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研究課題名（和文）Revealing the Role of Postnatal Theta Rhythm in Establishing Memory Consolidation Mechanism during REM Sleep

研究課題名（英文）Revealing the Role of Postnatal Theta Rhythm in Establishing Memory Consolidation Mechanism during REM Sleep

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研究成果の概要（和文）：私たちは、発達中のマウスの学習と記憶が年齢に敏感であることを発見しました。生後 26 日（P26）の子マウスは、P17 と比較して、恐怖条件付けおよび想起（24 時間遅延）中のすくみ行動の有意な増加を示しました。　シータリズムの強度が低く、レム睡眠の時間が短い夢のないマウスは、そのような年齢に依存した学習と記憶の強化を示さなかった（未発表の結果）。　我々は、17 歳以降の新生仔マウスの睡眠パラメータ（EEG/EMG）と深部脳局所電場電位（LFP）を測定する技術を開発しました。　手術による子犬の成長・発育への影響は確認されておりません。

研究成果の学術的意義や社会的意義
Our results suggest that in neonatal mice, theta oscillations integrate various brain regions for fear learning and memory. Our established EEG/EMG/LFP recording method can be used to study mice models for developmental disorders in children.

研究成果の概要（英文）：We found that learning and memory are sensitive to age in developing mice. Mice pups at the age of postnatal day 26 (P26) showed a significant increase in freezing behavior during fear conditioning and recall (24 h delay) compared to P17. Dreamless mice that exhibit low strength theta rhythm and less time in REM sleep did not display such age-dependent enhancement of learning and memory (unpublished results). We developed techniques to measure sleep parameters (EEG/EMG) and deep brain local field potentials (LFP) in neonatal mouse pups aged starting P17. We confirmed no effect of surgery on the growth and development of pups.

研究分野：sleep memory

キーワード：Sleep　Memory　Theta rhythm　Dreamless

１．研究開始当初の背景 (Background): In mammals, sleep occurs in cycles of rapid eye movement (REM) and non-REM (NREM) sleep. Each sleep stage is characterized by local and global synchronous neuronal activities that play critical roles in memory consolidation (review by Jesse J. Langill, Frontier in cellular Neurosci. 2019). For example, during NREM sleep, boosting slow oscillations enhances memory (Marshall et al., Nature 2006), whereas in REM sleep, disrupting theta rhythm impairs long-term memory storage (Boyce et al., Science 2016). Dreamless is the right mouse model to investigate the REM-dependent memory consolidating mechanism because they sleep less in the REM stage and show a low theta power during REM sleep (Funato et al., Nature 2016).

At the neonatal stage, although mice spent most of their time in REM sleep, theta in the hippocampus mature by P26 (Wills et al., Science 2010). Moreover, hippocampal-dependent memories formed in the neonatal stage are rapidly forgotten (infantile amnesia). Long-term memory storage starts at P30 onwards (Akers et al., Learn Mem. 2012). Hence, storing long-term memory in the hippocampus develops near the theta maturation period in the neonatal stage suggesting a possible role of theta in establishing memory consolidation mechanism. However, REM-theta role in establishing a memory consolidating mechanism memory is unknown (Key specific question).

２．研究の目的 (Purpose): An intellectually disabled child typically shows weak memory storage. This study will recognize the developmental role of theta oscillation during REM sleep in establishing memory storage mechanisms. The normal development of sleep-dependent memory consolidation mechanism is unknown.

３．研究の方法 (Methods): All animal experiments were approved by the University of Tsukuba Institutional Animal Care and Use Committee. Animals were maintained in a home cage, which was maintained at 23 degrees Celsius ambient temperature and 12h light /dark cycle with ad libitum access to food and water following institutional guidelines.

Contextual fear conditioning: Experiments were performed on dreamless ($NALCN^{Drl/+}$) pups and their wild-type littermates when they stayed with their mother. The development of contextual fear memory was assessed at postnatal days P17, P26, P30, P35, and P40. The contextual fear conditioning protocol was the same as previously used for neonates (Akers et al., 2014). For strong contextual fear conditioning, five foot shocks (0.5 mA, 2 s duration, 1 min apart) were delivered after 2 min. Mice were removed from the chamber 30 seconds after the last shock. During the test, mice were placed in the chamber for 5 min. Behavior was recorded by overhead cameras. Freezing behavior (i.e., absence of movements except breathing) was measured using an automated scoring system.

EEG/EMG/LFP electrode implantation in neonates' brains: From each cage, 2-3 pups were used

for surgery and 2-3 pups from the same cage were used as naïve control. Neonatal pups at P10 were chronically implanted with EEG/EMG electrodes and one tetrode under 1-2% isoflurane anesthesia. A miniature EEG screw connected with a thin copper wire was gently inserted into the surface of the skull. A thin tetrode made after twisting four platinum-iridium wires was lowered in the hippocampus and kept above CA1. A pair of thin EMG wires are inserted into the neck muscle. After surgery, the pups were placed in a heater until they had fully recovered from the anesthetic and were then returned to the mother and littermates.

Measurement of sleep (EEG/EMG) and LFP: One week later the pups were connected to a recording cable for measurement of sleep (EEG/EMG) and local field potential (LFP) for 4 hours. All EEG, EMG, and LFP waveforms were processed offline using a MATLAB-based program. The sleep-wake stage was scored in 4 second epochs using the criteria mentioned (Kumar et al., 2015).

Novel object recognition (NOR) task: All mice pups were ear-notched at P10 for identification purposes. Surgery and naïve mice were handled and habituated to the behavioral chamber. Handling and habituation consisted of 2 min of handling followed by placement into the behavioral chamber for 5 min. NOR was divided into one training phase followed by a test phase. The training phase consisted of placing the mouse into the chamber containing two copies of a single object. The training phase lasted for 10 minutes after that the mouse was removed and placed back into the home cage. Following a delay period of 24 hours, mice underwent a 5-minute test phase where they were placed in a chamber containing one previously encountered object and a novel object. Total object exploration measurements considered the complete test phase lasting 5 min.

Body weight and brain weight measurement: Surgery and Naïve pup's body weight was measured at P10, P11, P12, P13, P14, P16, P27 and P60. At the end of the study brain weight of surgery and naïve mice were compared.

４．研究成果 (Results): We found age-dependent contextual fear learning in neonatal mice. During fear conditioning, P26/P30 mice displayed an increase in freezing behavior compared to P17 (Figure 1A and 1B). Also, in memory recall (1-day interval), P26/30 mice performed better (Figure 1C). These data identify a precise temporal window for the enhancement of fear learning and memory. However, dreamless mice did not display such enhancement in freezing behavior (unpublished results, data not shown). It is possible that lower theta spectral power in dreamless affected fear learning and REM-dependent memory consolidation (Boyce et al., 2016). However, we did not know whether dreamless neonatal pups show reduced spectral power of theta wave during REM sleep, previously reported in adults (Funato et al. 2016). Recording sleep and LFP in neonatal pups remains challenging. Hence, we first established a method to implant EEG/EMG and LFP electrodes in P10 pups and examine the effect of surgery on the normal growth of the

pups by measuring body weight regularly. Data from the neonatal and adult stage body weight of surgery and naïve mice indicated no significant effect of surgery on body weight (Figure 2 C). Brain weight of surgery and adult mice also did not significantly differ (Figure 2A and 2B). To investigate normal learning and memory formation in surgery and naïve pups, a novel object recognition task was performed.

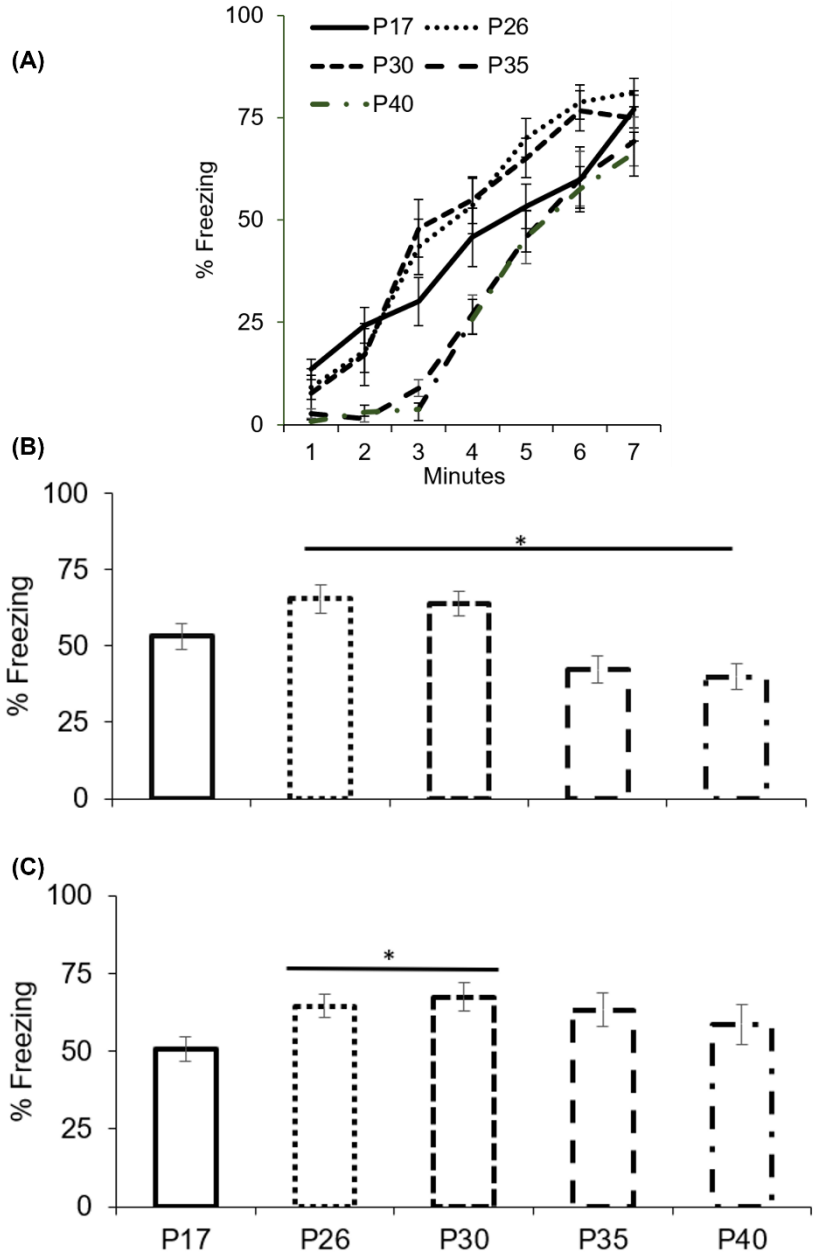

Fig 1. Fear learning and memory during postnatal development days P17 to P30. Freezing (A) 1 min bin (B) total inter shock interval period (5mins) during contextual fear conditioning (c) Freezing during (5min) recall with 24 hours delay

Both surgery and naïve pups spent significantly greater object interaction time with novel objects at the P19, P27, and P31 stages (Figure 3, upper naïve; bottom, surgery). This data suggests that there were no effects of surgery on the learning and memory of neonatal pups.

We also found that dreamless mice started exhibiting a change in EEG spectral power in the theta and delta range from neonatal age (unpublished results, data not shown).

Wild type mice (P17) EEG/EMG traces during sleep (NREM and REM sleep) and awake stage is shown in figure (Figure 4.)

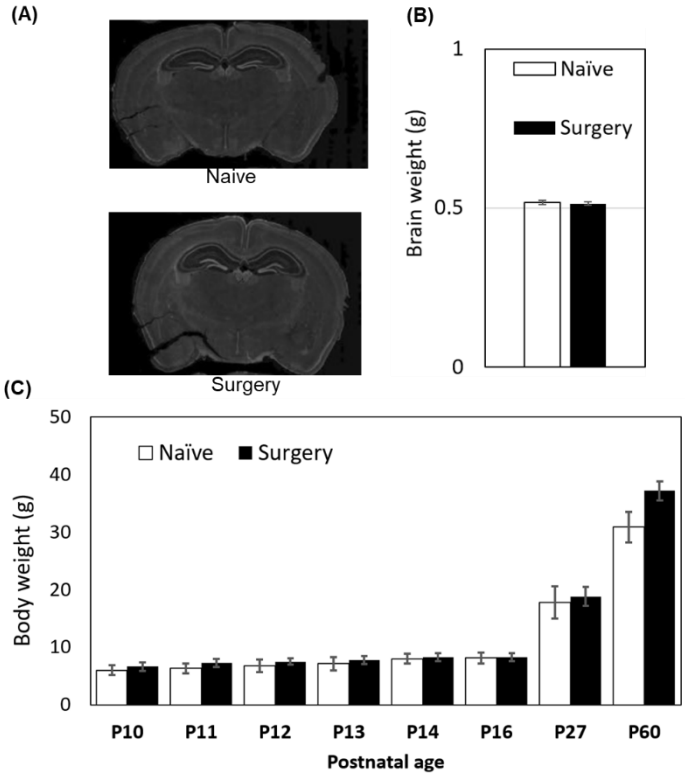

Fig 2. P10 surgery has no effect on (A) brain morphology, and (B) brain weight when they are adult, P60 (C) body weight.

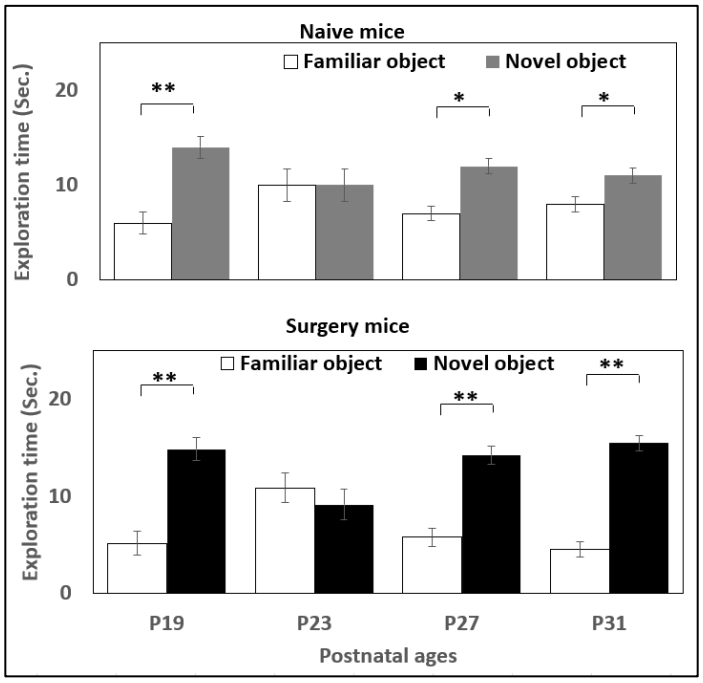

Fig 3. P10 surgery has no effect on novel object recognition memory (upper panel, Naïve mice; lower panel, surgery mice).

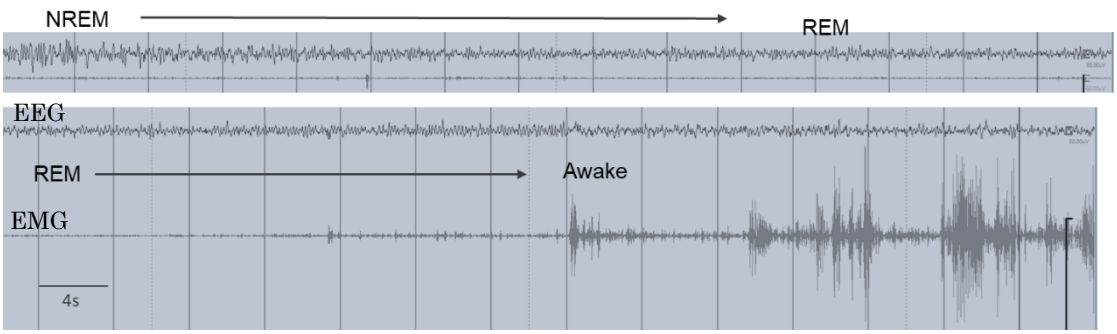

Fig 4. P17 mouse EEG and EMG traces during NREM, REM sleep and Awake stage

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　2件／うちオープンアクセス　0件）

１．著者名 Kim SJ, Hotta-Hirashima N, Asano F, Kitazono T, Iwasaki K, Nakata S, Komiya H, Asama N, Matsuoka T, Fujiyama T, Ikkyu A, Kakizaki M, Kanno S, Choi J, Kumar D, Tsukamoto T, Elhosainy A, Mizuno S, Miyazaki S, Tsuneoka Y, Sugiyama F, Takahashi S, Hayashi Y, Muratani M, Liu Q, Miyoshi C, Yanagisawa M, Funato H.	４．巻 612
２．論文標題 Kinase signalling in excitatory neurons regulates sleep quantity and depth	５．発行年 2022年
３．雑誌名 Nature	６．最初と最後の頁 512-518
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41586-022-05450-1	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Deependra Kumar*, Masashi Yanagisawa*, Hiromasa Funato*	４．巻 under review
２．論文標題 Sleep-dependent memory consolidation in young and aged brains	５．発行年 2024年
３．雑誌名 Aging Brain	６．最初と最後の頁 under review
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Deependra Kumar , Tomoyuki Fujiyama , Takeshi Kanda , Chika Miyoshi , Miyo Kakizaki , Masanori Sakaguchi , Hiromasa Funato , Masashi Yanagisawa
２．発表標題 NALCN haploinsufficiency causes memory loss in adolescent mice
３．学会等名 EMBO-Workshop, Molecular and Physiological basis of behavioral/cognitive defects in Neurodevelopmental disorders（国際学会）
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１．発表者名 Deependra Kumar, Tomoyuki Fujiyama, Takeshi Kanda, Chika Miyoshi, Miyo Kakizaki, Masanori Sakaguchi, Hiromasa Funato, Masashi Yanagisawa
２．発表標題 Learning and memory deficit in adult dreamless mice
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４．発表年 2022年

１．発表者名 Deependra Kumar , Tomoyuki Fujiyama , Takeshi Kanda , Chika Miyoshi , Miyo Kakizaki , Kaspar Vogt , Masanori Sakaguchi , Hiromasa Funato , Masashi Yanagisawa
２．発表標題 Uncovering the ontogenesis of sleep-dependent memory consolidation mechanism
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４．発表年 2023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
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８．本研究に関連して実施した国際共同研究の実施状況

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