学位論文内容の要旨

（Summary of dissertation）

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Anatomical Study on Neuronal Circuitry in Medial Prefrontal Cortex and Development of a New Device for Optical Study

（内側前頭前野の神経回路に関する解剖学的研究
および光学的研究のための新規デバイス開発）

[Background and Objectives]

The medial prefrontal cortex (mPFC) is critically involved in top-down control of a wide variety of higher order functions, including cognition and emotional learning. Among various long-range inputs to the mPFC, the connection with the basolateral amygdala (BLA) is particularly important for emotional regulation. Since how projection neurons in the mPFC respond to these inputs is also strongly shaped by inhibitory interneurons, it is important to elucidate interneuron population and how they connect with the BLA; however, anatomical and physiological evidence is still limited. Furthermore, to understand how neuronal ensembles in the mPFC-BLA circuits process a multitude of information, it is necessary to capture neuronal activities in large volume with high resolution. Capturing the calcium transients associated with neuronal activity requires volume imaging methods with subsecond temporal resolution. Such speed is a challenge for a conventional two-photon laser-scanning microscope (TPLSM) system, because it depends on serial focal scanning in three dimensions. To this end, one of the most promising solutions is to generate an axially elongated Bessel focus, which enables volume imaging with a single scan. Thus, I have undertaken an anatomical and optical study of the mouse mPFC-BLA circuit and challenged myself to develop an easy-to-use Bessel beam generator, which substantially extends axial focus.

[Materials and Methods]

Anatomical experiments Adult C57/BL6N mice aged more than 2 months were used. To characterize the BLA-mPFC connections, retrograde and anterograde neuronal labeling was performed. For tracer injection, mice were anesthetized with 1%–2% isoflurane. For retrograde labeling, FluoroGold was injected into the BLA or the mPFC. For anterograde labeling, either of adeno-associated virus (AAV) vector encoding enhanced green fluorescent protein (EGFP) or GCaMP7b was injected into the BLA. After allowing for expression, mice were transcardially perfused with 4% paraformaldehyde (in 0.1 M phosphate buffer, pH 7.2) for 10 min. After postfixation, 50-μm-thick sections were prepared. Tracer signals were enhanced by immunofluorescence for FluoroGold or EGFP. Subcellular structures labeled with tracer were characterized with immunofluorescence for presynaptic and postsynaptic molecules. To identify three mutually exclusive subtypes of interneurons, immunofluorescence for somatostatin (SST) or parvalbumin, or fluorescent *in situ* hybridization for serotonin receptor 3a mRNA, was performed. Images were taken with an Olympus confocal microscope FV1000.

in vivo calcium imaging Following mice aged more than two months were used: C57/BL6N mice, *Thy1-G-CaMP7-DsRed* mice, and *SST-Cre* mice. Several lines of AAV vectors encoding genetically encoded calcium indicators or Designer Receptors Exclusively Activated by Designer Drug (DREADD) were used as follows: For imaging from BLA boutons in the mPFC, AAV1-hSyn-jGCaMP8s or AAV2-hSyn-hM3Dq-mCherry was injected into the BLA. For imaging from SST neurons, AAV-hSyn-FLEX-jGCaMP7s, AAV-hSyn-FLEX-YCaMP1s or AAV1-hSyn-DIO-hM3Dq-

mCherry was injected into the mPFC. All imaging data were obtained using a Nikon A1R-MP+ TPLSM system equipped with a 25x water-immersion objective lens and a Ti:Sapphire laser.

Designing and testing of the light-needle creating device The light-needle creating device composed of a custom-made concave axicon and a plano convex lens was placed at a filter turret of a TPLSM system. The concave axicon refracted an incident Gaussian beam on its conical surface and converted it into an annular beam at the focus of the convex lens. Volumetric imaging using the present device was evaluated by using fluorescent beads and paraformaldehyde-fixed brain slices, which were prepared from *Thy1-H-YFP* mice and treated with optical clearing reagents. Applicability to *ex vivo* and *in vivo* calcium measurements was examined using *Thy1-G-CaMP7-DsRed* and *SST-Cre* mice.

[Results]

In Chapter 1, I first showed that the BLA was connected to both the prelimbic and infralimbic cortices of mPFC with distinct laminar connections. Furthermore, SST neurons were identified as the principal interneurons in the mPFC by immunofluorescence and *in situ* hybridization analysis. Finally, I captured calcium responses from BLA terminals and SST neurons using a conventional TPLSM system. From a series of experiments, I identified targets for improvement in the conventional system. In Chapter 2, I developed a light-needle creating device composed of a concave axicon lens and a convex lens, aiming to capture calcium responses from SST neurons *in vivo*. This device was installed to a filter turret in an existing TPLSM system, and successfully extended the depth of field beyond 200 μm. This system enabled a single Bessel scan to capture structural information from thick biological specimens around 250-μm-thick, yielding comparable images to those obtained with the Gaussian volume scanning. However, zooming effect and peripheral distortion was observed in a distance-dependent manner. A combination with major tissue clearing protocols and immunofluorescence allowed 2D Bessel scan to visualize subcellular structures such as dendritic shaft and spines. Although the present system provided high spatial and temporal resolutions for capturing spontaneous and evoked calcium transients from acute brain slices, the spatial resolution dropped considerably *in vivo*.

[Discussion]

Observations in Chapter 1 substantiate the previous findings on the wiring diagram in the mPFC. The mPFC and the BLA were reciprocally connected and that this connection was biased towards distinct subregions and layers. The present results also support that the composition of inhibitory interneuron in the mPFC was distinct from that in the sensory cortex; There were fewer parvalbumin neurons and more SST neurons, which received excitatory inputs from BLA neurons. In Chapter 2, I developed a new system, which allowed a conventional TPLSM to perform a volumetric imaging in a single frame scan. When tested on fixed slices and acute slices, it yielded high spatial and temporal resolutions; However, it was difficult to capture neuronal activity at high resolution *in vivo*, suggesting that it requires further optimization, including reduction of light scattering and improvement of signal-to-background ratio.

[Conclusion]

The present findings confirm the layer- and region-specific organization of BLA–mPFC circuits, and provide anatomical and physiological evidence that helps understand how these connections can drive specific circuits in the mPFC. In future studies, I have to consider the role of other interneurons and their interaction in the mPFC, and to identify possible cell ensembles that might be recruited together and interact during specific behaviors. The present device can be easily installed to an existing TPLSM system, enabling Bessel beam-based volumetric imaging. This easy-to-use optical needle generator can be widely used to investigate three-dimensional cellular structures and network dynamics. In the future, I will improve the present system to address important biological questions.