様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

平成　２９　年　　６　月　　１　日現在

機関番号：　８２４０１
研究種目：　挑戦的萌芽研究
研究期間：　2015～2016
課題番号：　１５Ｋ１４３５７

研究課題名（和文）Optogenetic dissection of hypothalamic modulation of hippocampal memory

研究課題名（英文）Optogenetic dissection of hypothalamic modulation of hippocampal memory

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交付決定額（研究期間全体）：（直接経費）　　3,000,000円

研究成果の概要（和文）：海馬の求心性および遠心性は、学習および記憶における彼らの役割に関してよく特徴付けられている。 恐らく、最も理解されていない接続のいくつかは、視床下部と背側DGおよびCA2サブフィールドとの間のものであろう。 ここで我々は、これらの視床下部核から海馬への入力の正確な時間的制御を得るために上顆粒核を標的とする領域特異的Creドライバマウス系を使用する光学原性行動アプローチを使用した。 海馬におけるこれらの入力の操作は、CA2予測が社会的行動を変化させ、DG入力が空間的挙動を変化させる、海馬行動のタスク特異的調節を明らかにした。

研究成果の概要（英文）：The afferent and efferents of the hippocampus have been well characterized in regards to their role in learning and memory. Perhaps some of the least understood connections however, are those between the hypothalamus and the dorsal DG and CA2 subfields. Here we used an optogenetic behavioral approach that uses a region specific Cre-driver mouse lines targeting the supramammillary nucleus to gain precise temporal control of the inputs from these hypothalamic nuclei to hippocampus. Manipulation of these inputs in the hippocampus revealed task specific modulation of hippocampal behavior, with the CA2 projections altering social behavior and the DG inputs altering spatial behavior.

研究分野：　neuroscience

キーワード：　hypothalamus　hippocampus　social behaivor

１．研究開始当初の背景

(1) While the hippocampus is a crucial structure for spatial, contextual and episodic memory, the last thirty years has seen disagreement concerning its role in social behaviors in rodents (Thor & Halloway 1982, Kogan et al 2000; Bannerman et al 2002; von Heimendahl et al 2012). More recent data however has suggested that the small and largely unstudied CA2 region of the hippocampus may play a key role specifically in social memory and social aggression (Hitti & Siegelbaum 2014; Stevenson & Caldwell 2014; Wersinger et al 2007). Blocking CA2 pyramidal cell transmission leads to social memory loss (Hitti & Siegelbaum 2014) while deletion of the vasopressin 1b receptor, highly expressed in CA2, lead to deficits in social memory and a loss of aggressive behavior in male mice (Wersinger et al 2007,2008). One possible explanation for these findings is the fact that dorsal CA2 receives strong and specific input from two nuclei of the hypothalamus, the paraventricular nucleus (PVN), which contains neurons expressing the neuropeptides vasopressin and oxytocin, and the supramammalliary nucleus (SuM), which contains neurons expressing Substance P (Cui et al 2012; Figure 1). Given the importance of these neuropeptides in regulating systematic responses to social cues and stresses, the possibility they have a direct impact on hippocampal memory is intriguing. Direct evidence testing these hypotheses is scant. Non-specific lesions of the fimbria/fornix, which contains the axons running from these nuclei to the hippocampus, results in social memory deficits (Maaswinkel et al 1996), however a precise and careful dissection of the role of the hypothalamic-hippocampal projection pathways in social behaviors has yet to be carried out.

２．研究の目的 The afferent and efferents of the hippocampus have been well characterized in regards to their role in learning and memory. Perhaps some of the least understood connections however, are those between the hypothalamus and the dorsal CA2 subfield, a region recently implicated in social and aggressive behavior. Here we propose an optogentic behavioral approach that uses two region specific Cre-driver mouse lines targeting the paraventricular nucleus and supramammalilary nucleus respectively, to gain precise temporal control of the inputs from these hypothalamic nuclei to dorsal CA2. Terminal stimulation or silencing of this inputs in the hippocampus will address the role of these specific hypothalamic inputs to the hippocampus in controlling social memory and aggressive behavior.

３．研究の方法

Our experiments are designed to use optogenetic approaches to characterize and dissociate the role of the projections from the SuM to the hippocampus in social and spatial behavior. We first addressed how stimulation and silencing of the SuM inputs into CA2 impact social and spatial behavior. To obtain specific optogenetic channel expression in the terminals of these projection neurons we will use a csf2rb2-Cre BAC transgenic line generated in our lab. We will inject into the SuM of adult male cre mice viruses we have produced (AAV-DJ/-DIO ChR2/EYFP or ArchT/EFYP), that allow robust Cre-dependent expression of the light-responsive activating (ChR2) or silencing (ArchT) channels, while simultaneously implanting optic fibers bilaterally above the CA2 pyramidal cell field. Cre expressing male littermates injected with an EYFP-only virus will serve as our control group. Preliminary data confirms that three weeks following surgery robust expression of the channels can be visualized in the axonal terminals located in dorsal DG and CA2, suggesting local light stimulation should be effective in activating or silencing these neurons.

First we will express ChR2/EFYP or EFYP alone and address how the activation of SuM inputs to CA2 impact social memory. We will use a standard protocol to assess social memory, placing a juvenile mouse in the cage with the subject mouse and recording the duration of the interaction, a reflection of the familiarity of the animals (Kogan et al 2000). After a 1 hour delay the protocol is repeated, either with the identical juvenile (‘familiar mouse’), which results in decreased interaction if social memory is intact, or a new juvenile (‘novel mouse’), which results in higher levels of interaction.

Next, we pursued a parallel line of similar experiments as described above, but with optogenetic activation targeting the fiber terminals in the dentate gyrus. Specifically, we examined whether optogenetic activation or inhibition of the SuM-DG subcircuits had different effects on the behavioral performance of the mouse in spatial working memory, fear memory, and social memory tasks.

Finally, to visualize the anatomical segregation of the CA2 and DG projecting neurons in the SuM we employed brain tissue clearing by the ScaleS technique. High-resolution 3D imaging revealed strong direct projections from SuM to the hippocampal subfields of dentate gyrus (DG) and CA2 from distinct neuronal populations. Further, combining tissue clearing and anti-cfos immunohistochemistry we will label task specific activation of these distinct subsets of cells.

４．研究成果

(1) Anatomical segregation and task specific activation of CA2 and DG projecting SuM neurons.

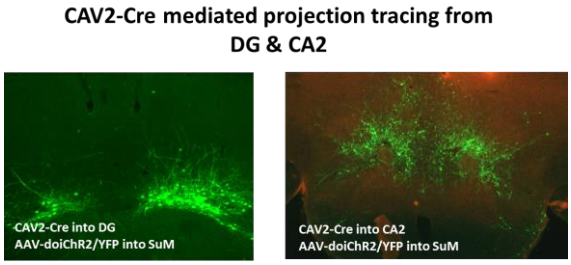

Figure 1.

As shown in Figure 1 above, projection specific viral retrograde labeling of CA2 and DG projecting neurons in the SuM delineated two anatomically distinct subpopulation.

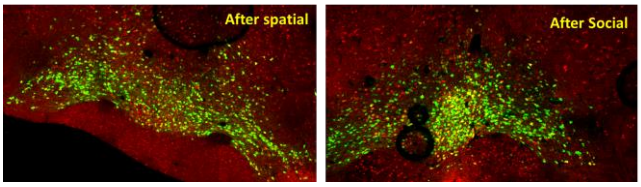

Figure 2. **Red: anti-cfos, green: anti-cre**

Further, as seen in Figure 2, spatial learning tasks led to cFos expression (red signal) in predominantly in the DG projecting cells, will social novelty led to expression primarily in the CA2 projecting populations. These results suggest the existence of two parallel circuits, both dealing with novelty, but in distinct cognitive domains. To understand these circuits better we next employed terminal specific optogenetic manipulations.

(2) Optogenetic stimulation of SuM terminals in CA2 impairs social memory.

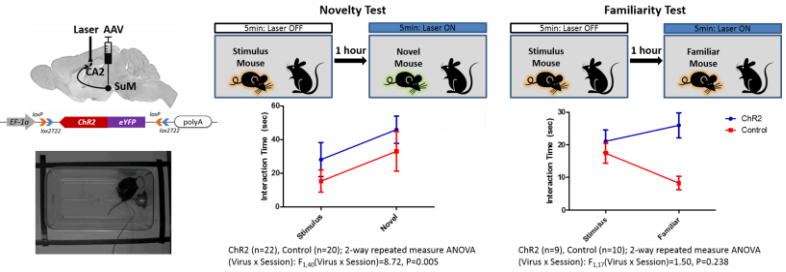

Figure 3. Laser stimulation during recall session.

As shown in Figure 3, optogenetic stimulation of the SuM terminals in CA2 during the recall phases of a direct interaction social memory task impaired the behavior of the subject mice. During stimulation mice treated a famailar mouse like it was novel, suggesting a role of these projections in driving the social novelty/exploration response. Parallel experiments, using the same protocol and stimulation parameters targeting the terminals in the DG had no effect on behavior (data not shown). This supports our hypothesis of two parallel pathways.

Further, using the pharmacogenetics DREADD system to specifically inhibit the activity of CA2 projecting neurons in the SuM impaired the natural response of the mouse to a novel juvenile (see Figure 4 below).

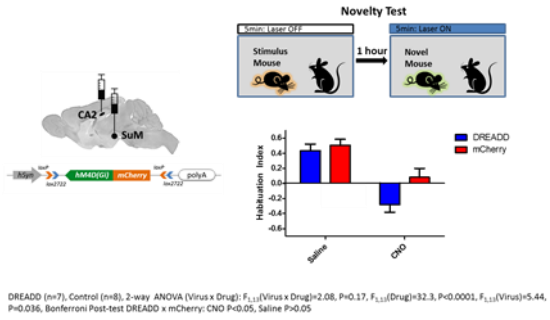

Figure 4. Inhibition of CA2 projecting SuM neurons impairs social novelty response.

(3) Terminal manipulation in the DG but not CA2 impair spatial novelty response

To examine the role of SuM->HPC transmission in spatial behavior we employed a simply behavioral tasks, tracking the exploration of mice in reaction to changes in the spatial configuration of familiar cues. As shown in Figure 5 we found that terminal inhibition in the DG impaired the novelty response, however the identical stimulation in CA2 had absolutely no effect.

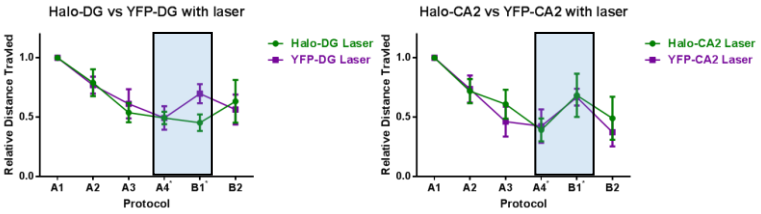

Figure 5. Blue boxed indicate sessions with laser stimulation.

The control mice in each experiment, shown in purple, increase their exploration following cue rearrangement (B1 session). Inhibition of DG terminals impairs this response (green plot, left graph), while inhibition in CA2 has no effect (green plot, right graph).

(4) A role for the DG projection, but not the CA2 projection, in spatial working memory.

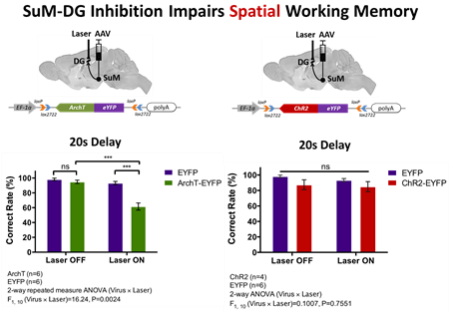

Figure 6.

In addition we are using a third behavioral paradigm, the DNMS T-maze, to examine the role of these circuits in spatial working memory. As shown in Figure 6, inhibition of the DG terminals impairs behavior in this task (left graph), while stimulation has no effect (right). Parallel experiment with CA2 terminal manipulations found absolutely no change in behavior (data not shown).

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）
none

〔学会発表〕（計　4　件）

① Optogenetic dissection of selective information routing by a hypothalamo-hippocampal circuit. Y Tao, S Chen, AJY Huang. ME Wintzer, D Polygalov, R Boehringer, J Chen, TJ McHugh. SFN Annual Meeting, November 13, 2016, San Diego, CA, USA.
② Optogenetic dissection of selective information routing by a hypothalamo-hippocampal circuit. Y Tao, S Chen, AJY Huang. ME Wintzer, D Polygalov, R Boehringer, J Chen, TJ McHugh. Japanese Neuroscience Annual Meeting, July 20, 2016, Yokohama, Kanagawa.
③ Supramammillary input to Hippocampal CA2 modulates social memory, ME Wintzer, AJY Huang, Roman Boehringer, Denis Polygalov, Lily MY Yu, Rebecca Piskorowski, Vivien Chevaleyre, TJ McHugh, SFN Annual Meeting, October 21, 2015, Chicago, IL, USA
④ Hypothalamic input to Hippocampal CA2 modulates social memory, ME Wintzer, AJY Huang, Lily MY Yu, TJ McHugh, Japanese Neuroscience Annual Meeting, July 28, 2015, Kobe, Hyogo

○取得状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等

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