REVIEW ARTICLE

Diversity of learning-induced synaptic plasticity at dorsal CA1 synapses: a possible underlying mechanism of contextual memory formation

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Abstract

We expose various kinds of context in our everyday lives and hence contextual memory is critically important for us. Since hippocampus is a major area for processing contextual memory, it needs to encode different components of various contexts such as 'what', 'where' and 'when', etc. However, the underlying mechanism by which the hippocampus differentially encodes various kinds of contextual information is interesting but not yet revealed completely. Since long-term potentiation (LTP) had been discovered and believed to be mechanism of learning, many studies were conducted to investigate the underlying detailed mechanism of memory formation by using various LTP induction protocols. By combining HSV-mediated *in vivo* gene delivery with *in vitro* patch-clamp recordings, it was found that LTP can induce plastic changes such as delivery of GluA1-containing AMPA receptor to the dorsal CA1 synapses of the hippocampus and also the causal relationship between this AMPA receptor delivery and learning was discovered. Then it was found that learning induced synaptic plasticity with diverse pattern highlighting the importance of this diversity in contextual memory formation. Here, we mostly review the studies which used hippocampal-dependent learning paradigm (inhibitory avoidance task) for better comparison and mapping of the concept about how learning induced diversity of synaptic plasticity at dorsal hippocampal CA1 synapses and how this diversity may become responsible as an underlying mechanism of contextual memory formation. We found that contextual learning induces synaptic plasticity at CA1 neurons with synapse-specific diversity, input-specific diversity, subregion- or subfield-specific diversity, episode-specific diversity but only a few studies revealed their functional significance. For complete understanding of learning-induced diversity of synaptic plasticity, it demands further studies for investigation of their functional correlation to learning performance by using new highly promising approaches like optogenetic techniques and cell-specific self-entropy analysis, etc.

Key words: Diversity, Synaptic plasticity, Contextual memory, Dorsal CA1 synapses.

Introduction

The hippocampus is critically involved in encoding and retrieval of contextual memory by working together with adjacent entorhinal cortex and parahippocampus. Different areas of hippocampus especially CA1, CA3 and dentate gyrus are important in processing different components and stages of contextual memory formation.[1] In these areas, contextual learning induces synaptic plasticity.[2,3] This synaptic plasticity displays physiological properties like associativity, input-specificity, and persistence that are highly suggestive of an information storage device.[1] Recent studies suggested that contextual memory was possibly encoded by diverse pattern of synaptic plasticity at dorsal CA1 region of hippocampus (Fig. 1).[4,5,6]

Lømo discovered long-term potentiation (LTP) in 1966 as a possible mechanism of learning.[7] Since selective blockade study of long-term potentiation (LTP) induction by NMDA receptor antagonist which impaired hippocampal learning, [8] LTP has been considered as a cellular model of hippocampal memory.[9] Later, by using inhibitory avoidance (IA) task as the learning paradigm of hippocampal contextual memory, *in vivo* field EPSC recording study showed that IA learning intrinsically induces LTP in CA1 region of hippocampus.[10] Moreover, we previously proved that

hippocampal learning required synaptic delivery of AMPA receptors into the CA3-CA1 synapses, suggesting causality of GluA1-containing AMPA receptor delivery and the learning.[2] Selective erasure of LTP by optogenetic approach further revealed temporal window for contextual memory.[11] So, in a process of memory formation, as there is no electrode in the brain to elicit LTP induction, what will be endogenous trigger and/or the mechanism to induce the learning-dependent LTP?

Since acetycholine (ACh) induced specific bursts[12] and formed LTP in CA1 region of hippocampal slices[13], it was hypothesized to be the required endogenous trigger for learning-dependent LTP induction. By using IA training and *in vivo* microdialysis study of ACh release, we found that cholinergic trigger induced learning-dependent synaptic plasticity at both excitatory and inhibitory synapses. Since bilateral blockade of the plasticity at either excitatory or inhibitory synapses severely impaired the learning, the learning seems to require the diversity of synaptic inputs onto CA1 pyramidal neurons.[3]

This finding of diversity of learning-induced changes in various synapses encouraged further studies to reveal the importance of diversity of learning-induced synaptic plasticity in contextual memory formation. To establish the memory of a context, we need to process and encode different components of the context such as "what", "when", "where", etc. Since the hippocampus plays an important role in contextual memory formation, it will need to encode different components of various kinds of contexts which we expose every day. So how the hippocampus differentially processes various kinds of information? Diversity of synaptic plasticity during memory formation may be possible mechanism for this function of hippocampus.

Mathematical approach regarding synaptic plasticity has been carried out in the recent years. By using cell-specific self-entropy analysis after IA training, contextual learning dramatically diversified both excitatory and inhibitory postsynaptic responses in both hemispheres, whereas the training did not promote synaptic diversity in either hemisphere in ventral CA1 neurons. Nonstationary fluctuation analysis further showed a molecular evidence of plasticity increasing the number of AMPA or $GABA_A$ receptor channels after IA training. [4] *In vivo* monitoring of multiple-unit spike activity of CA1 neurons before, during, and after exposure to different episodes also showed that a particular experience induced episode-specific super burst activity which may further promote episode-specific synaptic diversity and ripple-like events.[5] Moreover, learning-induced plasticity at dorsal CA1 synapses of two main input pathways (Schaffer's collateral and temporoammonic pathway) also revealed heterogeneity along the proximodistal axis of CA1 region.[6] Although various studies using different methods has shown various patterns of learning-induced synaptic plasticity, their results need to be reviewed together systematically to highlight the role of synaptic diversity for learning. Here, we mostly review the studies which used hippocampal-dependent learning paradigm (IA task) for better comparison and map the concept of how learning induced the diversity of synaptic plasticity at dorsal hippocampal CA1 synapses. By reviewing these studies, we suggest that this diversity may become responsible as an underlying mechanism of contextual memory formation.

Knowledge of diversity of learning-induced synaptic plasticity at dorsal CA1 synapses of hippocampus will be very helpful in understanding how contextual memory is processed and how it will be affected in many hippocampal-related neurodegenerative diseases like Alzheimer's disease.

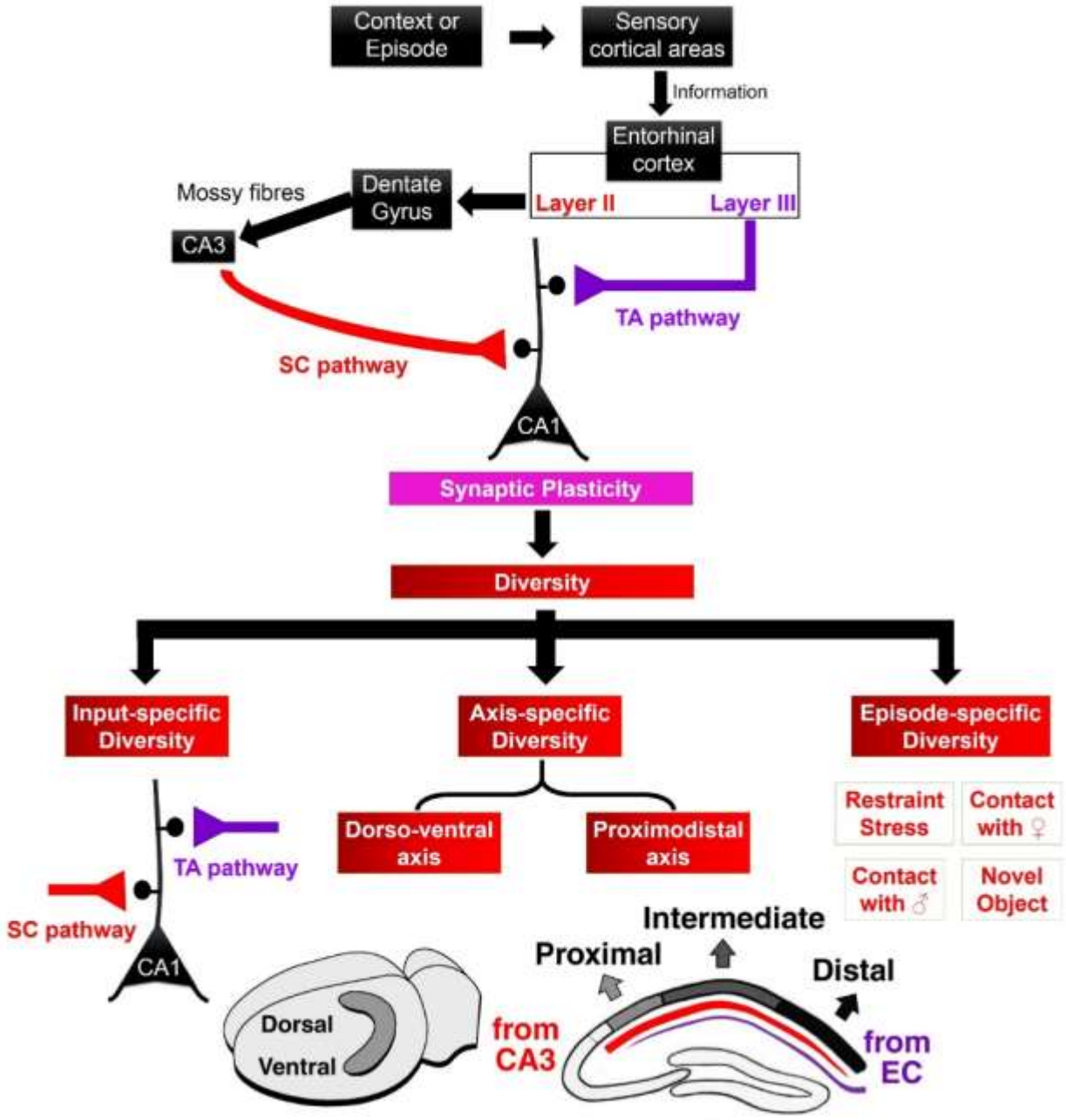

Fig. 1. Outline of information flow during contextual memory formation and diversity of learning-induced synaptic plasticity. SC: Schaffer. TA: temporoammonic. CA: cornu ammois. EC: entorhinal cortex.

Learning Paradigm of Contextual Memory

One of the most commonly used behavioral tasks to investigate learning-induced synaptic modification in the hippocampus is inhibitory avoidance (IA) task. The IA training apparatus (length: 33 cm, width: 58 cm, height: 33 cm) consisted of a two-chambered box that contained a lighted safe side and a dark shock side, separated by a trap door (Fig. 2). In this paradigm, rats are allowed to cross from an illuminated box to a dark box where an electric foot shock is

delivered. Thus, rats learn to avoid the dark box and stay in the lighted one, which they would normally not prefer.[2,10] The tendency to avoid the dark box, therefore, indicates the acquisition of contextual memories. The rats avoided entering the dark box when it was associated with a mild electric shock (IA-trained), but not those given foot shock without any contextual experience (unpaired), or those allowed to simply explore the experimental cage (walk-through). Untrained control rats were kept in their home cages and were not exposed to the IA apparatus. In this review, we mostly focus on the studies which used IA training as learning paradigm for better comparison of learning-induced synaptic plasticity at hippocampal CA1 region.

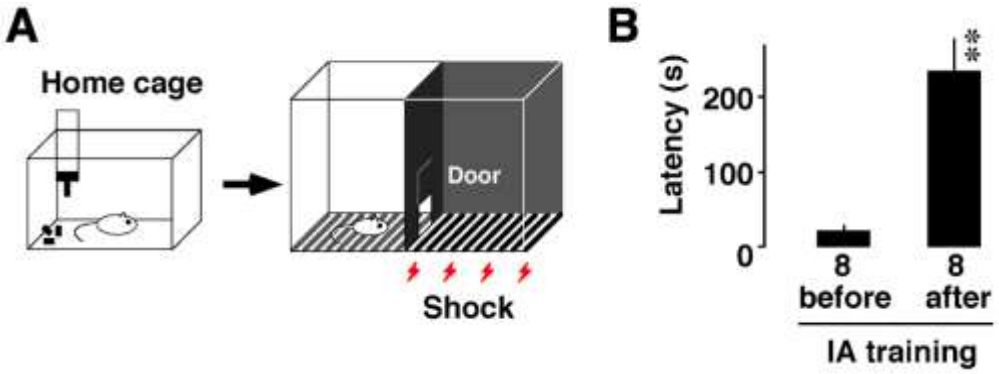

Fig. 2. (A) Schema of inhibitory avoidance (IA) task. On the day of IA training, rats were placed in the light side of the box. When rats moved into the dark side box, we closed the door and applied electrical foot shock (1.6 mA) for 2 sec. The rats were returned to the home cage soon after the shock. **(B)** Even one training, rats remember the episode well, spending much longer time in the light box after training.[3]

Role of Synaptic Plasticity in Memory Formation

After discovery of long-term potentiation (LTP) activity in the dentate area of the hippocampal formation[7], many researchers focused on LTP induction study, its underlying mechanism and its possible functional significance. Then LTP has been considered to be a potential mechanism of information storage, possibly memory formation.[9] During LTP induction, GluA1-containing AMPA receptors were delivered to the synapses. [14,15] By combining HSV-mediated *in vivo* gene delivery with *in vitro* patch-clamp recordings, we reported the causal relationship between GluA1-containing AMPA receptor delivery and learning. We found that contextual learning was successfully suppressed by bilateral expression of AMPA receptor delivery blocker (MPR-DD).[2] Moreover, inhibition of AMPA receptor trafficking by crosslinking immobilization approach markedly impaired LTP expression as well as contextual learning.[16] Therefore, these studies suggested that learning may induce synaptic plasticity by AMPA receptor delivery to the synapses and it is a possible underlying mechanism for contextual memory formation.

Diversity of Synaptic Plasticity at Different Synapses

Since our daily activities involve many different contexts, hippocampus, which is a critical area for contextual memory formation[17], needs to process and encode various components of different contexts we experienced. Here, we propose that diversity

of learning-induced synaptic plasticity may be a possible solution for this complicated function of hippocampus. We previously reported that contextual learning induced synaptic plasticity not only at excitatory synapses but also at inhibitory synapses of CA1 region.[2,3] Learning-induced synaptic plasticity at both excitatory and inhibitory synapses also exhibited temporal dynamics along with increase in diverse self-entropy levels, suggesting differentially encoding of different pieces of memory. Moreover, these temporal dynamics were also observed in pre-synaptic plasticity after IA training.[18] By using optogenetic technique as a powerful tool to study diversity of learning-induced synaptic plasticity, a recent study showed that contextual fear conditioning enhanced synaptic transmission between CA3 engram cells and CA1 engram cells by inducing both pre- and post-synaptic plasticity. But such enhancement was not found in other pair types of synapses.[19] From these experiments, we can clearly see that contextual learning can induce diverse pattern of synaptic plasticity at specific synapses with different timing.

By forming both afferent and efferent connections between CA1 and entorhinal cortex (EC), both regions interplay during memory formation. Various kinds of information from different cortical area are directed to perirhinal and entorhinal cortices. Then these traces of information flow from entorhinal cortex to hippocampal CA1 neurons through two major pathways, the indirect route named tri-synaptic pathway and the direct route called temporoammonic (TA) pathway.[20] Both projections converge on single CA1 pyramidal neurons; tri-synaptic pathway making CA3-CA1 synapses in the stratum radiatum via Schaffer's collateral whereas direct pathway forming TA-CA1 synapses in the stratum lacunosum moleculare.[21]

Our recent study has shown that contextual learning induced pathway-specific diversity of synaptic plasticity. IA training significantly strengthened post-synaptic plasticity at CA3-CA1 synapses of proximal and intermediate CA1 regions whereas such strengthening occurred at TA-CA1 synapses of intermediate and distal CA1 regions (Fig. 3). Surprisingly, we also found out a similar diverse pattern of learning-induced pre-synaptic plasticity at both CA3-CA1 and TA-CA1 synapses (Fig. 4).[6] These findings suggest that input-specific diversity of learning-induced synaptic plasticity may be responsible for different functional role of these inputs in establishing the memory of a particular context.

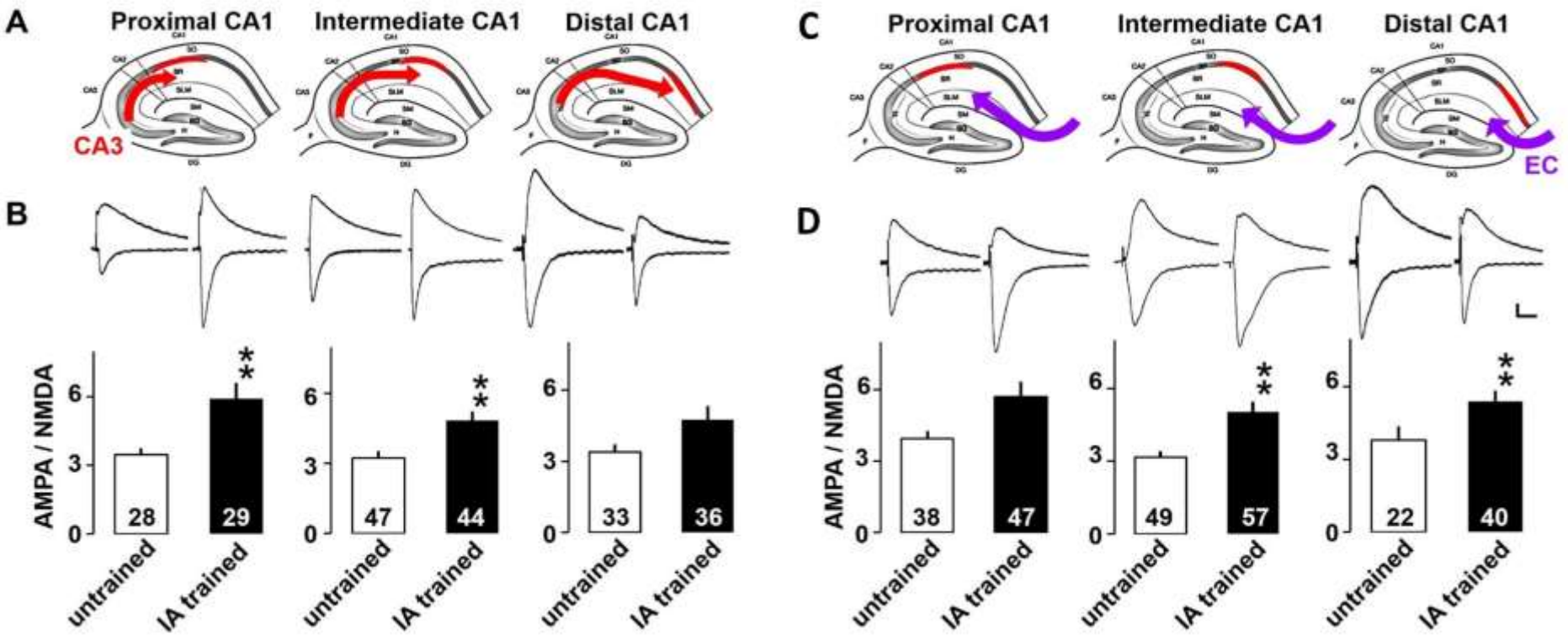

Fig. 3. Diversity of Post-synaptic plasticity at CA3-CA1 and ECIII- CA1 synapses. (A) The proximal, intermediate and distal CA1 subfields of the dorsal hippocampus where the AMPA/NMDA ratios of CA3-CA1 synapses were recorded. (B) IA training significantly increased the mean AMPA/NMDA ratio at CA3-CA1 synapses in the proximal and intermediate regions. The upper insets show representative traces. The number of cells in each group is shown at the bottom of each bar. (C) The proximal, intermediate and distal CA1 subfields of the dorsal hippocampus where the AMPA/NMDA ratios of ECIII-CA1 synapses were recorded. (D) IA training significantly increased the mean AMPA/NMDA ratio at ECIII-CA1 synapses in the intermediate and distal regions. The upper insets show representative traces. The number of cells in each group is shown at the bottom of each bar. Vertical and horizontal scale bars represent 20 pA and 40 ms, respectively. Error bars indicate + SEM. $^{**}P<0.01$ *vs* untrained.

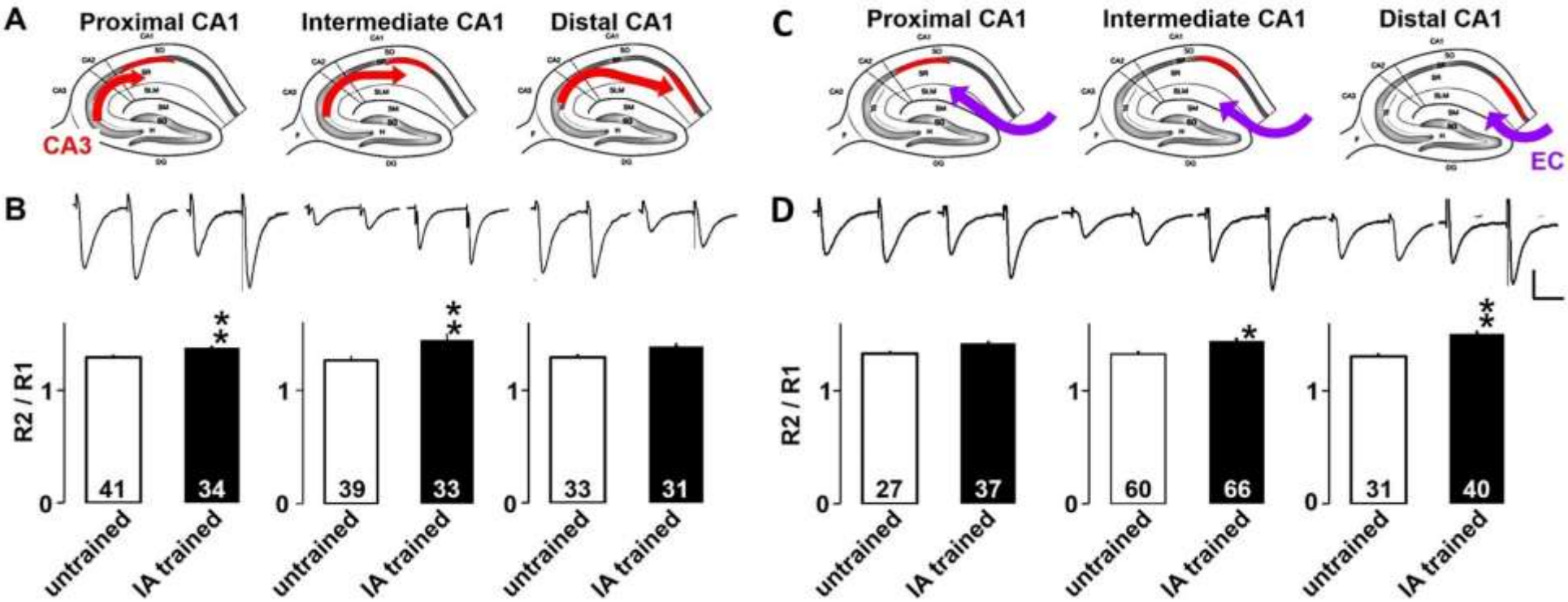

Fig. 4. Pre-synaptic plasticity at CA3-CA1 and ECIII-CA1 synapses. (A) The proximal, intermediate and distal CA1 subfields of the dorsal hippocampus where PPR of CA3-CA1 synapses were recorded. (B) IA training significantly increased PPR at CA3-CA1 synapses in the proximal and intermediate regions. A higher ratio suggests a lower release probability of glutamate. The upper insets show representative traces. The number of cells in each group is shown at the bottom of each bar. (C) The proximal, intermediate and distal CA1 subfields of the dorsal hippocampus where PPR was recorded at ECIII-CA1 synapses. (D) IA training significantly increased PPRs at ECIII-CA1 synapses in the intermediate and distal regions. A higher ratio suggests a lower release probability of glutamate. The upper insets show representative traces. The number of cells in each group is shown at the bottom of each bar. Vertical and horizontal scale bars represent 50 pA and 50 ms, respectively. Error bars indicate + SEM. **P*<0.05, ***P*<0.01 vs untrained.

Diversity of Synaptic Plasticity along Dorsoventral Axis of CA1 Region

Based on different input and output connections, there are different functional roles of dorsal and ventral hippocampus.[22] Dorsal hippocampus processes spatial and contextual memory[23,24], whereas ventral hippocampal is concerned with stress responses and emotional behavior.[25,26] Our recent study found that IA training increased AMPA receptor-mediated responses at dorsal CA3-CA1 synapses in both hemispheres, whereas ventral CA3-CA1 synapses did not show the plasticity in either hemisphere. Further nonstationary fluctuation analysis revealed that IA training significantly increased the postsynaptic number of both open AMPA receptor Na^+ channels and GABA receptor Cl^- channels at dorsal CA1 synapses without affecting single channel current, whereas the training did not affect the ventral CA1 synapses. By analyzing self-entropy level to quantify the learning-induced synaptic diversity, we found that contextual learning increased self-entropy levels in dorsal CA1 synapses but not in ventral CA1 synapses. Since learning performance was clearly impaired by the bilateral microinjection of plasticity blockers in dorsal, but not ventral CA1 subfields, contextual learning differentially induced synaptic plasticity at dorsal CA1 synapses probably encoding different traces of contextual memory.[4]

Diversity of Synaptic Plasticity along Proximodistal Axis of CA1 Region

Hippocampal CA1 region can be subdivided into distal, intermediate and proximal CA1, showing functional heterogeneity. Proximal CA1 cells have higher spatial specificity and play a crucial role in spatial memory formation. In contrast, distal CA1 cells process non-spatial, object-related information, such as odor-based memory.[27] The integration of object-related information with spatial and temporal contexts induced sequence coding activity, which is highest in the intermediate CA1 region.[28] We recently reported that IA training induced postsynaptic plasticity at CA3-CA1 synapses, but not at ECIII-CA1 synapses, in the proximal CA1 region, where spatial information is predominantly processed. In contrast, the training strengthened the ECIII-CA1, but not the CA3-CA1 synapses in the distal CA1 region, where non-spatial, object-related information processing predominantly occurs. However, unlike those two regions, the intermediate CA1 region showed postsynaptic plasticity at both CA3-CA1 and ECIII-CA1 synapses after IA training.[6] The intermediate CA1 region receives TA pathways from both the lateral and medial EC; therefore, many pyramidal neurons in this region seem to integrate spatial, temporal, or object-related information.[28] Consequently, it is possible that synaptic plasticity in this region might be necessary for establishing contextual memory by assembling information from Schaffer's collaterals and two TA pathways.[6]

Bilateral microinjection of plasticity blocker into the three CA1 regions revealed the functional role of this diverse postsynaptic plasticity. Blockage of plasticity in the proximal or intermediate CA1 regions clearly prevented learning. In contrast, blockage in the distal CA1 region attenuated, but did not completely prevent learning. Therefore, synaptic plasticity in proximal and intermediate region may play a more important functional role in IA learning.[6] Taken together, the present and previous results suggest a functional heterogeneity along the proximodistal axis.

Episode-specific Diversity of Synaptic Plasticity

In our recent study in which rats were allowed to experience one of the four episodes: restraint stress, social interaction with a female or male, or observation of a novel object, we recorded multiple-unit firings of CA1 neurons. By extracting and analyzing super-burst firings,

silent periods and ripple firing events for each episode, it was found that experience clearly diversified multiple features of individual ripple firings in an episode-specific manner, sustained for more than 30 min in the home cage. Moreover, by analyzing both miniature excitatory post synaptic current (mEPSC) and miniature inhibitory post synaptic current (mIPSC) events in identical CA1 neurons, we further found episode-induced synaptic plasticity and created episode-specific diversity 40 min after the episodic experience.[5] Since episode promoted plasticity at excitatory and/or inhibitory synapses with episode-specific manner, the diversified synapses may mediate to create diversified ripple-firings after episode.

Conclusion

Although many studies have shown that contextual learning induces synaptic plasticity at CA1 neurons with synapse-specific diversity, input-specific diversity, subregion- or subfield-specific diversity, episode-specific diversity, etc., there are only a few studies which revealed functional significance of these diverse patterns of learning-induced synaptic plasticity. To determine whether diversity of learning-induced synaptic plasticity is the underlying mechanism of contextual memory formation, it demands further studies focusing on investigation of its functional correlation to learning performance. New approaches like optogenetic techniques and cell-specific self-entropy analysis are highly promising candidates for better understanding of diversity of learning-induced synaptic plasticity. Upgrading the knowledge of how synaptic diversity participates in memory formation will be helpful in diagnosing the pathology of hippocampal-related neurodegenerative diseases like Alzheimer's disease and exploring the potential targets for treatment.

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