Spatial dependency of inhibitory components in hippocampal CA1 area: analysis using optical imaging method with voltage-sensitive dye

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Abstract — In many recent reports, investigators have described that the hippocampus has many inhibitory interneurons and non-uniform voltage-sensitive K\textsuperscript{+} channel density. However, the distribution and amplitudes of these inhibitory components remain unclear. In this study, we used optical imaging with voltage-sensitive dye to identify the location dependency of these inhibitions.

Keywords — Hippocampus, Inhibitory interneuron, Dendritic integration, Optical imaging, Voltage-sensitive dye

1. Introduction

The hippocampal CA1 area network has many complex inhibitory interneurons \cite{1} that play a crucial role in physiological function and information processing \cite{2, 3}.

Our earlier study found that back-propagating action potential (BPAP) is differently modulated by synaptic inputs depending on the differences of a relative timing of pre- and post-synaptic activation. When BPAP is paired with EPSP at proximal sites from soma, it is amplified and propagated to the distal sites of dendrites. However, when BPAP is paired with feedforward IPSP, it is attenuated.

Spike-Timing Dependent Plasticity (STDP), one form of synaptic plasticity, is changed by BPAP modulation \cite{4}. This property suggests that STDP might be changed not only by the relative timing of pre- and post-synaptic activations, but by the change of BPAP amplitude: inhibitory interneurons can be involved in some of these modulations of BPAP and STDP.

Hoffman et al. showed that hippocampal CA1 dendrites have non-uniform A-type voltage-gated K\textsuperscript{+} channels \cite{5}, but the location-dependency of these inhibitory components, which are feedforward, feedback IPSP, and voltage-gated K\textsuperscript{+} channels, remains unknown.

Therefore, in this study, we undertook analysis of the dynamics of dendritic-layer-specific feedforward and feedback inhibitory components using optical imaging with voltage-sensitive dye, which revealed the spatial spread and the temporal change of membrane potential.

2. Methods

Slice preparation and staining

Experiments were performed on hippocampal slices prepared from 4–5 week-old male Wister rats, which had been decapitated under deep anesthesia. The brains were maintained at room temperature in an experimental submerged chamber with artificial cerebro-spinal fluid (ACSF). The ACSF was identical to the cutting solution, but it contained 2.4 mM CaCl\textsubscript{2} and 1.2 mM MgSO\textsubscript{4}.

Membrane activities were measured as optical signals obtained using optical imaging with voltage-sensitive dye. The staining method was identical to that described by Tominaga et al. \cite{5}. Each slice was stained with 100 \muM Di-4-ANEPPS into a plexiglass ring with a fine-mesh membrane filter in an interface chamber. After staining, slices were returned to ACSF and recovered for an additional 30 min.

Optical imaging

We used a high NA 20\times objective lens (Olympus Corp.) and a 0.5\times camera adapter. The final magnification of the system was 10 \times. Light from halogen light sources (150 W \times 2; Moritex Corp.) was used. The camera (MiCAM Ultima; Brain Vision Corp.) recorded fluorescence signals with an excitation filter (\textlambda = 535\pm45 nm) and a dichroic mirror (\textlambda = 565 nm) and an absorption filter (\textlambda > 610 nm). This CMOS-based camera had adequate spatial resolution (10 \times 10 \mu m\textsuperscript{2} / single point) and adequate temporal resolution (100 Hz).

The hippocampal CA1 area network has many complex inhibitory interneurons \cite{1} that play a crucial role in physiological function and information processing \cite{2, 3}.
Inhibitory interneurons play a key role in information processing in the local neural network of hippocampal CA1 area. This study examined the effects of feedforward and feedback inhibition using optical imaging with voltage-sensitive dye and pharmacological applications. Results show changes of the inhibitory effect dynamics along dendrites. These results suggest an important question to explore information processing on dendrites: How do inhibitory inputs affect dendritic integrations depending on input locations and timings?

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References