博士論文概要

Cluster-type topographic neuronal organization of the hippocampus for encoding memory

(記憶における海馬神経細胞のクラスター型構築)

人間総合科学研究科感性認知脳科学専攻
Cho Jiyeon (チョジヨン)

Memory is vital to our daily existence. It is thought that memory is initially processed within the hippocampal-entorhinal cortex before being consolidated permanently in the neocortex. A large number of studies have performed electrophysiological, genetic, molecular, and anatomical studies to assess hippocampal-dependent memory processes and have provided substantial evidence that the hippocampus is dedicated to long-term memory. Nonetheless, understanding how memory is anatomically represented within the hippocampal neuronal network is still lacking.

Based on electrophysiological unit recording studies, it has been suggested that the functional organization within the hippocampal network is widely distributed (i.e., random organization), since neighboring cells do not necessarily encode for similar information (e.g., adjacent place fields). However, several studies provided contrary evidence, namely, that a topographic functional organization may exist, by showing that the ensemble of neighboring hippocampal cells could have overlapping place fields (Eichenbaum et al. 1989; Hampson et al. 1999; Wood et al. 2000; Leutgeb et al. 2005). Recent anatomical studies also support the existence of a topographic organization in the hippocampus, by showing that a preferential occurrence of biased synaptic interconnectivity between neighboring cells (Deguchi et al. 2011; Takahashi et al. 2012; Druckmann et al. 2014).

The consensus that the hippocampus is randomly organized may or may not be correct, due to technical limitation of conventional techniques used to investigate

hippocampal functional organization, such as electrophysiological recording methods. To reveal functional organization, a sufficiently large number of hippocampal cells or cell populations should be observed at the same time. However, electrophysiological methods only allow a very limited number of simultaneously recorded cells (typically < 100 cells) as well as narrow recording distance (< 50 μm). Thus, it is challenging to observe the spatial distribution of all active cells in an entire population of the hippocampus under electrophysiological investigation.

IEGs are immediately expressed upon presentation of a stimulus in a wide range of brain areas including the hippocampus and are activity-dependent (the higher the neuronal firing rates, the higher the expression). Thus, IEGs (such as *Arc*, *c-fos*, and *Zif268*) have been used widely as a marker of neuronal activation. Further, mapping the spatial and temporal distribution of IEG expression (IEG imaging methods) allow one to detect all active cells simultaneously in an entire neuronal population at the cellular level. Thus, the use of the IEG imaging method has allowed the examination not only of neuronal activity but also the functional neuronal organization in the hippocampus, as well as whole brain areas. In this regard, IEG imaging methods have emerged as an alternative way of mapping functional organization, in addition to electrophysiological methods. Indeed, a few studies reported positive evidence supporting a topographic functional organization in the hippocampus (Guzowski et al. 1999; Vazdarjanova and Guzowski 2004). However, these studies were still limited since they only partially observed active populations, not the entire hippocampus.

Using IEG (Zif268) imaging methods, our laboratory has investigated the spatial distribution of the entire, active neuronal populations in the hippocampus following several hippocampus-dependent behavioral tasks. Our laboratory found new evidence of a topographic functional organization in the hippocampus, by showing that dorsal hippocampal pyramidal cells formed distinct ensembles, which were spatially clustered,

with a few , active Zif268 neighboring cells following either spatial (Nakamura et al. 2010; Pavlides et al. 2019) or episodic memory task (sequential order olfactory discrimination; Cho et al. Submitted). Our previous findings suggest that a cluster-type organization may exist in the hippocampus for memory. Given these findings, I propose that a cluster-type topographic functional organization is a principal cellular component of encoding memory in the hippocampus and a cluster is a basic unit of functional organization.

The first aim of the present studies was to determine whether a cluster-type functional organization is indeed a universal anatomical arrangement in the hippocampus for memory. To address this question, hippocampal functional organization was determined in animals following contextual fear conditioning, which is a well-established, hippocampus-dependent learning. Long-Evans male rats were fear conditioned and tested for long-term, contextual fear memory, 24 h later. The rats were isolated for 1.5 h, to suppress basal IEG expression, and sacrificed. Their brains were processed for Zif268 immunohistochemistry. Immediate early gene (Zif268) imaging methods were used to investigate the topographic distribution pattern of neuronal activation in the dorsal hippocampus. The distribution of Zif268 positive cells including the total number of cells and clusters, the average number of cells within a cluster, and the distance between clusters were calculated and analyzed to determine the cellular arrangement of hippocampal cells for contextual fear memory. The spatial distribution of Zif268 IR (+) cells were further analyzed using distance-based methods, to determine whether the observed patterns (e.g., clustering) are not occurred by chance. In brief, the results showed a significant increase of Zif268 expression in the CA1 field of the hippocampus following contextual fear conditioning and Zif268 positive cells in fear conditioned animals are distinctly spatially clustered with a few (2 ~ 3) adjacent cells at a significant level (i.e., not occurred by chance). The results indicate that contextual fear memory is also organized in the spatial form of cell clusters, similar to spatial and episodic memory we tested

previously. Although the present results support my hypothesis that a cluster-type organization occurs universally in the hippocampus for encoding memory, practical evidence that shows how clusters may be established and maintained is still missing.

Previously, I have shown that post-learning sleep is critically involved in hippocampus-dependent memory consolidation (Cho et al. 2018). Further, our laboratory has demonstrated that hippocampal cells involved in the original experience are reactivated again in sleep (Pavlides and Winson 1989). Disrupting the reactivation of cells in sleep gave rise to memory impairment (Yang et al. 2014). Thus, I hypothesize that the reactivation of hippocampal cells in post-learning sleep may contribute to cluster formation. Further, if the cell clusters identified in the hippocampus are indeed a basic, functional unit of memory, preventing post-learning sleep should negatively affect cluster formation. As of now, there are no studies that investigate whether the cells or cell groups which may be activated in sleep following learning experiences have a specific pattern of spatial distribution such as clustering. Thus, investigating cluster formation following post-learning sleep manipulation is the first study of showing possible physiological mechanisms that underlie a topographic organization in the hippocampus. Accordingly, the second aim of the present studies was to investigate whether post-learning sleep affects cluster formation. To address this question, hippocampal functional organization was determined following contextual fear conditioning in animals which were kept awake or allowed to sleep. Long-Evans male rats were fear conditioned following which they were kept awake or allowed to sleep for a 4 h time window, which I (Cho et al. 2018) and others (Graves et al. 2003; Smith and Rose 1996) previously confirmed to be critical for memory consolidation. The next day, animals were tested for their long-term fear memory and their brains processed for Zif268 imaging analysis. Spatial distribution of Zif268 positive cells in the dorsal hippocampus was verified using IEG imaging methods and further analyzed using distance-based methods, to determine whether the observed patterns (e.g.,

clustering) are not occurred by chance. The results show that sleep deprivation impairs not only long-term fear memory but cluster formation in fear conditioned animals. Sleep animals showed a distinct pattern of spatial clustering with a few (2 ~ 4) adjacent Zif268 positive cells, while Awake animals showed less clustering. The present results strengthen my hypothesis that a cluster-type organization exists in the hippocampus and also indicate that post-learning sleep may be involved in the strengthening of cluster formation.

Following the proposal by Semon (1920) of memory engram, many studies have attempted to reveal where and how memory is internally represented in the brain. Indeed, recent studies reported that a specific neuronal population in the hippocampus is simultaneously activated following spatial or episodic events (Pastalkova et al. 2008; Ziv et al. 2013). Additionally, it was shown that these cell populations or ensemble activities are indeed associated with memory (Garner et al. 2012; Liu et al. 2012; Ramirez et al. 2013; Matsuo 2015). However, none of the studies have investigated the spatial distribution patterns of these cells or cell populations. As described earlier, most studies, which investigated how the neuronal network in the hippocampus is functionally organized for memory, indicate that the hippocampus is not topographically but rather randomly organized. However, a few studies suggested that it is topographic. The lamellar-type organization proposed by Andersen et al. (1971) offer plausible explanation for a topographic organization in the hippocampus. Recent studies support the feasibility of this idea, by showing that the projections to either the CA1 or CA3 field were not evenly distributed (Brivanlou et al. 2004; Druckmann et al. 2014). The present results are in agreement with these observations and also offer strong evidence that a specific type of functional organization exists in the hippocampus – that is cluster-type organization.

Besides, as mentioned earlier, it has been suggested that the hippocampal ensemble activities during learning in the awake state are reactivated during post-learning sleep, then reinstated during retrieval (Ólafsdóttir et al. 2018) and the beneficial

effect of post-learning sleep depends on the neuronal reactivation of cell groups which were activated during learning (Diekelmann 2014). The present results support this idea, by showing that disrupting post-learning sleep gave rise to less clustering as well as memory impairment, compared to undisturbed sleep conditions following a learning experience.

In summary, the present results extend the previous findings in our laboratory that a cluster-type topographic neuronal organization indeed occurs in the hippocampus to encode memory thus confirming the proposed hypothesis that a cluster-type topographic functional neuronal organization is a principal cellular arrangement in the hippocampus. Further, the present results suggest that cell clusters may be established during post-learning sleep.

However, a number of important questions remain unanswered which would require further investigation, to precisely and thoroughly understand how a cluster-type organization or cell clusters mediate memory processing in the hippocampus. Using an optogenetic approach in combination with pharmacogenetic (such as tTA system) and recent *In vivo* calcium imaging methods may break new ground and allow us to gain a better understanding of how the hippocampus is functionally organized to encode memory.

Although memory is one of the primary brain functions in both animals and humans, we still know little about how it is implemented in the brain. Hence, adding new knowledge of a cluster-type functional neuronal organization could help us understand how memory is processed in the brain and expand our thinking about how the neuronal network in the brain works together to execute higher cognitive functions such as memory.