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Monosynaptic excitatory transmission from the hippocampal CA1 region to the subiculum

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Abstract

The subiculum is a major output region of the hippocampus, receiving inputs from the CA1 region. We obtained paired patch-clamp recordings from synaptically coupled pairs of CA1 pyramidal cells (CA1PCs) and subicular principal cells (SubPCs), using rat hippocampal organotypic slice cultures. A single action potential in a presynaptic CA1PC evoked a unitary excitatory postsynaptic current in a SubPC ($EPSC_{CA1\rightarrow Sub}$). The failure rate of the transmission was remarkably low (0.08). Paired-pulse depression in SubPCs was apparent when an interval of presynaptic action potentials was shorter than 50 ms. When trains of action potentials were induced in a CA1PC, $EPSC_{CA1\rightarrow Sub}$ was significantly depressed with increasing spike frequency (20-100 Hz). Thus the unitary monosynaptic transmission from a CA1PC to a SubPC is reliable, and depressed in response to frequent inputs, suggesting that the may function as a low pass filter to provide the downstream brain regions with appropriate information.

1. Introduction

The subiculum plays an important role in spatial memory and motivation [1, 2, 3, 4]. It is also involved in the pathogenesis of temporal lobe epilepsy [5, 6], and mental illnesses such as Alzheimer's disease [7, 8, 9], Addison's disease [10] and schizophrenia [11, 12]. Principal cells of the subiculum (SubPCs) receive inputs from those of the CA1 region (CA1PCs) [13, 14, 15, 16]. Both SubPCs and CA1PCs are involved in spatial information processing as "place cells" [17, 18, 19, 20]. Several studies on the synaptic transmission from the CA1 region to the subiculum have been reported [21, 22, 23, 24] using extracellular stimulation protocols that activate multiple neighboring inputs from CA1PCs simultaneously, but the precise properties of the unitary monosynaptic transmission from a single CA1PC to a SubPC have not yet been provided. Thus on the functional point of view, the subiculum has been less investigated than the other hippocampal regions such as the CA1 and the CA3 regions.

Paired recording from connected cell pairs is a powerful and versatile technique to examine a functional communication between synaptically coupled neurons [25]. To analyze the properties of the monosynaptic transmission precisely, we have recorded from connected pairs of CA1PCs and SubPCs in rat organotypic hippocampal slice cultures. As only one CA1PC is stimulated, potential complications arising from synaptic cooperativity and pooling of neurotransmitter is avoided. A single action potential in a CA1PC induced a monosynaptic

excitatory current in a SubPC ($EPSC_{CA1\rightarrow Sub}$) with few failures. The $EPSC_{CA1\rightarrow Sub}$ was significantly depressed when presynaptic action potentials were frequently evoked. Thus the subiculum may prohibit frequent signals from propagating out of the hippocampus.

2. Material and methods

2.1. Slice culture preparation

All experiments were carried out in slice cultures prepared from P5-7 day-old rat pups. All animal procedures were approved by the Animal Care and Use Committee at Kobe University Graduate School of Medicine (Permit Number: P130808). Entorhinal-hippocampal slice cultures were prepared as previously described [26]. Briefly, entorhinal-hippocampal slices were sectioned at 400 μm, attached to glass coverslips using clotted chicken plasma (Japan Biotest, Saitama, Japan), placed in sealed test tubes with serum-containing medium, and kept in a roller-drum incubator at 36 °C for 14–21 days.

2.2. Electrophysiology

Cultures were transferred to a recording chamber mounted on an upright microscope, (AxioExaminer, Zeiss, Jena, Germany) and superfused with an external solution (pH 7.4) containing 148.8 mM Na^+, 2.7 mM K^+, 149.2 mM Cl^-, 2.8 mM Ca^{2+}, 2.0 mM Mg^{2+}, 11.6 mM HCO_3^-, 0.4 mM $H_2PO_4^-$, 5.6 mM D-glucose and 10 mg l^{-1} phenol red (pH 7.4). All experiments were performed at 34°C. Recordings were obtained from CA1 pyramidal cells (CA1PCs) and subicular principal cells (SubPCs) of the hippocampus with patch pipettes (2–5 MΩ) using an EPC 10 amplifier (HEKA Elektronik, Lambrecht, Germany). Pipettes were filled with a solution containing 135 mM K-gluconate, 5 mM KCl, 10 mM Hepes, 1 mM EGTA, 2 mM Mg-ATP, 5 mM creatine phosphate (CrP), 0.4 mM GTP, 0.07 mM $CaCl_2$, 1 mg l^{-1} biocytin, pH 7.2. The actual membrane potentials were corrected for the liquid junction potential. Presynaptic action potentials were evoked by injecting depolarizing current (1 ms, 3.2 nA) at 0.1 Hz unless otherwise mentioned. Series resistance (typically between 5 and 15 MΩ) was regularly monitored and the cells were excluded if a change of more than 20% occurred.

2.3. Drugs and chemicals

ATP, CrP, EGTA, GTP and CNQX were purchased from Sigma-Aldrich (MO, USA). Biocytin was from (Life Technologies, NY, USA).

2.4. Data acquisition and analysis

Electrophysiological signals were filtered at 10 kHz, digitally recorded using PATCHMASTER software (HEKA Elektronik, Lambrecht, Germany) and stored on a hard disk for later analysis. The amplitude, latency and kinetics were determined as described elsewhere [27]. To quantify the synaptic responses evoked by each action potential during a train, the peak amplitude of the response was measured from the baseline directly preceding each EPSC. The standard deviation of the latencies was used to calculate the jitter. Numerical data in the text were expressed as means ± S.E.M. ANOVA with Fisher's least significant difference test was used to compare values.

2.5. Biocytin labeling

For staining cells, 0.1 % biocytin (Life Technologies, NY, USA) added to the pipette solution. After experiments, slices were fixed in 4 % buffered paraformaldehyde. Biocytin was visualized with an avidin-biotin peroxidase complex (Vector Laboratories, CA, USA) using diaminobenzidine.

3. Results

3.1. Properties of Unitary $EPSC_{CA1→Sub}$

To characterize the monosynaptic transmission between a CA1PC and a SubPC, we employed the whole-cell patch-clamp recording from a pair of a CA1PC and a SubPC in rat hippocampal organotypic slice cultures (Fig. 1A,B). The border zone between the CA1 region and the subiculum was identified by abrupt widening of the CA1 pyramidal cell layer and it was confirmed that the density of SubPCs in the layer is lower than that of CA1PCs [16, 28, 29, 30]. Recordings were obtained from CA1PCs and SubPCs about 100 μm apart from the border zone. We recorded from 46 SubPCs and 71 CA1PCs, and obtained 23 synaptically coupled pairs of a CA1PC and a SubPC. The probability to obtain a connected pair was 32.4%. Among them 20 pairs were good for later analysis. All SubPCs in 20 recorded pairs regularly fired action potentials in response to a depolarizing current injection (Fig. 1C). When single presynaptic action potentials were evoked in a CA1PC, fast inward currents were induced in a SubPC (Fig. 1D, Table 1). These fast inward currents were completely blocked by bath application of CNQX (10 μM) an AMPA/ kainate receptor antagonists (data not shown, n=12). Evidence for a monosynaptic property of the unitary excitatory postsynaptic currents (EPSC) is provided by the low variability in latency (jitter: 0.85 ± 0.01 ms, n=20) between the peak of presynaptic action potential and the onset of postsynaptic response (Fig. 1D) and the one-to-one transmission at high frequencies (Fig. 3). When presynaptic action potentials were evoked at 0.1 Hz, the failure rate of postsynaptic responses was low (0.08 ± 0.02, n=20, Fig. 1D). The mean amplitude of the unitary EPSCs from a CA1PC to a SubPC ($EPSC_{CA1→Sub}$) of all pairs was 44.1 ± 7.6 pA (n=20, Table 1) with variability in the amplitude from pair to pair (from 14.0 to 122.2 pA). This variability may derive from the difference in the number of functional synapses in each pair. We found no accompaniment of an outward inhibitory postsynaptic current with the unitary $EPSC_{CA1→Sub}$. No monosynaptic response ($EPSC_{Sub→CA1}$) was observed in a CA1PC while the unitary $EPSC_{CA1→Sub}$ was recoded (n=20). It is not likely that mutual, reciprocal synaptic connections are organized between CA1PCs and SubPCs..

3.2. Paired-pulse depression of the monosynaptic transmission between a CA1PC and a SubPC

When two presynaptic action potentials were evoked at an interval less than 50 ms, the second unitary $EPSC_{CA1→Sub}$ was depressed compared to the first one (Fig. 2A). The paired pulse ratio was calculated as a ratio of the amplitude of the second $EPSC_{CA1→Sub}$ to that of the first $EPSC_{CA1→Sub}$. The paired pulse ratios decreased significantly when the intervals were shorter than 50 ms ($p=0.63$ at 50 ms-interval; $p<0.01$ at 25 ms-interval; $p<0.01$ at 10 ms-interval: ANOVA with Fisher's least significant difference test from data at 100 ms-interval, n=16, Fig. 2B).

3.3. Frequency-dependent properties of unitary $EPSC_{CA1→Sub}$ in response to a train of presynaptic action potentials

CA1PCs fire at low frequencies less than 1 Hz, but at high frequencies up to 40 Hz [31] when rats enter the place field of a CA1PC [32, 33, 34, 35]. To investigate how the synaptic transmission is modulated in a SubPC when a CA1PC fires action potentials at high

frequencies, we recorded the unitary $EPSC_{CA1\rightarrow Sub}$ in a SubPC during trains of 10 presynaptic action potentials in a SubPC at 10, 20, 40 and 100 Hz (Fig. 3A). As firing frequencies were increased, the amplitudes of unitary $EPSC_{CA1\rightarrow Sub}$ decreased sharply (Fig. 3B). The ratios of the amplitude of the 10th $EPSC_{CA1\rightarrow Sub}$ to that of the first $EPSC_{CA1\rightarrow Sub}$ were 0.87 ± 0.09 at 10Hz, 0.55 ± 0.08 at 20Hz, 0.31 ± 0.06 at 40Hz and 0.20 ± 0.07 at 100Hz (n=20).

4. Discussion

Prior investigations reported several functional properties on the synapses between the CA1 region and the subiculum [21, 22, 23, 24]. In these studies, stimulation of multiple presynaptic inputs was applied with an extracellular electrode to record the synaptic responses. In the present study, by the paired patch-clamp recording from connected pairs of CA1PCs and SubPCs, the precise properties of unitary monosynaptic excitatory currents ($EPSC_{CA1\rightarrow Sub}$) were characterized for the first time.

The $EPSC_{CA1\rightarrow Sub}$was reliably induced by each presynaptic action potential with less failure rate (0.08) than that of the Schaffer collateral unitary EPSC (0.53) [36], and that of the mossy fiber unitary EPSC (0.83) [37]. Furthermore the paired pulse ratio of the unitary $EPSC_{CA1\rightarrow Sub}$ (0.84 at 50 ms-interval) is smaller than that of the Schaffer collateral EPSC (1.21; 1.54 at 50 ms-interval) [38, 39] and that of the mossy fiber EPSC (2.54 at 50 ms-interval) [37]. Thus the synapses between CA1PCs and SubPCs have higher probability of glutamate release than two major hippocampal glutamatergic synapses, the mossy fiber synapse and the Schaffer collateral synapse. At rest CA1PCs fire typically at low frequencies less than 1 Hz in vivo [18, 40]. Together with the finding that no inhibitory synaptic responses were not detected with monosynaptic $EPSC_{CA1\rightarrow Sub}$ (n=20, Fig.1), point to point information into the subiculum could be reliably transferred to the downstream regions such as the entorhinal cortex. In contrast to our finding, paired pulse facilitation of the synaptic responses onto the subiculum was reported [41, 42]. This discrepancy might be attributed to a difference in the stimulation protocols or the experimental preparations.

CA1PCs can fire at high frequencies up to 40 Hz as an intermittent burst when an animal enters the place field [31, 40, 42]. Unitary $EPSC_{CA1\rightarrow Sub}$ was significantly depressed by presynaptic spikes from CA1PCs at frequencies higher than 10 Hz (Fig. 3B). Synapses filter the flow of information between neurons by activity-dependent modification of neurotransmitter release. Thus the facilitating synapses with a low initial probability of neurotransmitter release function as high-pass filters, whereas the depressing synapses with a high initial probability of release act as low-pass filters [43, 44]. The reliability and the frequency-dependent modulation of the synaptic transmission from the CA1 region to the subiculum might endow the subiculum with a function as a low pass filter to prevent frequent signals from propagating out of the hippocampus. This possibility needs to be studied further.

5. Conclusions

We characterized for the first time the properties of the unitary monosynaptic transmission between a CA1PC and a SubPC, using the rat hippocampal organotypic slice cultures. The monosynaptic transmission is reliable and significantly depressed when frequent presynaptic spikes arrive. The subiculum might function as a low pass filter in the hippocampal circuit. This possibility need to be studied further to clarify the functions of the subiculum and its involvement in the pathogenesis of the neurological and psychiatric disorders.

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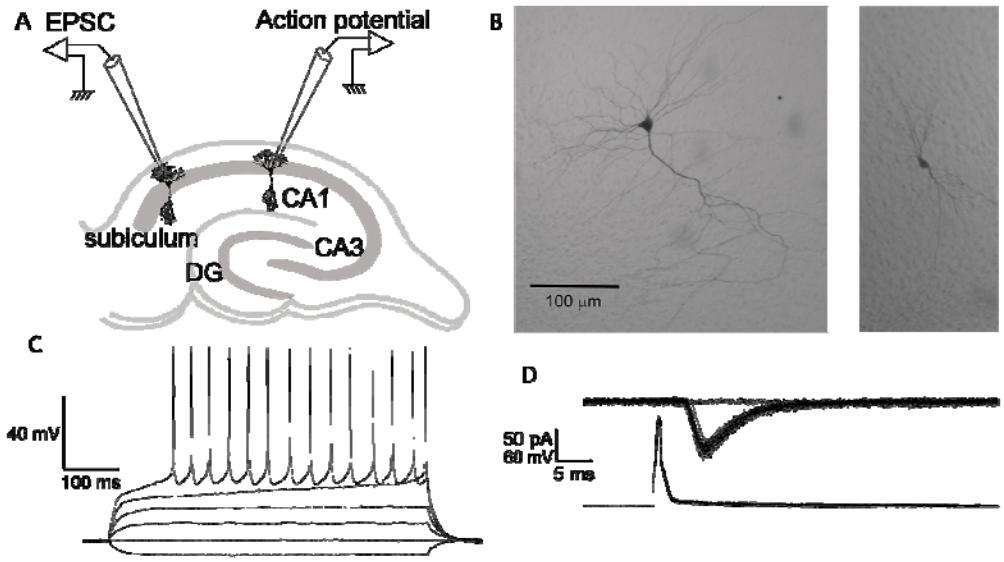

Fig.1: Monosynaptic responses recorded from a synaptically coupled pair of a CA1PC and a SubPC. (A) Schematic diagram illustrating the position of the recording pipettes in the hippocampal slice. (B) A SubPC (left) and a CA1PC (right) labeled with biocytin. (C) Membrane potentials recorded from a typical SubPC in response to different current pulses injected for 600 ms shown superimposed with an increment of 20 pA from -20 pA to 80 pA. (D) Single action potentials at 0.1 Hz (lower) in a CA1PC induced unitary EPSCs in a SubPC at -70 mV.

Parameters (Unit)	Value	Data Counts
Amplitude (pA)	44.1±7.6	n=20
Paired-Pulses Ratio	0.84±0.02	n=20
Latency (ms)	2.88±0.24	n=20
Risetime 20-80% (ms)	1.05±0.09	n=20
Decay Tau (ms)	5.47±0.47	n=20
Resting Membrane Potential (mV) SubPC	-70.5±1.4	n=25
Resting Membrane Potential (mV) CA1PC	-69±1.4	n=27

Table 1: properties of monosynaptic EPSCs recorded from connected pairs of CA1PCs and SubPCs

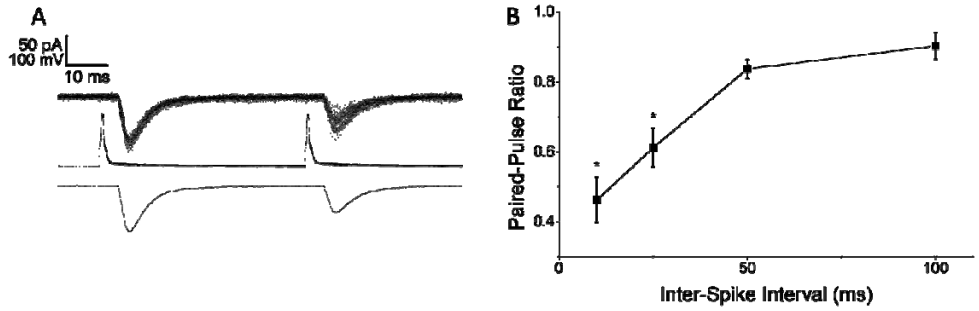

Fig. 2: Paired-pulse depression of unitary $EPSC_{CA1\rightarrow Sub}$. (A) $EPSC_{CA1\rightarrow Sub}$ in a SubPC (top) in response to paired action potentials (middle) in a CA1PC at an interval of 50 ms. Averaged paired pulse response is shown at the bottom. (B) A plot of paired-pulse ratios against the intervals of presynaptic action potentials. Each point represents the mean of 16 pairs. Error bars indicate S.E.M. Asterisks show a significant difference from data at 100 ms-interval (ANOVA with Fisher's least significant difference test, $p<0.05$).

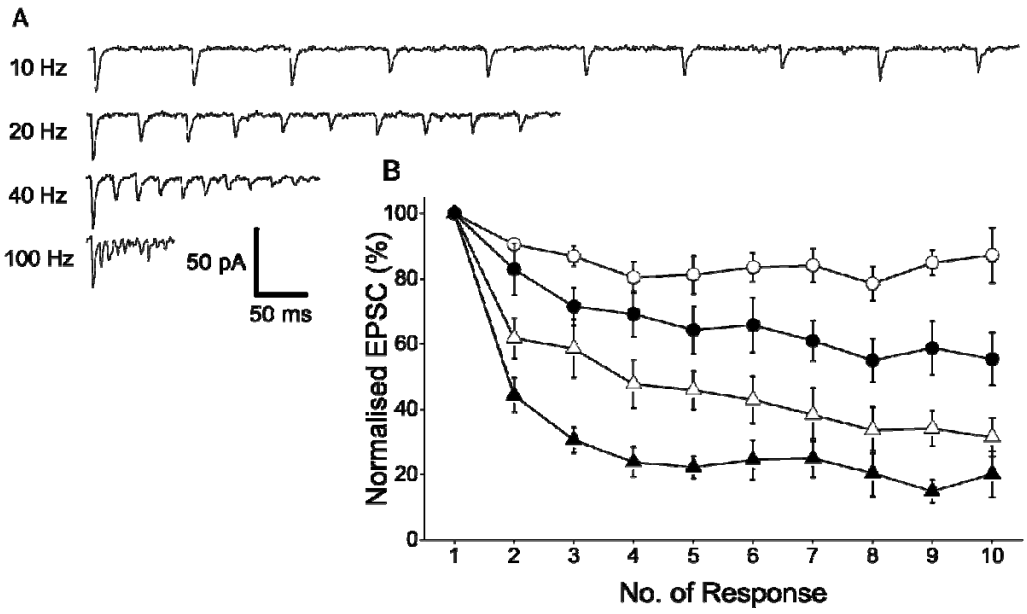

Fig. 3: Unitary $EPSC_{CA1\rightarrow Sub}$ during trains of action potentials at high frequencies. (A) Unitary $EPSC_{CA1\rightarrow Sub}$ in a SubPC during trains of 10 presynaptic action potentials in a SubPC at different frequencies (10-100 Hz). Each trace is the average of 4 to 6 sweeps. (B) Ratios of the amplitude of each unitary $EPSC_{CA1\rightarrow Sub}$ to that of the first one in response to a train of 10 action potentials at 10 Hz (open circle), 20 Hz (filled circle), 40 Hz (open triangle) and 100 Hz (filled triangle). Each point shows the mean of 20 pairs and error bars indicate S.E.M.