of the hippocampus and project almost exclusively to layers II/III, with some invasion of layer I and layer IV of the PCC [2, 9]. However, how these connections relay signals from the subicular complex to PCC remains unknown. One of the major reasons for this may be that we do not know global functional connections between the subiculum and PCC. Con-

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ventional electrophysiological techniques have some limitations, particularly in their spatial resolution in the analysis of neuronal circuits: only a restricted number of electrodes can be placed in the tissue at a time. A new method would be required by which we can make simultaneous recordings with higher spatial resolution from many individual neurons that often distribute over a wide area in the brain.

In the present study, we have employed a high-speed optical recording technique using a voltage-sensitive dye [10–14] to visualize and characterize the spatiotemporal pattern of neural activities from a wide area in a brain slice that includes the subicular complex and PCC. We succeeded to record a global propagation of neural activity from the subicular complex to PCC and could partially characterize the synaptic properties in the propagating pathway. The results suggested that the propagation requires an signal enhancement in the transition region between the subicular complex and PCC. The signal enhancement seemed to be initiated by glutaminergic synapses and usually inhibited by GABAergic synapses.

MATERIALS AND METHODS

Slice preparation. Male Wistar rats (3–5 week; SLC, Japan) were used. The animals were decapitated under deep anesthesia with ethyl ether. The brain was rapidly desected out and kept for 10 min in an ice-cooled standard (artificial CSF) solution which contained (in mM): NaCl 128, KCl 1.7, KH_2PO_4 1.24, MgSO_4 1.3, CaCl_2 2.4, NaHCO_3 26, and glucose 10, and was saturated with a mixture of 95% O_2 and 5% CO_2 ; the pH was 7.4. The brain was cut into slightly oblique coronal slices with a microslicer (400 μm in thickness; DTK 1500, Dosaka, Japan), and each slice included a portion of the subicular complex and PCC. They were placed for 1–2 h in an incubation chamber. The slices were stained with a voltage-sensitive dye, RH 482 (Nippon Kanko-Shikiso Kenkyusho, Japan), at 0.1 mg/ml for 15 min, and kept in the incubation chamber to recover for 30 min. Then, the stained slice was transferred to a recording chamber mounted on an inverted microscope (IMT-2, Olympus, Japan). The chamber was continuously perfused with oxygenated standard solution at the rate of 2–5 ml/min. All experiments were done at room temperature except otherwise specified. In some experiments, the perfusing solution contained 5 μM bicuculline methbromide (BIC), 10 μM 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX), 50 μM D(–)-2-amino-5-phosphonopentanoic acid (AP5), or 0.2 μM tetrodotoxin (TTX). These drugs were obtained from Research Biochemi-

cal Inc. (Natick, MA, USA).

Optical recordings. An inverted microscope equipped with a 12 V/100 W halogen lamp and an interference filter (690 ± 30 nm) was rigidly mounted on a vibration-isolation table (Visolator AYN-1209K4, Meiritsu Co., Japan). To minimize bleaching of the dye and photodynamic damage to the slice, a shutter in the light path to the slices was opened only for the duration needed to measure the optical response. We used an optical recording system (HR Deltaron-1700, Fujix, Japan) that consisted of an area sensor with 128×128 photodiodes and a data processing unit. Slices were viewed with a $\times 4$ or $\times 10$ objective lens. Each pixel received light from a 25×25 μm sample area, and the whole area of measurement was equal to 3.3×3.3 mm on the (Fig. 1B). In each trial, an initial background signal were first recorded 10–20 ms prior to electrical stimulation and stored as a reference image. Recording images were taken at 0.6 ms/frame and obtained by subtracting the reference image and magnifying the difference ($\times 400$). They were multiplexed and digitized into 8-bit signals. Sixteen trials were averaged to improve the signal-to-noise ratio. We usually saved 512–1,024 sequential frames (ca. 300–600 ms) as one record. Each frame was printed out with a color video copy processor with pseudocolors (256 colors; red indicates high, green middle, and blue low magnitudes of the optical signal; Mitsubishi, CP 2500, Japan) (Fig. 1A). To analyze the time course of the signal at a local area, data from each pixel were retrieved, analyzed and plotted by the program Microcal Origin 5.0. The latency of the signal was measured as the time from the stimulus onset to the onset of an evoked optical signal, and the amplitude its highest peak.

Stimulation and field potential recording. Bipolar metal electrodes or glass pipettes were used as stimulation electrodes, and were placed at positions in the postsubiculum, approximately 2.88 and 2.9 mm from the dorsal and medial edges, respectively (Fig. 1B). Electrical pulse stimulation (0.1 to 0.5 mA in intensity, 0.05 to 0.1 ms in duration) was applied every 15 s with a constant current stimulator (Nihon Kohden, SEN-3301, Tokyo). Field potentials were recorded with a glass pipette filled with a standard solution (5–10 M Ω) and connected via AgCl–Ag to a high input-impedance, compensated preamplifier. Electrical signals were ac-coupled (time constant, 0.6 s), monitored, and digitized into 12 bits at a sampling rate of 10–20 kHz by the pCLAMP system (Axon Instrument Inc., CA, USA). Data were retrieved and processed with Microsoft Excel 5.0 software. After recording, slices were fixed in 4%

paraformaldehyde for 24 h, then cryoprotected with 30% sucrose for 24 h. Forty-micrometer-thick sections were cut on a cryomicrotome, mounted on glass slides coated with a 0.2% gelatin solution, and stained with 1.8% neutral red.

RESULTS

Imaging activity propagation from the subicular complex to PCC

To reveal the functional neural connections between the subicular complex and PCC, we visualized the propagation of neural activity following subicular stimulation using real-time imaging with a voltage-sensitive dye. Figure 1 shows a typical example of a series of optical images indicating the propagation of neural activities in a slice. Essentially the same results were obtained from 11 out of 98 slices. In the remaining 87 slices, propagation was limited to a subicular region. Although it is controversial which propagation pattern reflects physiological response, we will first analyze the data from the slices showing successful propagation and discuss this problem later (see the last section in RESULTS).

Local neural excitation in the subicular complex was evoked by a single electric pulse (0.3 mA, 0.1 ms) which was followed by the propagation of neural activity along the superficial layers of the PCC. First, the activity propagated into the local area along the superficial layers of the subicular complex, up to 0.6 mm away from the stimulus site with a fast conduction velocity of about 0.216 ± 0.029 m/s ($n=11$) (Fig. 1A, 1.2–6 ms). The excitation, however, seemed to be restricted to a limited area from 6 to 18 ms (Fig. 1A, 6–18 ms). At 24 ms, a weak optical signal in the middle layers of the subicular complex becomes to be visible as indicated by the arrowhead in Fig. 1A, 24 ms. The intensity of the optical signal seen in the middle layers was gradually increased until reaching a maximal level by 42 ms (Fig. 1A, 24–42 ms). To identify the site of the signal enhancement, we marked the site by injecting a large current and examining it by a histological method (an arrow in Fig. 1B). It was located in the subicular complex close to the PCC, which is likely in the postsubiculum according to a neuroanatomical study [14]. At 48 ms after the stimulation, the enhanced activity in the middle layers moved into the superficial layers in the subicular complex. This process showed that the signal continued to increase until 54 ms in a localized area (Fig. 1A, 48–54 ms). During the next 90 ms, from 60 ms post-stimulus, the enhanced activity spread along two pathways; one towards the deep layers of visual cortex area by-

passing the corpus callosum, and the other along the superficial layers of the PCC. In this study, we did not analyze the former pathway but rather focused on the circuit of the subicular-PCC because the latter type of propagation was predominantly observed.

Propagation in the PCC was characterized by its slow velocity of about 0.054 ± 0.006 m/s ($n=11$) while sustaining strong activity (Fig. 1A, 60–150 ms). This activity propagated exclusively along the superficial layers of the PCC, and the velocity was four times slower than the first step of propagation. The propagation range in the overall steps was extended more than 5 mm in the slice, and that in the PCC was up to 4.7 mm. We have observed that activity along the superficial layers of the PCC sometimes extended into the superficial layers of the visual cortex. The propagation of neural activity lasted up to 200 ms.

Temporal pattern of optical signal at individual loci along the propagation path

To examine the spatiotemporal properties of neural activity shown in Fig. 1, we analyzed the time course profile of the optical signal at 14 pixels located along the propagation pathway (Fig. 2A). We found that the propagation of neural activity in the subicular-PCC circuit could be divided into three steps based on their activity pattern and conduction velocity.

First, there was fast propagating activity along the superficial layers of the subicular complex, which was evoked immediately after the electrical stimulation (Fig. 2B, trace 1–3). It seemed to consist of at least two components, a fast-transient with short latency and a sustained plateau lasting 30–35 ms followed by a slow decaying phase. We referred to this local propagation as the first step. Following the fast propagation of neural activity, the signal became to be very weak at locus 4 (Fig. 2B, trace 4). During activity propagation from loci 4 to 5, of which the distance was 168 μ m, the signal intensity was significantly enhanced following a long delay of 25 ms (Fig. 2B, C). The waveform of the activity indicated a slow rising time of 30–40 ms with a long duration of 110–120 ms. The enhanced signal continued to increase up to a maximal level at locus 6. We referred to the slow propagation process including signal enhancement, as the second step (Fig. 2B, trace 4–6). The enhanced signal then spread into the PCC and propagated slowly along its superficial layers maintaining strong activity. The signal was characterized by a similar waveform to that at locus 6 in the second step and a markedly slow conduction velocity (Fig. 2B, trace 5–14). We referred to the propagation of neural activity in the PCC as the third step of propagation. Figure 2C

A

B

Fig. 2. Time course of neural activity at individual pixels. **A:** Recording area and location of stimulation (Stim). Numbered dots indicate the location of pixels for the time course analysis shown in B. **B:** Time courses of neural activity at successive loci along the superficial layer (●, 1–14 in A). The dotted vertical line indicates the time for stimulus onset, and the number on it corresponds to those in A. **C:** Latency of signal plotted against distance from the stimulation site. Inverse of the slope of the curve estimates the propagation velocity of the signal. Fast propagation process (●) in the subiculum, long delay (●–■) near the signal enhancement region, and a slow propagation process (■) in the cingulate cortex are clearly seen. Data from Fig. 1.

shows distance dependency of the activity latency along the superficial pathway from the subicular complex to the PCC. It clearly indicates fast propagation in the first step, a long delay in the second step and slow propagation in the third step.

It is known that voltage-sensitive dyes measure changes in membrane potential, identical to intracellular recordings [16, 17]. It is also reported that the optical signal from voltage-sensitive dyes roughly matches the field potential in brain slice preparations [12, 13]. To reconfirm this in our preparations, we made simultaneous recordings of the field potential and optical signal at 4 different loci along the subicular-PCC pathway (Fig. 3). As seen in the figure, roughly matching are the time course profile of each set of data of the optical and electrical signals as well as the trend in amplitude changes between each series of data. Thus, it is reasonable to assume that the optical signals shown in the present study reflect electrical activities in the tissue. This may be rather surprising

considering that the two types of signals have different origins: the field potential represents local changes in extracellular population potentials, while the optical signal represents changes in the summation of intracellular potentials from a fixed pixel.

Detailed features of neural activity in the signal enhancement region

We noticed in the second step of propagation that the weakened activity was largely enhanced following a long delay (Fig. 2B, trace 4–6). To examine how such signal enhancement occurs and how the enhanced signal propagates into the PCC, we analyzed the spatiotemporal pattern of neural activity in the corresponding region in detail. The signal in this region seemed to propagate from the superficial layers to the middle to deep layers, and then propagate to the superficial layers through a distant bit path (Fig. 1A, 18–48 ms). Therefore, the analyzed area was determined as a rectangular region (336×490 μm) that in-

Fig. 1. Visualization of the propagation of neural activity in the subicular-to-cingulate cortex area. **A:** A series of optical images of neural activity recorded from 1.2 to 150 ms after an electric pulse stimulation (0.3 mA, 0.1 ms). Each frame was taken at the rate of 1 frame/0.6 ms. The time after stimulation is indicated at the upper left in each image (in ms). Signal intensity is indicated as fractional changes in optical absorbance relative to the background (%). The

color in each pixel indicates the intensity change of optical signals; red is strong, green medium and blue background. **B:** Optical recording area (open square) and a bright field image of a counter-stained slice with neutral red. Stimulating electrode was placed onto the subicular complex (asterisk). Arrow shows the site of signal enhancement in the subicular complex. PCC, posterior cingulate cortex; S, subiculum; VC, visual cortex; d, dorsal; l, lateral; m, medial; v, ventral.

Fig. 3. Comparison of field potentials with optical signals. Stimulation site and recording loci (1–4) are shown in the lefthand panel. **Upper**, represent optical signals; **lower**, corresponding field potentials. The numbers on the traces indicate the recording loci as in the lefthand panel.

cludes deeper layers below loci 4, 5, and 6 (Fig. 2A). The region, which is adjacent to the PCC and called the transition zone here, was subdivided into 40 blocks (5×8) (Fig. 4A), and the signal at the center of each block (3×3 pixels) was measured (Fig. 4B).

To determine the temporal sequence of the signal propagation, we first analysed latency-distance relationships along the layer and depth. Optical signals at different depths (Fig. 4B, a–h) seemed to propagated slowly along the layer of the cortex (Fig. 4B, 4→5→6) with comparable propagation rates (Fig. 4C) except for the slower rate of the propagation in the superficial layers (Fig. 4C, ■). There was a trend that the propagation rate in the middle layer was fastest, deep layer moderate, and superficial layer slowest (Fig. 4E). It is notable that there is a large delay in the middle layer of column 4 (Fig. 4E, ■). More importantly, the signal started from the superficial layers of column 4 and ended at 6-a in the superficial layer of column 6 (Fig. 4E). Thus, the signal propagated into the middle layer from the superficial layer in the same column (column 4) with a large delay (ca. 13 ms) between the layers. The signal at the middle layer spread along the layers and then the signal propagated to both superficial and deep layers (Fig. 4E, ▲, +).

Next, we analyzed the peak amplitude of the activity in this region. Interestingly, the smallest signal was observed in the superficial layer in column 4 and largest just below the superficial layer in column 6 (Fig. 4F), a similar to the latency behavior (Fig. 4E). The signal amplitude in the superficial and middle layers gradually increased with propagation along the layers, while little change in the amplitude was observed in the deep layer (Fig. 4D). Significant increases in the amplitude was visible from superficial

to middle layers in column 4 and from middle to superficial layers in column 6, while decreases in the amplitude was observed towards the deep layer in all columns (Fig. 4F).

Taken together the analysis on latency and amplitude, we can postulate the following signal sequence: a small signal starts at the superficial layer in column 4 and propagates to a middle layer region accompanied by an increase in amplitude. Then it spreads along the layer with a moderate increase in amplitude, and finally the signal amplitude increases again during propagation from the middle to superficial layers; in the middle of this travelling, the signal amplitude reaches to its maximal level (Fig. 4F, +). A putative travelling pathway of the signal is schematized in Fig. 4G.

One of important results in this section was the large delay in propagation from the superficial to middle layers in column 4 (Fig. 4E, ■). It is possible that this delay includes synaptic processes. To check this possibility we applied $10 \mu\text{M}$ CNQX, a non-NMDA glutaminergic receptor antagonist, and found that the drug completely blocked the signal propagation at a certain position in the middle layer (Fig. 5B, trace d). This position nearly corresponds to the place of the long delay observed in the control experiment (see control data in trace d). It is highly likely that the first excitatory synapses responsible for the signal enhancement are located around this position, and the major receptor governing the transmission is non-NMDA glutaminergic receptors.

Contribution of GABAergic inhibition to signal enhancement and propagation

We next examined the involvement of inhibitory

Fig. 4. Spatiotemporal profile of activities in the transition region from the subiculum to the PCC. A: Optical recording area (a checker board area of $336 \times 490 \mu\text{m}$) composed of 40 blocks (5×8). **B:** Time courses of the optical signal at the center of each block (3×3 pixels). The numbers, 4, 5 and 6 correspond to the loci 4, 5 and 6 in A. a–h

indicate the depth level from the surface. **C, D:** Latency and amplitude of signals as a function of distance along the layers from locus 4. **E, F:** Latency and amplitude of signals (horizontal axis) are plotted against depth (vertical axis). **G:** Schema of signal travelling path. Data from Fig. 1.

synapses in the signal propagation. The application of $5 \mu\text{M}$ bicuculline (BIC), an antagonist for GABA_A receptor had little effect on the signal propagation.

However, completely different results were obtained when the drug was applied to the preparations without showing signal propagation into the PCC. As men-

Fig. 5. Effect of CNQX on signal propagation. **A:** Optical recording area indicated by an open square with stimulation (Stim) and recording loci (1, 2 and a-g). **B:** Time course of signals along the layer (1-2) and depth (a-g). Thin curves indicate control signals and thick curves that with 10 μ M CNQX.

Fig. 6. Restoration of signal enhancement and propagation by bicuculline. **A:** Optical recording area indicated by an open square (1.25 $\times$ 1.25 mm) (left) and its enlargement (right) with stimulation (1*) and recording loci (2-5 and a-e). **B:** Time course of signals along the layer (1-5) and depth (a-e). Thin curves, control; thick curves, with 5 μ M bicuculline.

tioned in the first section of RESULTS, many preparations (87 out of 98 slices) did not show signal propagation into the PCC devoid of the characteristic signal enhancement in the second step of propagation (Fig. 6B, control traces). Signal propagation in such preparations exhibited only the first step, vanishing at a po-

sition (Fig. 6B traces c, d) similar to that observed in the case of CNQX blockade (Fig. 5B, traces c,d); that is, between the middle and deep layers. Even stronger stimulation could not induce either signal enhancement or propagation to the PCC. There seems to be a critical difference between the slices showing propa-

Fig. 7. Analysis of components of the signal in the synaptic relay region. Stimulation site and recording loci (a–f) are shown in the righthand panel. Time courses of signals are plotted against depth (vertical axis) (a–f). **1:** Control records from the slice without signal enhancement and propagation to the PCC. **2:** Restoration of signal enhance-

ment by 5 μ M bicuculline. **3:** Inhibition of the signal enhancement by 10 μ M CNQX. **4:** Suppression of slow transient by 50 μ M AP5. **5:** Elimination of fast transient by TTX (this experiment was successively carried out with the same slice). **6, 7:** Application of only AP5 affected bicuculline-restored signals little.

gation to the PCC and those not showing it in this synaptic region. The latter slices may have damage in the excitatory synaptic mechanism or involve strong inhibition. To check the latter possibility, we applied 5 μ M BIC to the slices that failed to show signal enhancement and propagation. Surprisingly, all 48 tested slices restored the characteristic signal enhancement and propagation to the PCC as shown in Fig. 6B. The restored activity by BIC showed typical three steps of propagation and signal enhancement indistinguishable from the successful propagation without BIC. This result strongly suggests the crucial involvement of inhibitory neurons in signal propagation from the transition zone to the PCC.

We further examined the involvement of other components in the optical signals by using the BIC-treated slices. When 10 μ M CNQX was applied to a BIC-treated slice (Fig. 7, column 2), the signal pattern returned to a pattern (column 3) similar to the control without BIC (control, column 1). One important observation was that the signal vanishing point is very similar between the control and CNQX-treated responses. In control and CNQX-treated traces at the superficial layers (a, b, c), we noticed two components, fast and slow transients, that seemed to be insensitive to CNQX. The slow transient could be eliminated by the addition of 50 μ M AP5, a NMDA recep-

tor antagonist, and the fast transient completely vanished with the addition of tetrodotoxin (TTX). It is suggested that the fast transient represented synchronized action potentials induced by the direct stimulation of fibers in the subicular complex, and that the slow transient summated synaptic potential generated by the NMDA receptors. However, the application of only AP5 had little effect on the bicuculline-restored signals (column 6,7) except for slightly shortening the duration of signals, by ca. 10 ms. Here, we reconfirmed the previous hypothesis that the major synapse responsible for the following signal propagation is the non-NMDA glutaminergic synapse.

DISCUSSION

The present study has clearly demonstrated that a single electrical stimulation to the subiculum complex can induce long-range signal propagation to the PCC, sometimes extending to the visual cortex. Analyses of the signal propagation led to three main conclusions. First, a signal enhancement occurs in a specific region of the subicular complex, which is located in a relatively invariant area in the transition zone from the subicular complex to the PCC. Second, electrically evoked signals could not propagate into the PCC without the signal enhancement. Finally, the enhance-

ment and following propagation of the signal are mainly mediated by non-NMDA glutaminergic synapses that may usually be strongly inhibited by a GABAergic inhibitory system.

Signal enhancement via synaptic relay.

One of the most intriguing observations in this study was the significant enhancement of the signal in the transition zone between the subicular complex and the PCC. This process appeared in two different pathways; one was from the superficial to middle layers in a subicular complex region, the other was from the middle to superficial layers in a subicular complex region closer to the boundary between the subicular complex and PCC (Fig. 4G).

Previous anatomical studies using tracer injections into the subiculum indicated that the axons of subiculum neurons projected to layers I, II and V of the post-subiculum, but most were in layer V [15]. Extracellular stimulation of superficial layer cells can evoke burst discharges in the deep layer [18, 19]. Our data seem to be consistent with those electrophysiological observations, assuming that the first signal enhancement reflects the electrically measured bursting activity in the deep layer. From our data, the signal intensity was gradually increased in the middle to deep layers, lasting about 20 ms (Fig. 1A, 24–48 ms). We consider that this signal enhancement is very likely caused after synaptic transmission, and probably mediated by polysynaptic connections because there was a considerably long delay before signal enhancement. Additional support for this idea is that neurons in the deep layer of the subicular complex are richly interconnected with excitatory synapses [1, 19].

The second signal enhancement process from the middle to superficial layers showed a relatively faster propagation velocity (0.12 m/s) and gradual increases in the signal intensity without changing the waveform (Fig. 4B, column 6; Fig. 4F, +). These properties are somewhat different from the first enhancement, suggesting that the second enhancement may involve different mechanisms in its process. A recent study on the neocortical layer V pyramidal neurons using simultaneous dual- and triple-patch pipette recordings has demonstrated that the apical dendrites are capable of initiating regenerative potentials [20]. Action potentials are initiated in the axon initial segment and propagate actively into apical dendritic trees. The signal of this back-propagation is enhanced by dendritic Ca^{2+} - and Na^{+} -dependent potentials, in particular, during bursts of action potentials [20]. We assume that the signal enhancement observed in the pathway from the middle to superficial layers might be mediated by such an active back-propagation of action potentials

Fig. 8. Signal delay region in the transition zone. Signal delay region was estimated from 5 slices showing propagation to PCC based on the analysis as in Fig. 4E. The hatched area indicates SDs around the mean coordinate: 2.2 ± 0.175 mm from the dorsal edge, 2.3 ± 0.175 mm from the medial edge.

and/or by a more complicated neural circuits. In the former case (back propagation), the signal propagation to the PCC might occur by means of dendro-dendritic interactions. Morphological evidence for such interactions has been reported in the limbic system [21]. Further study is needed to check this possibility.

Inhibitory circuit may control activity propagation into the cingulate cortex. In the present study, many preparations (87 out of 98 slices) lacked the characteristic signal enhancement in the second step and following propagation. In such cases, the signal rapidly decayed and vanished around the middle to deep layers in the subiculum; the vanishing point seeming to be located in the region of the long delay in a successful propagation (Fig. 8). However, when the inhibition was suppressed by bicuculline, the signal enhancement instantly appeared (Fig. 6) and signal propagation along the pathway of the subicular-PCC could readily be restored. Interestingly the signal vanishing region seemed to be nearly identical to that of CNQX, both in the bicuculline-treated slices and in the slices showing successful propagation. These results indicate that excitatory and inhibitory synapses are converged in a relatively invariant region in the transition zone. As the excitatory synaptic mechanism is primarily important for the signal enhancement and following propagation, the balance between excitatory and inhibitory actions on a fixed region may be important for signal transmission.

Recent electrophysiological studies on the subicular complex have demonstrated the presence of two distinct types of neurons in this region, identified as

bursting and nonbursting cells. Both cell types receive excitatory and inhibitory inputs, inhibition being more pronounced in nonbursting cells and excitation more pronounced in bursting cells [19]. The failure of signal enhancement frequently observed in the present study may have arisen from insufficient activation of the bursting neurons, probably because of mechanical damage to the neural circuits during the procedure of slice preparation. Alternatively, the success of signal enhancement may have arisen from damage to the inhibitory circuits. This latter mechanism seems to be more likely because, in every case tested, bicuculline could restore signal enhancement and propagation to the PCC. One of possible mechanisms for the restoration by bicuculline may be that the activity of nonbursting neurons was enhanced by the suppression of inhibitory synapses. It is reported that nonbursting cells in the subiculum were able to burst under a condition of decreased inhibition during picrotoxin exposure [19]. Presumably, in intact brain, the signal enhancing ability of the subicular neuron is suppressed by inhibitory circuits, then certain inputs from other areas come into the signal enhancing region and disinhibit the inhibitory circuits making the signals from hippocampus propagate into the PCC. Further analyses should be done to prove this hypothesis.

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