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研究課題名（和文）Rescuing impaired learning in a mouse model for autism

研究課題名（英文）Rescuing impaired learning in a mouse model for autism

研究代表者
ＭＩＤＤＬＥＴＯＮ　ＳＴＥＶＥＮ（Middleton, Steven）

国立研究開発法人理化学研究所・脳神経科学研究センター・研究員

研究者番号：６０５２６７９７

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研究成果の概要（和文）：私はまず、ワーキングメモリーの課題に重要な2つの脳領域（海馬と前頭前野）について、健常マウスと自閉症モデルマウスの違いを詳細に明らかにすることを目的としました。健常なマウスでは、シャープウェーブ・リップルという脳内で起こる特定のタイプの活動が、学習に関して二重の役割を担っていることを発見しました。正解と不正解、この2つの領域をつなぐ脳内ネットワーク内の活動は、試行の結果に依存して系統的に変化する。しかし、仮説2に基づき、自閉症モデルマウスで同じ実験を行うと、ネットワークが過度に硬直し、鋭角波リップルがニューロン間の結合を適切に再構成できないことがわかり、学習が損なわれていることが説明された。

研究成果の学術的意義や社会的意義
This project demonstrates that by using large scale recording of neural activity and using the appropriate analytical techniques to understand the interaction of multiple brain regions, we can better understand disease states. Moreover, the results and follow up work aim to explore better treatments

研究成果の概要（英文）：This project aims to reverse the impaired learning in a mouse model of autism. To address this I first aimed to characterize in detail the differences between normal and the autism model mice, in two specific brain areas (hippocampus and prefrontal cortex) that are important in working memory tasks. Here, I found in line with the original first hypothesis, that in normal healthy mice, a specific type of activity that occurs in the brain called sharp-wave ripples have a dual role with respect to learning. When animals make a correct vs an incorrect decision, activity within the brain networks that connect these two areas change in a systematic way, dependent on the trial outcome. However, in line with hypothesis 2, when the same experiments are done in the autism model mice the networks appear to be overly rigid, meaning that sharp-wave ripples cannot reconfigure the connections between the neurons appropriately, explaining why learning is impaired. The remaining data is being analysed

研究分野：Neurosciecne

キーワード：Hippocampus　Prefrontal cortex　Memory　Autism　Sharp-wave ripples

１．研究開始当初の背景

The original idea behind this project stemmed from the fact that despite our ability to study and observe neuronal activity in unprecedented detail, using techniques such as high density recording and imaging, little progress has been made in reversing disease states in animal models. In most cases, research has focused on a specific brain area, where interventional approaches have been targeted which typically show little success in reversing disease related changes. This project uses a disease model of autism, which I previously demonstrated shows decreased rates of learning and poor performance in spatial working memory tasks. By using a combination of newly developed high density recording techniques and analytical approaches that can track changes across multiple brain regions, the idea was to understand exactly the nature of the changes in this autism model compared to healthy control subjects, and additionally to experimentally reverse these changes to observe improved behavioral performance in memory dependent tasks.

２．研究の目的

Since my original study on this transgenic mouse line identified changes in sharp-wave ripples, which are known to be extremely important for memory the objectives of the study were centered on these specific events. The first goal was to record activity from both the hippocampus (where sharp-wave ripples are generated) and the prefrontal cortex, which is known to play a key role in decision making and thus is important for the behavioral task used, as animals must make a choice. The hippocampus is also key to this task, as the spatial map that exists in the hippocampus is used both for navigation and basing the upcoming directional decision on, based on past experience. Specifically, I focused on neural activity around and during sharp-wave ripples in both structures to understand if in control animals there were differences in activity following a correct and incorrect behavioural choice. Once, this had been established the next step would be to perform the same experiments in the autism model mice and identify how these patterns of activity differed from the control mice. This would not only give a clear indication of the specific circuit level deficits in this particular transgenic disease model, but would also identify a potential target that might be experimentally manipulated to allow for a targeted rescue of neural activity, and ultimately might lead to improved memory performance in these animals.

３．研究の方法

High density neural activity was recorded from both the prefrontal cortex and hippocampus while animals were performing the spatial working memory task outlined in the original proposal. Specifically, this was carried out as animals were still learning the task, in order to establish the network activities that support learning and how this process is altered in the disease state. To analyze this data and better understand the underlying processes, a combination of principal component and independent component analysis was used to identify cell assemblies, both within single regions (the hippocampus and prefrontal cortex, separately), but also across regions. These were then tracked across the whole learning process, allowing us to identify the changes that occur in response to a correct versus incorrect behavioral choice. Additionally, we were also able to look at various properties of the cell-assemblies (a collection of neurons that are involved in a specific memory) formed such as size and stability, which we could then compare between the two groups of animals. Finally, as outlined originally by using the inhibitory DREADD system (which is a virally driven technique that allows for expression of a channel in specific sets of neurons that is activated by a drug (CNO) and allows for the modulation of neural activity) which was targeted to the hippocampus, neural activity was down-regulated with the aim of reversing some of the disease related changes associated with the SCN2A transgenic mouse line.

４．研究成果 research results

The experiments related to this project have revealed several key findings that support the initial hypotheses that were originally proposed. By recording from large populations of

neurons and LFP (Figure 1) from both the prefrontal cortex and hippocampus during the learning phase of the task, we can see clear evidence of differences in the way information is coded between the control and transgenic animals.

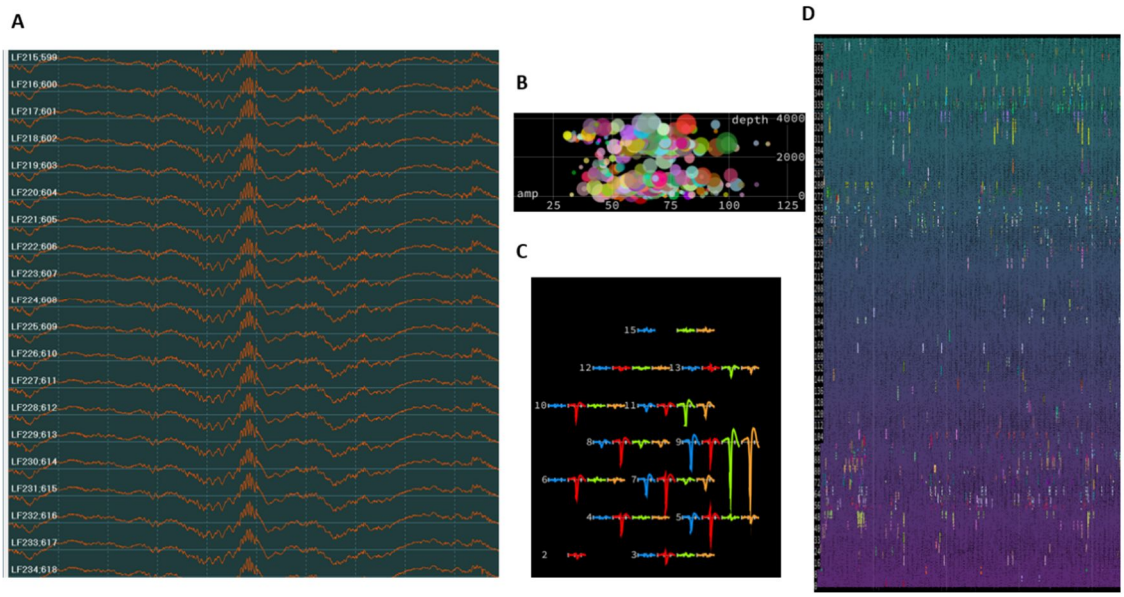

Figure 1 – **A)** local field potential activity recorded from hippocampus from approximately 20 recording sites, showing a sharp-wave ripple (center). **B**) An example of a recording from a single session, showing the distribution of neurons recorded across the 384 sites of the probe. **C**) Examples of units recorded across 14 sites from the same probe. **D**) A 200 ms trace of data showing all the recording sites, with the isolated neurons colour coded to show their activity and location on the Neuropixel probe.

Firstly, rather than simply looking at simple firing rates during the trial, but instead comparing how the firing rates vary over time during the trial, we can observe changes which may underlie the phenotype. These changes become more evident when the neurons activity is weighted by how variable across trials, to give less emphasis to those neurons whose firing rate remains constant throughout (Figure 2).

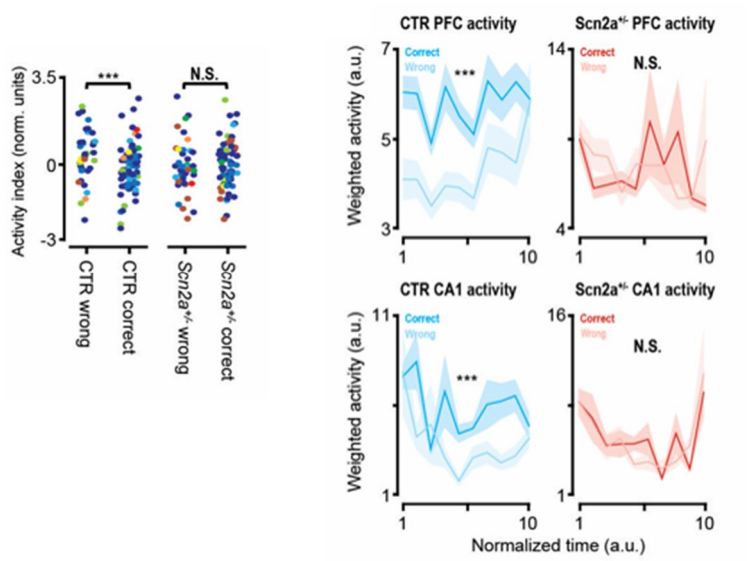

Figure 2 – Left panel shows all neurons activity as a function of trial outcome, with there being a clear difference in the control group depending on success. There was no significant difference in neuron activity in the SCN2A group. Right panel shows how both hippocampal and prefrontal neuron activity varies throughout the trial with their being a clear difference in the control group (blue) between correct and incorrect trials. This however wasn't observed in the transgenic group.

Further analysis used to identify cell assemblies has shown that it's evident that the neural networks are being shaped in a consistent manner, dependent on trial outcome in control

animals. What this means, is that correct trails are acting to reinforce the cell-assembly responsible for driving this task specific performance, whereas errors act to reconfigure the network, most likely in an attempt to improve future performance. However, when the same analysis was applied to the SCN2A animals these changes were not evident suggesting that the networks themselves are too rigid and incompatible with the fast changes required to support learning (Figure 3). In addition to this analysis demonstrated that there were clear differences in how sharp-wave ripples were synchronizing the neurons across regions, between the control and SCN2A groups.

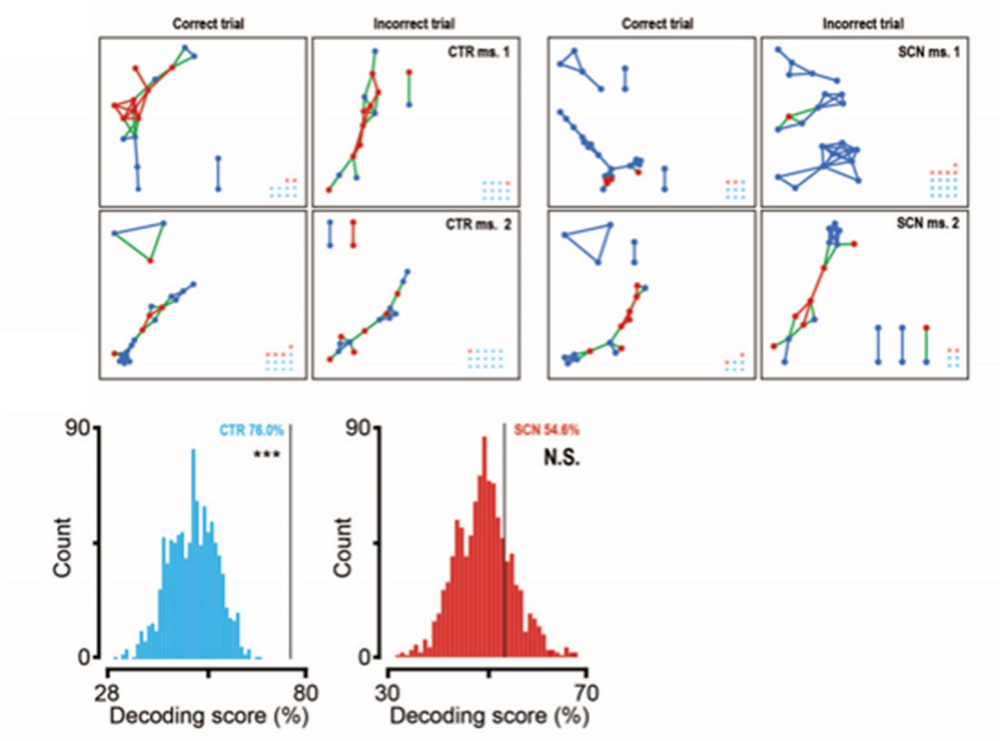

Figure 3- Top, shows examples from single trials showing cell assemblies that are active during that particular trial, with blue dots showing hippocampal neurons, red dots being prefrontal neurons, blue lines show a pair of highly correlated hippocampal neurons, red lines shows the same for prefrontal neurons and green lines represent a mixed PFC-HPC neuron pair. These connectivity maps are calculated per trial, and the differences between them are compared as a function of the trial success.
Below, this change in network connectivity is used to decode (predict) trial outcome. Obviously if these changes are reproducible depending on success or failure of the trial, then decoding with a high rate of success as an be seen for the control group (blue). However, if the way that the network reconfigures is random then decoding will be close to chance levels, which is the case for SCN2A animals (red).
All of these data, together with a more detailed analysis of sharp-wave ripples and the activity of the neurons during these periods led to the use of the inhibitory DREADD in the hippocampus, which was used to modulate the excitability of hippocampal neurons during the task. This data is currently being analyzed.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Chen Shuo、He Linmeng、Huang Arthur J. Y.、Boehringer Roman、Robert Vincent、Wintzer Marie E.、Polygalov Denis、Weitemier Adam Z.、Tao Yanqiu、Gu Mingxiao、Middleton Steven J.、Namiki Kana、Hama Hiroshi、Therreau Ludivine、Chevaleyre Vivien、Hioki Hiroyuki、Miyawaki Atsushi、Piskorowski Rebecca A.、McHugh Thomas J.	４．巻 586
２．論文標題 A hypothalamic novelty signal modulates hippocampal memory	５．発行年 2020年
３．雑誌名 Nature	６．最初と最後の頁 270～274
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41586-020-2771-1	査読の有無 有
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１．著者名 Middleton Steven J.、McHugh Thomas J.	４．巻 43
２．論文標題 CA2: A Highly Connected Intrahippocampal Relay	５．発行年 2020年
３．雑誌名 Annual Review of Neuroscience	６．最初と最後の頁 55～72
掲載論文のDOI（デジタルオブジェクト識別子） 10.1146/annurev-neuro-080719-100343	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 －

１．著者名 Adaikkan Chinnakkaruppan、Middleton Steven J.、Marco Asaf、Pao Ping-Chieh、Mathys Hansruedi、Kim David Nam-Woo、Gao Fan、Young Jennie Z.、Suk Ho-Jun、Boyden Edward S.、McHugh Thomas J.、Tsai Li-Huei	４．巻 102
２．論文標題 Gamma Entrainment Binds Higher-Order Brain Regions and Offers Neuroprotection	５．発行年 2019年
３．雑誌名 Neuron	６．最初と最後の頁 929～943.e8
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.neuron.2019.04.011	査読の有無 無
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 －

１．著者名 Middleton Steven J.、McHugh Thomas J.	４．巻 29
２．論文標題 Memory: Sequences Take Time	５．発行年 2019年
３．雑誌名 Current Biology	６．最初と最後の頁 R158～R160
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.cub.2019.01.024	査読の有無 無
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〔学会発表〕　計0件

〔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