Mechanisms of memory consolidation in sleep

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研究成果の概要（和文）：本研究では、 睡眠中に恐怖記憶が固定される際に、様々な遺伝子カスケード（MAPK, PKAなど）がどのようなタイムコースで活性化されるのかについて解析した。その結果、文脈的恐怖条件づけ直後の睡眠中に海馬でPKAがリン酸化されていることが明らかになったものの、最初の4時間と次の4時間の間に差はなかった。睡眠中の動物で海馬の背側部のPKAのリン酸化を薬理学的に阻害すると恐怖記憶に障害が起こるのに対し、睡眠剥奪動物でPKAを活性化すると、睡眠剥奪による恐怖記憶の阻害が見られず正常に記憶が固定された。さらに睡眠中のPKA産生に果たすsharp wave ripplesの役割について検討を進めた。

研究成果の概要（英文）：In the proposed studies we tested whether various gene cascades (e.g., MAPK, PKA) are activated specifically in sleep for the consolidation of fear memory, along with the time-course of the activation. Of these, we found that there was an increase in PKA phosphorylation during sleep in the hippocampus of contextual fear conditioned animals, however, the results approached but were not statistically significant. We did not find a differential time-course of PKA activation at the 0-4h vs 4-8h time points. Inhibition of PKA in the dorsal hippocampus produced a disturbance in fear memory, while activation of PKA in sleep-deprived animals reversed the sleep-deprivation suppression of fear memory. We are currently testing whether sharp wave ripples (SPWr), which occur during learning and in sleep, modulate memory consolidation by their effects on PKA expression. We observed that eliminating SPWr suppressed PKA phosphorylation in the hippocampus and in turn disrupted long-term fear memory.

研究分野：　Behavioral Neuroscience

キーワード：　sleep　memory　hippocampus　amygdala　fear conditioning　sharp wave ripples　PKA phosphorylation

１．研究開始当初の背景

Sleep plays a significant role in memory consolidation. Further, the beneficial effects of sleep on memory are thought to occur within a time window of approximately 0-4h following a learning experience. Memory consolidation also involves gene expression and protein synthesis and a number of the genes involved have been identified. In the proposed studies we investigated mechanisms of memory consolidation in sleep. More specifically, we looked at possible gene cascades (e.g., cAMP/PKA) that may be occurring specifically in sleep for the consolidation of fear memory. In a first set of experiments we analyzed changes in PKA expression/phosphorylation following fear conditioning along with its time course and brain specificity. In a second set of experiments we pharmacologically suppressed/ enhanced PKA activation in sleep and determined the effects on long-term fear memory. In a final study (which was prompted by our previous findings), we are investigating whether sharp wave ripples (SPWr), which occur during learning and again in slow wave sleep (SWS) may be the physiological mechanism that activates PKA for the consolidation of memory.

２．研究の目的

The objective of the proposed studies was to determine possible molecular mechanisms underlying memory consolidation in sleep. There were two specific aims. The first of these was to investigate possible gene cascades that may be activated specifically in sleep for the memory consolidation to occur. Further, we aimed to determine a possible time course and brain site specificity for these molecular changes. A second objective was to directly manipulate one of the gene cascades, namely PKA, in sleep and see that it affected long-term fear memory. The findings from the first two studies led us to a third objective, namely, to investigate possible physiological conditions that may be occurring in sleep that may activate PKA and thus memory consolidation. One such mechanisms we believe is SPWr.

３．研究の方法

During the funding period, we performed three sets of experiments. (1) Initially we determined that sleep deprivation disrupts fear memory consolidation. This was a necessary first step to test our hypothesis. (2) We then tested the effects of fear conditioning in PKA expression along with brain site specificity and time course of the changes. (3) In a third set of experiments, we infused PKA antagonists/agonists directly into the dorsal hippocampus during sleep/awake to determine effects on long-term fear memory. In a final set of experiments we have been testing whether SPWr observed in sleep may affect PKA expression in the hippocampus and long-term fear memory. Further, we are examining whether enhancing PKA expression pharmacologically could reverse SPWr suppressive effects on fear memory.

４．研究成果

1. We confirmed that sleep deprivation suppresses long-term fear memory as measured 24h and 48h later. We observed that both rapid-eye-movement sleep as well as total sleep deprivation were effective in suppressing fear memory.

2. Contextual fear conditioning enhanced the pPKA/PKA ratio, however, the effects approached but did not reach statistical significance. This was mainly true for the dorsal, but not the ventral hippocampus, the amygdala or the frontal cortex. This suggests a site specificity for PKA activation, although again the results only approached significance. We did not observe a differential time course for the PKA enhancements in that they were seen at both the 0-4h and 4-8h intervals following fear conditioning. A possible explanation for these results is sampling of fresh brain tissue, which may not be totally accurate for the intended brain cite. We modified the tissue collection method by performing brain punches or micro-dissecting the hippocampus in subregions, however, the tissue sample was not sufficient to perform the Western blot analysis. This will have to be confirmed at a later time.

3. In the third set of experiments we directly manipulated PKA levels pharmacologically by injecting the PKA antagonists (Rp-cAMPs) directly into the hippocampus during sleep (0-4h interval) following contextual fear conditioning. In fear conditioned animals allowed to sleep, Rp-cAMPs suppressed fear memory at 24h retest, in comparison to vehicle injected controls. In

contrast, injection of the PKA activator (Sp-cAMPs) in sleep deprived animals reversed the sleep-deprivation induced memory deficits. The results from these studies have been published recently - Cho, et al. 2018).

4. In ongoing experiments, we observed that elimination of SPWr suppressed contextual fear memory at 24h. Further, it suppressed the pPKA/PKA ratio observed in sleep (0-4h) following fear conditioning Fig. 1. Injection of Sp-cAMPs reversed the SPWr elimination suppression of fear memory. These studies are almost completed and manuscript is being prepared for publication.

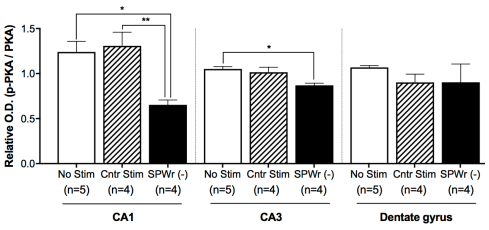

Fig. 1. Effects of SPWr elimination on PKA expression in the dorsal hippocampus SPWr suppression significantly reduced the pPKA/PKA ratio.

５．主な発表論文等

（研究代表者、研究分担者及び連携研究者には下線）

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６．研究組織

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