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

令和　元 年　 ５ 月 ３１ 日現在

機関番号：８２４０１
研究種目：若手研究(B)
研究期間：2017～2018
課題番号：１７Ｋ１８３６７

研究課題名（和文）The coordinating roles of ITGb3 for adult neurogenesis-mediated homeostatic synaptic scaling and innate anxiety

研究課題名（英文）The coordinating roles of ITGb3 for adult neurogenesis-mediated homeostatic synaptic scaling and innate anxiety

研究代表者
ＰＡＲＫ　ＹＵＮＫＹＵＮＧ（PARK, YUNKYUNG）

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：５０５６８８６３

交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：癌または免疫関連疾患の研究の分野で集中的に研究されている細胞接着タンパク質であるインテグリンは、それらのサブユニット特異的機能および脳内の存在は、ほとんど知られていないままである。本研究では、脳海馬の興奮性ニューロンで発現しているインテグリン（ITG）β3が補償適応メカニズムによって脳波の恒常性を維持することが明らかになった。
このことから、ITGβ3 の異常調節や不在することで、脳波のパワーの過剰興奮といった異常なネットワーク活動が生じると予想している。 一般的な不安障害は、 こうした補償適応メカニズムの欠如によって引き起こされるとも考えられる。

研究成果の学術的意義や社会的意義
This study reports the physiological roles of neuronally expressed ITGβ3 for mature hippocampal circuit in a cell-type specific manner for the first time. It will also help future studies in the field of stress, anxiety disorder, drug addiction and autism, in which ITGβ3 have been implicated.

研究成果の概要（英文）：Integrins are cell adhesion proteins that have been intensively invesitgated in the field of cancer or immune-related disease research. Yet, their subunit-specific function and presence in the bran remain largely unknown. In this study, integrin(ITG)β3-dependent cellular and network mechanisms underlying anxiety-like behavior have been explored in mature hippocampal circuit.In CA3, both pre and post ITGβ3 mediate the excitatory basal synaptic transmission between mossy fiber from dentate gyrus and thorny excrescence of pyramidal neurons which, in turn, modulates the frequency and power of network oscillation. Interestingly, its modulatory mechanism in mossy fiber is more crucial for maintaining the homeostasis or adaptation between older and newer granule cells in dentate gyrus during ongoing adult neurogenesis, requiring its presence throughout the critical periods of early development.

研究分野：neuroscience

キーワード：ITGβ3　anxiety　network oscillation　synaptic plasticity　homeostasis

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９、ＣＫ－１９（共通）

１．研究開始当初の背景

(1) ITGβ3 is known to mediate homeostatic synaptic scaling in cultured hippocampal neurons. Synaptic scaling is a form of homeostatic synaptic plasticity found in vitro, in which global activity perturbation generates compensatory changes at individual synapses in a multiplicative manner that preserves the relative differences in synaptic strengths across synapses: Synaptic AMPA receptors are increased (scale up) by chronic inactivation or decreased (scale down) by chronic activation of network.

(2) Recent in vivo studies using null mice indicate a link between ITGβ3 and emotional behaviors such as innate anxiety.

(3) Although it is tempting to speculate that ITGβ3-mediated homeostatic synaptic scaling might be the underlying cellular mechanism for setting the anxiety level, the correlation between synaptic scaling in mature circuits of adult brain and anxiety-like behaviors has never been tested. More curiously, how might consequences of synaptic changes underlie the anxiety in living animals remain unclear.

(4) Anxiety-related information transfer occurs when enhanced output from ventral hippocampus in theta and gamma rhythmicity synchronizes its downstream brain regions to execute behaviors which reflect the anxious state of mice.

(5) In vivo studies which genetically modulated the neurogenesis of adult mice, demonstrated that adult-born granule cells in dentate gyrus (DG) of ventral hippocampus are involved in the level of innate anxiety. These new neurons sprout axons called mossy fibers (MF) and innervate CA3 pyramidal neurons, forming synapses between mossy fiber bouton (MFB) and CA3 thorny excrescence (TE). The birth-rate and bouton sprouting from immature MFs are bi-directionally modulated by chronic activity perturbation; Chronic activation by drugs such as picrotoxin (PTX) and serotonin or voluntary exercise increase birth rate and sprouting, whereas chronic inactivation by GABA or stress and aging decrease both. In contrast, mature TEs undergo structural changes in a compensatory manner upon chronic activity modulation; PTX (activation) decrease the size of TEs, whereas diazepam (inactivation) increases them. These events may play a role in modifying the excitability of mature DG neurons to maintain constant DG output in CA3. Such activity-dependent bidirectional modulation of adult neurogenesis and subsequent circuit reorganization to maintain homeostasis is highly reminiscent of homeostatic synaptic scaling studied in vitro as a compensatory adaptive mechanism. However it has not been tested whether ITGβ3 mediates these changes via homeostatic synaptic scaling mechanisms in vivo and how such processes might be related to modulating innate anxiety levels.

２．研究の目的

(1) The first aim is to test the hypothesis that ITGβ3 is a key player to dynamically adjust the synaptic strengths between MFB and TE via homeostatic synaptic scaling in CA3 circuit and that a series of these events will dynamically modulate the network oscillations in adult ventral hippocampus, by which anxiety-related information might be transferred.

(2) The second aim is to examine the primary locus of ITGβ3 function in neurons in DG-CA3 circuit for the above phenomena.

３．研究の方法

(1) This study examine the network oscillation and basal synaptic transmission using field recordings of DG-CA3 connection from in vitro acute slices prepared from ventral hippocampus of adult animals.

(2) Morphological analysis of structural change in TEs is combined with immunohistochemistry experiments. To visualize TEs, adeno-associated virus (AAV) serotype 9 vectors were constructed to deliver the spaghetti monster FLAG-P2A-mGFP gene under the transcriptional control of excitatory neuron-specific CaMKII promoter.

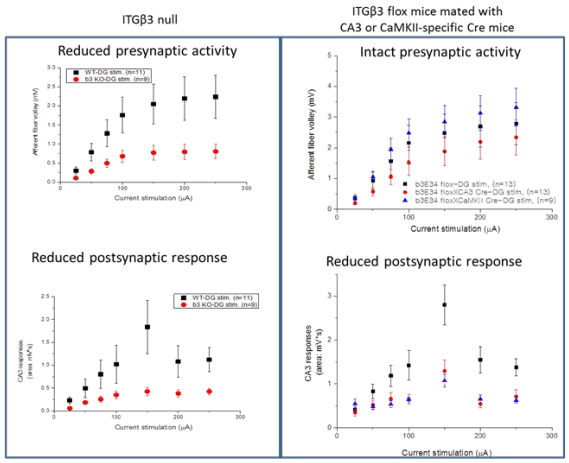
(3) To distinguish if locus of ITGβ3 is DG or CA3 or both, above measurements were

compared between wild-type, null and conditional ITGβ3 floxed mice, either crossed with transgenic mice expressing Cre or injected with viruses carrying Cre in a cell-type specific manner.

4．研究成果

(1) In our preliminary experiments, field recordings of DG-CA3 connections of 3 month old ITGβ3 null mice showed reduction in both afferent fiber volley activity and population output in CA3 (Left panel in Fig.1). However, conditional ITGβ3 floxed mice mated with mice expressing Cre in excitatory neurons of CA3 or both in CA3 and dentate gyrus showed reduction in postsynaptic output with intact mossy fiber volley activity unlike null mice (Right panel in Fig.1).

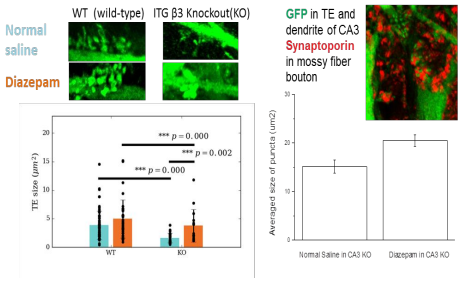
Figure 1. Excitatory population activity evoked by current injection in DG cell layer



(2) CA3-specific ITGb3 knockout mice also exhibited frequency shift of peak power toward gamma rhythmicity unlike null mice which showed reduction in overall power of theta and gamma oscillations with similar peak power in the theta-frequency range, compared to wild type mice.

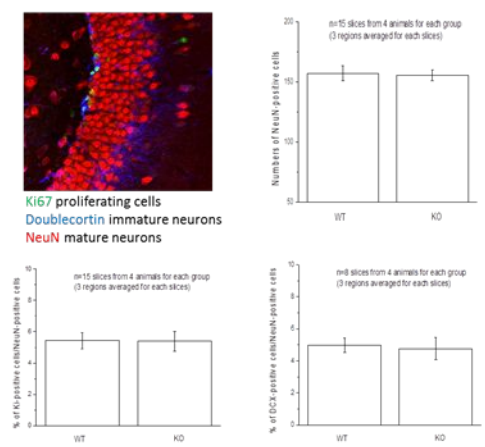
(3) The area of thorny excrescences of CA3-specific ITGb3 knockout mice was smaller than control but underwent the enlargement upon in vivo injection of diazepam for 2 weeks, which accompanied with enhanced synaptoporin (MF-specific synaptic vesicle protein) puncta size (Fig. 2). The presence of compensatory structural change, together with above findings, observed in these mice might indicate that ITGb3 in CA3 might be required to mediate the basal postsynaptic response between MFB-TE but dispensable to induce the homeostatic synaptic plasticity.

Figure 2. Morphological analysis of size changes in TE and synaptoporin puncta



(4) However, 3 month old CA3-specific knockout mice those were injected with both AAV- and retro-viruses which deliver Cre into mature and adult-born DG neurons around the age of 2 weeks, showed reduction in both afferent fiber volley activity and population output in CA3. They also exhibited reduction in overall oscillation power, mimicking the null mice. Together with above results, this finding might implicate that expression of ITGb3 in adult-born or immature DG neurons might be crucial to modulate the mossy fiber activity and oscillation power in the preserved frequency range.

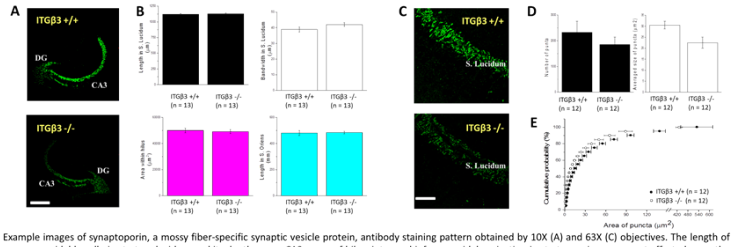
Figure 3. Proliferation rate in DG



(5) At present, a cellular mechanism for ITGb3 to modulate the mossy fiber activity and homeostatic synaptic plasticity remain

unclear. Based on immunostaining experiment result that counts the progenitor cells, immature neurons and mature neurons from wild-type and null mice, proliferation rate and numbers of total neurons in seem unaffected by ITGb3 (Fig.3). Also null mice did not show the difference in gross anatomy of mossy fiber (MF) projection such as the length in stratum lucidum of CA3 and the hilus area within DG (Fig. 4). Ruling out above two possibilities, the results showing the reduction in averaged size of synaptoporin punta in null mice (Fig. 4) together with its increase accompanying with TE enlargement upon chronic diazepam injection into CA3-specific knockout mice (Fig. 2) might suggest that ITGb3-dependent modulation of this protein might play an important role.

Figure 4. Mossy fiber gross anatomy is not altered but synaptoporin puncta size is decreased in ITGβ3 null mice.



Example images of synaptoporin, a mossy fiber-specific synaptic vesicle protein, antibody staining pattern obtained by 10X (A) and 63X (C) objectives. The length of suprapyramidal bundle in stratum lucidum and its depth across CA3, area of hilus, intra-and infrapyramidal projection in stratum oriens were not affected upon the loss of ITGβ3 (B). D shows that not the number of synaptoporin-positive puncta but its size is significantly different. In D, cumulative plots for synaptoporin puncta present in the field of view (x: 192.6 μm, y: 192.6 μm) shows that such change occurs in a multiplicative manner in ITGβ3 null mice. Scale bars in A and C indicates 250 μm and 40 μm, respectively

5．主な発表論文等

〔雑誌論文〕（計　　件）

〔学会発表〕（計　　件）

〔図書〕（計　　件）

〔産業財産権〕
○出願状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
出願年：
国内外の別：

○取得状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
取得年：
国内外の別：

〔その他〕
ホームページ等

6．研究組織

(1)研究分担者
研究分担者氏名：
ローマ字氏名：

所属研究機関名：

部局名：

職名：

研究者番号（8 桁）：

(2)研究協力者

研究協力者氏名：

ローマ字氏名：

※科研費による研究は、研究者の自覚と責任において実施するものです。そのため、研究の実施や研究成果の公表等については、国の要請等に基づくものではなく、その研究成果に関する見解や責任は、研究者個人に帰属されます。