様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）電顕三次元立体再構築に基づく脳の様々な領域におけるシナプス結合の共通原理の解明

研究課題名（英文）Synaptic organizational principles in a subset of brain regions

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：本研究では3次元電子顕微鏡を使用して脳のさまざまな領域の神経区画の構造、特にニューロンの樹状突起のユニークな構造と樹状突起棘の配置を明らかにした。 また、正常なマウスとパーキンソン病のマウスモデルで、生後1、3、6、22か月の樹状突起棘の形態の発達上の変化を調べ、脊椎密度は減少するが、野生型マウスでは年齢とともに平均頭体積が増加することを明らかにした。なお、パーキンソン病のマウスモデルでは、年齢に伴う脊椎の頭の体積の増加は見られなかった。

研究成果の学術的意義や社会的意義
本研究で、海馬、皮質、小脳では、樹状突起の直径とともに脊椎密度が増加することが明らかにした。 さらに、正常な野生型マウスの線条体で見られた平均脊椎頭部体積の年齢による増加はパーキンソン病のモデルマウスでは観察されなかった。

研究成果の概要（英文）：My research used three-dimensional electron microscopy to reveal the structures of neuronal compartments in different areas of the brain. I revealed the unique structures of neuronal dendrites and the placement of dendritic spines along the neuronal dendrites. Furthermore, in the striatum, I studied the developmental changes of dendritic spine morphology in 1, 3, 6 and 22 months of age in the normal mice and in the mouse model of Parkinson's disease. I found that the spine density decreases, but the average head volume increases with age in wild-type mice. However, the increase in spine head volume with age was not seen in Parkinson's disease mouse model. In summary, my research revealed the structure of dendritic spines in the hippocampus, cerebral cortex, striatum, cerebellum and the unique dendritic protrusion in the interpeduncular nucleus of wild-type mice; and the abnormality in the structures of dendritic spines in the striatum of Parkinson's disease mouse model.

研究分野：Neuroanatomy

キーワード：pine　Synapse　Dendrite　Parkinson's disease

１．研究開始当初の背景

It is believed that there are more than a billion of neurons in our brain. Light microscopic (LM) studies shows that the neurons in different areas of the brains do not look alike. For example, in area CA1 of the hippocampus, cortex and cerebellum; the neurons are organized in a laminar fashion such that their cell bodies are located in a defined region. However, in many other regions of the brain the cell bodies of the individual neurons are simply scattered without showing any definite laminar organization. Not only the organization of the cell body, but also the dendritic morphology varies among neurons. Several oblique dendrites branch from the main apical dendrite in case of the principal neurons in the hippocampus and cortex. The oblique dendrite possesses numerous spiny structures called as dendritic spines. In contrast, the interneurons in hippocampus and cortex are generally smooth and do not possess any spines. It is believed that the neuronal diversity is rather related to repertoire of computational task that the neuron must perform. Although the difference in gross neuronal morphology was already revealed by LM observation more than a century ago by the pioneering work of Camillo Golgi and Santiago Ramón y Cajal, still now we know very little on the precise subcellular structures of neurons and how their morphology is remodeled in various pathological conditions.

２．研究の目的

The research aimed to reveal the diversity of dendritic spines in the multiple spiny regions of the brain. Dendritic spines have attracted a special attention because they are the major site of the excitatory synaptic contacts in many regions of the brain. Previous transmission electron microscopic (TEM) studies have shown that the even in the CA1 of the hippocampus, the size of the dendritic spines varies by more than 55 folds. However, there is a lack of a systematic study which compares the spine morphology across different regions of the brain. In order to eliminate the bias caused by the age, individual animal variability and the sample preparation method, it is necessary to perform the comparative study of the spine morphology within the same animal. Furthermore, it is not known how these dendritic spines are organized across the dendritic shaft. The organizational principle of the dendritic spines across the dendritic shaft has an important consequence in terms of synaptic computation. Thus, the first aim of this research was to compare the spine morphology among proximal stratum radiatum of CA1 hippocampus, CA1 stratum lacunosum-moleculare, CA3 stratum lucidum, somatosensory cortex, striatum and cerebellum; and to decipher how they are organized along the dendritic shaft. Second, the research aimed to decipher the peculiar dendritic morphology in the interpeduncular nucleus of the brain. Third, the research analyzed how the spine morphology varies with age in the striatum and how it is remodeled in the Parkinson’s disease.

３．研究の方法

First, the brain samples were prepared from C57BL/6WT mouse for FIB/SEM imaging by adopting the well-established protocols (Parajuli et al., 2020a,b,c). Briefly, the mouse was perfused with 100 ml of 2% paraformaldehyde and 2% glutaraldehyde. The brain was removed from the skull and washed several times in 0.1M calcodylate buffer supplemented with 2mM Calcium chloride. The brain block was incubated in ferrocyanide-reduced osmium solution, followed by 1% tannic acid solution. The brain blocks were then stained with Osmium tetroxide and uranyl acetate solution. The brain blocks were dehydrated and embedded in resin. In the project to compare the morphology of dendritic spines in the striatum between wild-type and Parkinson's mutant mouse, A53T-BAC-*SNCA* transgenic mice was used. Forty to fifty nanometer serial EM images were obtained using focused ion beam/scanning electron microscopy (FIB/SEM). The images were obtained between 8000 to 15000 magnifications. Dendritic spines and the dendritic shafts from the resulting images were annotated to measure the relative dimensions of dendritic spines in different brain regions and to decipher their organizational principle across a dendrite.

４．研究成果

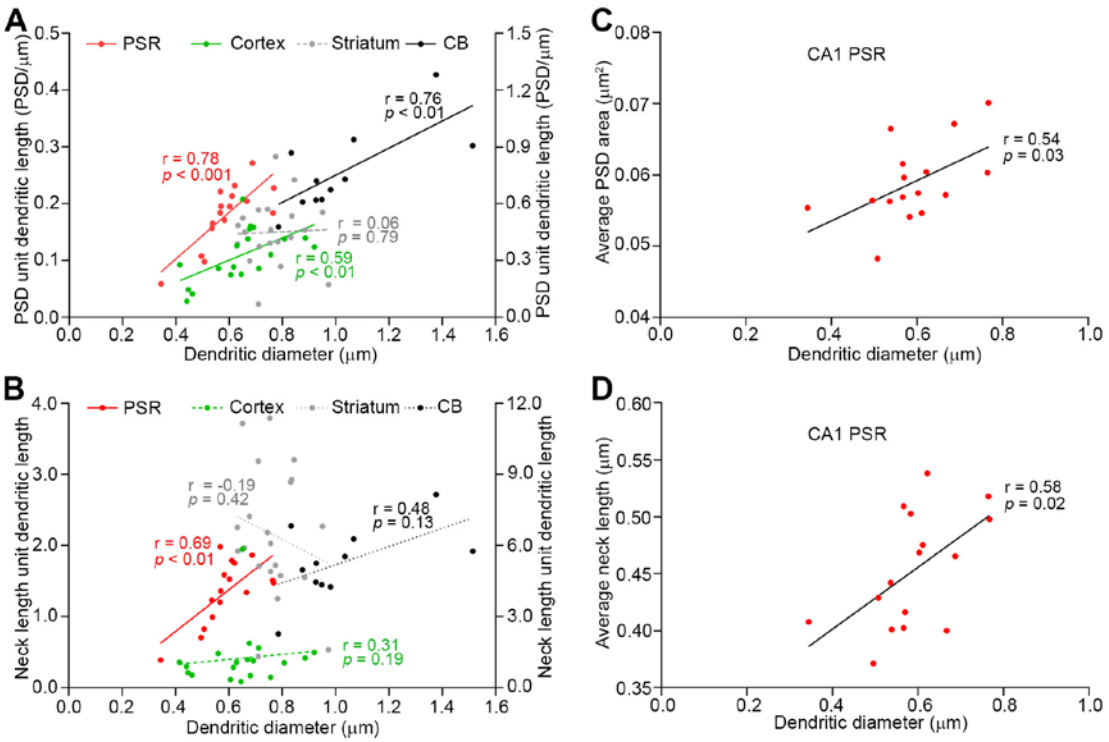
First, we compared the relative dimensions of the dendritic spines across different brain regions. The relative dimensions of the spines are tabulated below. The table shows that the dimensions of the spine head, spine neck and the density of spines in the dendritic shafts greatly differs according to the brain regions.

Brain region	Spines Dendrites (n)	Spine density (mean ± SD)	Head volume (mean ± SD, ratio, CV)	Neck length (mean ± SD, ratio, CV)	Neck diameter (mean ± SD)	Ratio of PSD area to neck length (mean ± SD)
CA1 PSR	552 23	3.04 ± 0.825	0.05 ± 0.045 124, 0.94	0.46 ± 0.246 38, 0.54	0.20 ± 0.087	0.19 ± 0.193
CA1 SLM	70 12	0.75 ± 0.360	0.12 ± 0.086 35, 0.71	0.49 ± 0.289 15, 0.59	0.25 ± 0.127	0.32 ± 0.323
CA3 PSR	8 ----	----	1.66 ± 1.988 155, 1.20	----	----	----
Cortex	221 19	1.14 ± 0.723	0.08 ± 0.101 159, 1.26	1.09 ± 0.561 20, 0.52	0.23 ± 0.121	0.13 ± 0.169
Striatum	403 21	1.94 ± 0.621	0.07 ± 0.109 204, 1.66	1.12 ± 0.556 106, 0.50	0.26 ± 0.104	0.11 ± 0.204
CB	824 11	7.10 ± 1.693	0.13 ± 0.035 11, 0.27	0.74 ± 0.300 27, 0.40	0.27 ± 0.054	0.17 ± 0.130

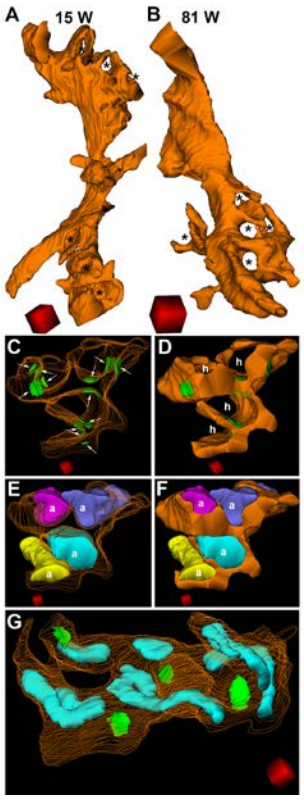
Units: Spine density: spines/μm, Head volume: μm^3, Neck length: μm, Neck diameter: μm

CA1 PSR: CA1 proximal stratum radiatum; CA1 SLM: CA1 stratum lacunosum-moleculare; CA3 PSR: CA3 proximal stratum radiatum; CB: Cerebellum. This data is published in eNeuro journal (Parajuli et al., 2020a).

Next, we showed that the dendritic spines are organized in an orchestrated fashion such that in the CA1 stratum radiatum, somatosensory cortex and cerebellum, the density of the spines gradually increase with the increase in the dendritic diameter. However, the same tendency was not seen in the striatum. This suggests that the organizational principle of the dendritic spines differs according to the brain regions.



Spines are organized in a regulated manner in the dendrites. A, PSD area unit dendritic length shows a significant positive correlation with the dendritic diameter in the CA1 PSR, cortex, and CB (see also Extended Data Fig. 3-1). However, in the striatum, the PSD area unit dendritic length does not correlate with dendritic diameter. B, Neck length unit dendritic length is positively correlated with the dendritic diameter in the CA1 PSR. The data from CA1 PSR, cortex, and striatum are plotted in reference to the left vertical axis, and the data from CB are plotted in reference to the right vertical axis in panels A, B. Average PSD area (C), and average neck length (D) show a positive correlation with the dendritic diameter in the CA1 PSR (Parajuli et al., 2020a).



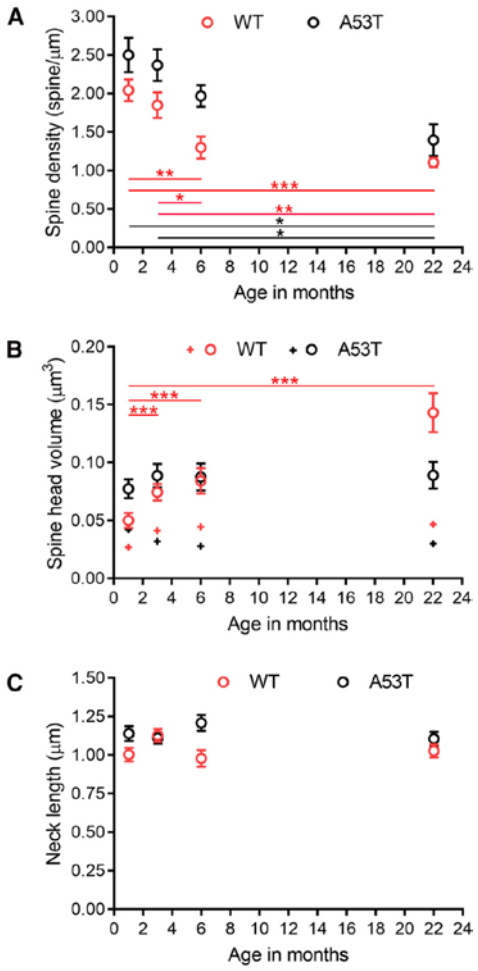
Next, we showed that the dendrites in the interpeduncular nucleus exhibits very unique U-shaped morphology with several grooves. This is a very interesting finding, because this type of U-shaped protrusions from the dendrites has not been reported before.

Three-dimensional reconstruction of crest synapse from 3 months **(A)** and 18 months **(B)** mice show numerous claw-shaped protrusions (shown by asterisks) extending from the IPN dendrite (orange). Raw traces **(C)** and three-dimensional rendering **(D)** of images obtained from 18 months mouse showing that each crest synapses (PSDs are shown by green) in pair (marked by white arrows in **C**) locate next to each other. Hollow spaces (h in **D**) are evident in the dendrite. Raw traces **(E)** and three-dimensional rendering **(F)** of dendrite showing that axons (a) traverse through hollow, claw-shaped spaces in the dendrites. G**,** Three-dimensional reconstruction of a dendrite (raw traces in orange) from 3

months old mouse shows that mitochondria (turquoise) are excluded from the site of crest synapses (green). Scale cubes: 1 μm in each sides in A and B, 0.5 μm in each sides in C-G. This data is published in Biochemical and Biophysical Research Communications journal (Parajuli et al.,2020b).

Next, we revealed that in the striatum, the spine density decreases but the spine head size increases with the age in the wild-type animal. However, similar tendency was not seen in the mutant mouse model (A53T-BAC-*SNCA*) of Parkinson's disease. This suggests that Parkinson's disease is accompanied by the developmental changes in the structure of the dendritic spines.

Morphologic alterations in spines in WT and A53T-BAC-SNCA mice with age. A, Age-related changes in spine density. The graph shows that spine density decreased with age in both the WT and A53T-BAC-SNCA mice. In WT mice, the spine density at 1 and 3 months was significantly lower compared with that at 6 and 22 months (**p = 0.003 between 1 and 6 months, ***p < 0.001 between 1 and 22 months, *p = 0.03 between 3 and 6 months, **p = 0.001 between 3 and 22 months; one-way ANOVA). In A53T-BAC-SNCA mice, there were fewer spines at 22 months compared with that at 1 and 3 months (*p = 0.01 between 1 and 22 months, *p = 0.01 between 3 and 22 months; Kruskal–Wallis test). B, Age-related changes in spine head volume. The average spine head volume increases with age in the WT (red open circle), but not A53T-BAC-SNCA (black open circle), mice. In WT mice, spine head volume at 1 month was significantly smaller than that at 3, 6, and 22 months (***p < 0.001 between 1 and 3 months, ***p < 0.001 between 1 and 6 months, ***p < 0.001 between 1 and 22 months; Kruskal–Wallis test). Median head volume is shown by the + symbol. C, Age-related changes in spine neck length. The spine neck length did not vary significantly with age in either WT or A53T-BAC-SNCA mice. Different levels of significance are denoted by the number of asterisks (*p <0.05, **p <0.01, ***p <0.001). This data is published in eNeuro journal (Parajuli et al.,2020C).



The result shown above are described in details in my following first-author papers from 2020.

- **Parajuli LK,** Urakubo H, Takahashi-Nakazato A, Ogelman R, Iwasaki H, Koike M, Kwon HB, Ishii S, Oh WC, Fukazawa Y, Okabe S. Geometry and the organizational principle of spine synapses along a dendrite. **eNeuro,** 2020, 7 (6), 1-19.
- **Parajuli LK*,** Wako K, Maruo S, Kakuta S, Koike M*. Unique synaptic topography of crest-type synapses in the interpeduncular nucleus. **Biochemical and Biophysical Research Communications,** 2020, 530 (1), 130-135. * corresponding authors
- **Parajuli LK,** Wako K, Maruo S, Kakuta S, Taguchi T, Ikuno M, Yamakado H, Takahashi R, Koike M. Developmental changes in dendritic spine morphology in the striatum and their alteration in an A53T alpha-synuclein transgenic mouse model of Parkinson's disease. **eNeuro,** 2020, 7 (4), 1-14.

５．主な発表論文等

〔雑誌論文〕　計6件（うち査読付論文　6件／うち国際共著　5件／うちオープンアクセス　5件）

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２．論文標題 a-Synuclein BAC transgenic mice exhibit RBD-like behaviour and hyposmia:a prodromal Parkinson's disease model	５．発行年 2020年
３．雑誌名 BRAIN	６．最初と最後の頁 249-265
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/brain/awz380	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Masashi Ikuno, Hodaka Yamakado, Hisako Akiyama, Laxmi Kumar Parajuli, Katsutoshi Taguchi, Junko Hara, Norihito Uemura, Yusuke Hatanaka, Katsumi Higaki, Kousaku Ohno, Masaki Tanaka, Masato Koike, Yoshio Hirabayashi, Ryosuke Takahashi	４．巻 28
２．論文標題 GBA haploinsufficiency accelerates alpha-synuclein pathology with altered lipid metabolism in a prodromal model of Parkinson's disease	５．発行年 2019年
３．雑誌名 Human Molecular Genetics	６．最初と最後の頁 1894-1904
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/hmg/ddz030	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 Yu-shin Sou, Soichiro Kakuta, Yuji Kamikubo, Kazue Niisato, Takashi Sakurai, Laxmi Kumar Parajuli, Isei Tanida, Hiromitsu Saito, Noboru Suzuki, Kenji Sakimura, Yusuke Maeda, Taroh Kinoshita, Yasuo Uchiyama and Masato Koike	４．巻 6
２．論文標題 Cerebellar Neurodegeneration and Neuronal Circuit Remodeling in Golgi pH Regulator-Deficient Mice	５．発行年 2019年
３．雑誌名 eNeuro	６．最初と最後の頁 1-22
掲載論文のDOI（デジタルオブジェクト識別子） 10.1523/ENEURO.0427-18.2019.	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Parajuli LK	４．巻 64 (5)
２．論文標題 Technical Toolbox for Decoding Synaptic Complexity	５．発行年 2018年
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掲載論文のDOI（デジタルオブジェクト識別子） 10.14789/jmj.2018.64.JMJ18-LN03	査読の有無 有
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1．著者名 Shuo Chen, Adam Z. Weitemier, Xiao Zeng, Linmeng He, Xiyu Wang, Yanqiu Tao, Arthur J. Y. Huang, Yuki Hashimotodani, Masanobu Kano, Hirohide Iwasaki, Laxmi Kumar Parajuli, Shigeo Okabe, Daniel B. Loong Teh, Angelo H. All, Iku Tsutsui-Kimura, Kenji F. Tanaka, Xiaogang Liu, Thomas J. McHugh	4．巻 359 (6376)
2．論文標題 Near-infrared deep brain stimulation via upconversion nanoparticle-mediated optogenetics	5．発行年 2018年
3．雑誌名 Science	6．最初と最後の頁 679-684
掲載論文のDOI（デジタルオブジェクト識別子） 10.1126/science.aaq1144	査読の有無 有
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1．著者名 Bishop HI, Cobb MM, Kirmiz M, Parajuli LK, Mandikian D, Philp AM, Melnik M, Kuja-Panula J, Rauvala H, Shigemoto R, Murray KD, Trimmer JS	4．巻 11:1
2．論文標題 Kv2 ion channels determine the expression and localization of the associated AMIGO-1 cell adhesion molecule in adult brain neurons	5．発行年 2018年
3．雑誌名 Front Mol Neurosci	6．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fnmol.2018.00001	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　0件）

1．発表者名 Parajuli Kumar Laxmi, Wako Ken, Maruo Suiki, Takahashi Ryosuke, Koike Masato
2．発表標題 Distinct age-dependent subcellular changes in a model mouse of Parkinson's disease as revealed by volumetric FIB/SEM imaging of striatum
3．学会等名 42nd Annual Meeting of the Japanese Neuroscience Society
4．発表年 2019年

1．発表者名 Parajuli Kumar Laxmi, Wako Ken, Maruo Suiki, Takahashi Ryosuke, Koike Masato
2．発表標題 Developmental changes of spine morphology in the striatum and its alteration in Parkinson`s disease mouse model
3．学会等名 ABiS Symposium Okazaki
4．発表年 2020年

〔図書〕　計2件

１．著者名 Ivar S. Stein, Travis C. Hill, Won Chan Oh, Laxmi K. Parajuli, Karen Zito	４．発行年 2019年
２．出版社 Springer Protocols	５．総ページ数 21
３．書名 Multiphoton Microscopy	

１．著者名 Ai Takahashi-Nakazato, Laxmi Kumar Parajuli, Hirohide Iwasaki, Shinji Tanaka, Shigeo Okabe	４．発行年 2019年
２．出版社 Springer Protocols	５．総ページ数 11
３．書名 Methods in Molecular Biology	

〔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