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研究課題名（和文）Hippocampal Organization of Spatial Map

研究課題名（英文）Hippocampal Organization of Spatial Map

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研究成果の概要（和文）：本課題では、異なるタイプの記憶の基盤となる海馬の機能的組織化（functional organization）と睡眠による影響、空間及び物体記憶に果たす内側（MEC）、外側嗅内皮質（LEC）の役割について解析した。恐怖記憶及び順序記憶に伴って活性化される神経細胞は海馬内にクラスター状に分布していることが明らかとなったことから、クラスター状の機能的組織化が海馬における記憶形成の基本であると結論された。また、光遺伝学的手法によりMECの神経活動を抑制すると空間記憶、LECの抑制では物体記憶に各々障害が見られることがわかった。これらの神経活動抑制が、海馬の機能的組織化に及ぼす影響についても解析した。

研究成果の学術的意義や社会的意義
It is known that the hippocampus is involved in memory. Very little is known of how memory is organized in this structure. We had previously shown a cluster-type organization for the encoding of spatial memory. The current studies provide evidence that all types of memories are encoded in clusters.

研究成果の概要（英文）：We undertook a set of experiments to determine: 1) functional neuronal organization in the hippocampus for the encoding of different types of memories; 2) effects of sleep on functional organization; and 3) possible differential role of medial and lateral entorhinal cortex (MEC, LEC) in spatial and object memory. We found that indeed, there was a formation of clusters in fear conditioned animals, in comparison to non-shocked controls. Further, sleep following conditioning enhanced cluster formation. In a second study, we found a similar cluster-type organization to encode sequential memory, suggesting that cluster-type is a fundamental principle for memory formation in the hippocampus. In a third study, we found that optogenetic suppression of neuronal activity in the MEC disrupted spatial memory while suppression of activity in LEC disrupted object memory. Effects on hippocampal neuronal activity of such suppression on hippocampal clustering are now being tested.

研究分野：Neuroscience

キーワード：hippocampus　med. entorhinal cortex　lat. entorhinal cortex　immediate early genes　sleep　cluster organization　memory　optogenetics

１．研究開始当初の背景 **Background**

It is well known that the hippocampus plays a major role in spatial navigation and episodic memory - that is, memory for discrete events. This has been based both on lesion/pharmacological, as well as unit recording studies. Following the discovery of 'place cells' in the hippocampus (O'Keefe and Dostrovsky, 1971), a question that has perplexed hippocampal neuroscientists is 'what is the functional neuronal organization within the hippocampus to encode memories'. Most recording studies have reported that place cells encoding for a specific part of space are not topographically organized, since adjacent cells do not necessarily have adjacent place fields. Thus, it has been concluded that the spatial or cognitive map is organized in a random fashion. This is very different than what has been reported in primary sensory cortex (e.g., visual cortex), where the neuronal organization is columnar. A major problem with recording studies is that they are limited to a few simultaneously recorded cells which does not allow a broad view of the spatial map. A similar observation (that is random organization) has been made for other types of memories, although a handful of studies have reported that some type of topographic organization may exist.

We have adapted a different approach to investigate functional neuronal organization by using activity dependent immediate early genes (IEG's; Zif268; Homer1a). IEG's are activated immediately by various stimuli and they are activity dependent - that is, the higher the cellular activity the higher these IEG's accumulate in cells. This allows the mapping of activity in the entire hippocampus, indeed in the entire brain.

Using IEG's we have previously shown that in animals performing a spatial task, neurons in the dorsal hippocampus formed clusters of a few active cells, spread across the dorsal CA1 and CA3 hippocampal fields (Nakamura, et al., 2010; Pavlides, et al., 2019).

The studies performed under the current funding asked the question: is there a topographic organization in the hippocampus for the encoding of different types of memories? Further, we tested whether sleep plays a role in the consolidation of memories. Lastly, we tested the hypothesis that the MEC/LEC play differential roles in spatial/object memory, using optogenetic inhibition of these areas. This will allow us to determine how entorhinal cortex may be providing the inputs for cluster formation. The findings from these studies have the potential of transforming our view of how the hippocampus is organized to encode for long-term storage of memory.

２．研究の目的 **Aim of the study**

We performed three studies to determine: 1) functional neuronal organization in hippocampus for encoding of different types of memories (contextual fear and sequential memory); 2) effects of sleep on functional organization; and 3) the role of the MEC/LEC in different - spatial vs object - types of memories and how these hippocampal afferents may participate in hippocampal functional organization.

In the first study, we tested the hypothesis that a similar organization (i.e., clusters) must exist in the hippocampus to encode all types of memories. Besides spatial information, the hippocampus is also involved in contextual fear memory. Animals were fear conditioned or used as non-shocked controls. 24h later, they were tested for fear memory and their brains processed for Zif268 expression. In a second part of the study, experimental animals that underwent conditioning and controls, were allowed to sleep or kept awake for approximately 4h, tested 24h later and their brains processed for Zif268 expression.

In a second study we tested functional organization in the hippocampus for sequential order memory, for which the hippocampus is also involved. Similar to the first study, we tested the hypothesis that a principle mechanisms of topographic organization must be operating in the hippocampus for all types of memories.

In a third study, we used optogenetic stimulation to suppress neuronal activity either in the MEC or LEC while the animals underwent exposure to a shock. They were then tested 24h later either for place or object memory. Previously, studies using mainly lesions suggested that the two entorhinal subdivisions played distinct roles in memory.

３．研究の方法 **Methods**

Study 1.
Experiment 1. Two groups of animals were used. Group 1 animals were contextual fear conditioned while Group 2 animals were used as non-shock controls. 24h later, the animals were exposed to the same context (no shocks); kept in an isolation chamber of 90min (peak Zif268 expression); sacrificed and their brains processed immunohistochemically for Zif268 expression. Hippocampal sections were captured at high magnification on a microscope and possible topographic neuronal organization of Zif268 expressing cells was analyzed.

Experiment 2. Following conditioning (or controls), animals were allowed to sleep for 4hrs or were kept awake during that time. There were four groups: 1) fear conditioned/sleep; 2) fear conditioned awake; 3) non-shocked controls sleep; and 4) non-shocked controls awake. 24h later, animals were placed in conditioning chamber, sacrificed after 90min and their brains processed for Zif268 expression.

Study 2.
Animals were conditioned in a sequential order discrimination task. They were presented with a set of seven different odors, and then presented with 2 odors in a probe. They had to chose the odor presented first in the sequence in order for them to receive a reward. Control animals received a reward regardless of which odor they chose - thus they did not have to remember a sequence. Animals were trained to a criterion of 80% correct on three consecutive trials for two days. Once they reached criterion, they were allowed to rest for 2d. They were then presented with 10 trials of the task and if they performed to criterion, they were placed in an isolation chamber for 90min, sacrificed and their brains processed for Zif268 expression. The brains were then analyzed for possible topographic organization.

Study 3.
Animals were injected with a virus (AAV encoding NpHR3.0) either in the MEC or LEC. Three weeks later they were also implanted with a probe for optogenetic stimulation and an electrode for recording unit activity. They were then trained on a plus maze which had an object placed at the end of one arm that was electrified to deliver a shock. Food was placed on top of the object which animals (that had been food deprived), were trained to retrieve. On the day of the experiment, animals underwent three trials during which they approached the object to retrieve food. Upon touching the object they would get shocked (0.3mA/1sec). One group of animals was optogenically inhibited during the acquisition trials, while a second group was not optogenetically stimulated. The animals were then tested 24h later for memory of the shock. For one group of animals the object was placed on the same arm as training, to test for object memory. For another group, the object was moved to a different arm, thus testing for place. Time to retrieve food and staying on the arms was recorded. The brains of these animals were also examined for effects of optogenetic stimulation on neuronal inhibition in MEC and LEC, using Zif268 expression.

４．研究成果 **Results**

Study 1. *(Cho and Pavlides, 2022. Hippocampal cellular functional organization for fear memory: effects of sleep. Hippocampus, 32, 839-856).*

A) Similar to our previous studies of spatial memory (Nakamura, et al., 2010; Pavlides, et al., 2019), we found a topographic organization (clusters) in the dorsal hippocampus of animals contextually fear conditioned, especially in the CA1 hippocampal field. This was true for both total number of cells as well as number of clusters. Surprisingly, clusters were also observed in the control animals, although to a lower level. This is most likely due to the fact that contextual fear memory has both a fear component as well as a spatial component.

B) In a second set of experiments, we found that sleep (in comparison to animals kept awake) following conditioning had an enhancing effect, on cell clusters. This supports our previous findings (Pavlides, and Winson, 1989; replicated by a number of labs) of a replay of neuronal activity in sleep following awake learning. We had previously also shown enhanced IEG expression in sleep following learning (for review, see Pavlides and Ribeiro, 2003).

Study 2. *(Cho, Arai and Pavlides, Submitted. Hippocampal functional neuronal organization for the encoding of episodic memory).*

Proof positive that 'topographic' cellular organization is a fundamental principle for memory encoding in the hippocampus, it is necessary to show that all functions involving this structure follow the same principle. We found that indeed, in animals performing a sequential order discrimination task (previously shown to be hippocampus dependent), there were clusters of active cells in the dorsal CA1 and CA3 fields. This was not the case in animals that performed a simple discrimination task which is not hippocampus dependent.

Study 3. *(Arai, Sypniewski and Pavlides, In Preparation. Differential role of medial and lateral entorhinal cortex for spatial and object memory).*

It has been reported previously that MEC/LEC play different roles in information processing - the former being more tuned to spatial, while the latter more tuned to object memory. Most of these studies, however, used lesions to permanently remove these areas. In the present study we showed that inhibiting MEC/LEC specifically at the time of memory acquisition with optogenetic stimulation (aimed precisely at each subregion), that indeed the MEC processes spatial while the LEC processes object information.

This will allow us to optogenetically stimulate (instead of inhibiting) unit activity to determine how the entorhinal inputs may play a role in the formation of clusters in the hippocampus and whether different hippocampal cells may form unique clusters or the same cells can switch function depending on task at hand. These are ongoing experiments that would require further analysis for conclusive results.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　1件／うちオープンアクセス　2件）

１．著者名 Pavlides, C., Donishi, T., Ribeiro, S., Mello, C.V., Blanco, W., and Ogawa, S.	４．巻 161
２．論文標題 Hippocampal functional organization: A microstructure of the place cell network encoding space.	５．発行年 2019年
３．雑誌名 Neurobiology of Learning and Memory	６．最初と最後の頁 122-134
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.nlm.2019.03.010.113	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Cho, J., Sypniewski, K.A., Arai, S., Yamada, K., Ogawa, S., and Pavlides, C.	４．巻 25
２．論文標題 Fear memory consolidation in sleep requires protein kinase A.	５．発行年 2018年
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掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/lm.046458.117.	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計8件（うち招待講演　1件／うち国際学会　7件）

１．発表者名 Arai, S., Sypniewski, K.A. and Pavlides, C.
２．発表標題 The role of the medial and lateral entorhinal cortex in place and object fear memory.
３．学会等名 The 44th annual meeting of the Japan Neuroscience Society（国際学会）
４．発表年 2021年

１．発表者名 Arai, S., Sypniewski, K.A. and Pavlides, C.
２．発表標題 The role of the medial and lateral entorhinal cortex in memory.
３．学会等名 The 43th Annual meeting of the Japan Neuroscience Society（国際学会）
４．発表年 2020年

１．発表者名
Arai, S., Sypniewski, K.A. and Pavlides, C.

２．発表標題
The Role of the Medial and Lateral Entorhinal Cortex in Episodic Memory

３．学会等名
Tsukuba Global Science Week 2020 Digital Poster Session（国際学会）

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2020年

１．発表者名
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Hippocampal Functional Organization in Contextual Fear Memory Task.

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４．発表年
2019年

１．発表者名
Pavlides, C. Cho, J. Arai, S. and Sypniewski, KA.

２．発表標題
Hippocampal functional neuronal organization for spatial and episodic memory.

３．学会等名
12th FENS Forum of Neuroscience（国際学会）

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2020年

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2018年

１．発表者名 Arai, S. and Pavlides, C.
２．発表標題 Hippocampal Functional Organization in Fear Memory.
３．学会等名 The 30th meeting of the Japanese Society for Behavioral Neuroendocrinology
４．発表年 2019年

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２．発表標題 Memory Consolidation in Sleep
３．学会等名 The Third Sino-Japan session: Symposium on the Frontier of Behavioral Neuroendocrinology.（招待講演）（国際学会）
４．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関