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研究課題名（和文）Molecular mechanisms of postnatal amygdalar neurogenesis

研究課題名（英文）Molecular mechanisms of postnatal amygdalar neurogenesis

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研究成果の概要（和文）：生後の扁桃体におけるニューロン新生については未だ不明な点が多い。申請者はこれまでに、生後に与えた母子社会的剥離による外的環境変化によって、扁桃体のニューロン新生が変化することを見い出した。しかし、そのサブタイプや起源についての詳細は明らかになっていない。本研究では、扁桃体における新生ニューロンの運命とその起源を推定するために、GFPラベルした神経幹細胞の母子社会的剥奪後における扁桃体系譜解析、およびシングルセルRNAシークエンス解析と行動解析を行うことで、扁桃体におけるニューロン新生の分化軌跡と新生ニューロンが担う生理的役割を検証した。

研究成果の学術的意義や社会的意義
As newborn cells, particularly during the postnatal period, are context-sensitive, they can be manipulated by environmental stimuli. Understanding the contributions of amygdalar neurogenesis after early-life stress will provide new insight into fundamental aspects of brain plasticity.

研究成果の概要（英文）：Neurogenesis in the postnatal amygdala remains unknown. We have previously found that neurogenesis in the amygdala was altered by environmental stimuli due to maternal and social deprivation (MSD) inflicted after birth. However, the details of its subtypes and origin are not clear. In this study, to infer the fate and origin of neonatal neurons in the amygdala, we performed amygdala lineage analysis of GFP-labelled neural stem cells (NSCs) after MSD, as well as single-cell RNA sequencing analysis and behavioral analysis to examine the differentiation trajectories of amygdalar neurogenesis and the physiological role of newborn neurons.

研究分野：Neuroscience

キーワード：Postnatal amygdala　Neurogenesis　Early-life stress　Lineage tracing

１．研究開始当初の背景

The generation of newborn neurons and their distribution in the brain is crucial for establishing and maintaining the neuronal network. Adult neurogenesis predominantly occurs in distinct neurogenic regions of the ventricular and subventricular zone around the lateral ventricles, which provide new neurons to the olfactory bulb through the rostral migratory stream and the subgranular zone of the hippocampus dentate gyrus, where they produce granular cells in the dentate gyrus. Although the amygdala is considered a non-neurogenic region, some reports described postnatal neurogenesis in the amygdala (Bernier et al., 2002; Jhaveri et al. 2019). In the mouse brain, the amygdala neurons are formed early in the ventro-caudal telencephalon during the embryonic stages and are well-developed at birth (Soma et al., 2008). After birth, the brain continues to develop within a certain period, particularly during the first two weeks of life, where environmental factors can influence the cell proliferation, migration, differentiation, and survival of newborn cells. However, the factors regulating their survival and maturation beyond the gestation period are unclear.

Since the amygdala modulates response to the changing environment and plays a role in the acquisition and consolidation of fear memory, it raises the key questions of how environmental influences can alter cell proliferation, survival, and maturation of newborn cells in terms of their origins, cell subtypes and differentiation potential during postnatal development. Indeed, a recent report showed the presence of neural precursors and the generation of new interneurons in the basolateral amygdala in adult mice (Jhaveri et al. 2019); however, amygdalar neurogenesis has been poorly studied in postnatal brains.

Previously, we demonstrated the effect of early-life stress by the maternal and social deprivation paradigm (MSD) on neural stem cell (NSC) population size and neurogenesis in the adult brain (Daun et al., 2020). Furthermore, we showed that early life stress exhibits distinct effects on the activity of the NSC-neurogenesis system in the adult brain, depending on the timing and duration of the stress. MSD during the stress-hyporesponsive period (SHRP) increased the size of the NSC population; however, the same stress extended beyond the SHRP had the opposite effects.

２．研究の目的

Recently, we found that early-life stress led to the occurrence of neurogenesis in the basolateral amygdala (**Fig. 1**). Thereby, we asked whether the newborn cells migrate from the neurogenic area or if there is a small population of the progenitor cells truly neurogenic, replacing existing cells in the amygdala. However, the identity of factors contributing to these effects remains to be determined, which leads to the conception of the study in greater depth on postnatal amygdalar neurogenesis. Since amygdalar neurogenesis is poorly studied in the postnatal brain, it would be intriguing to examine the factor(s) contributing to the enhancement of postnatal amygdalar neurogenesis after MSD.

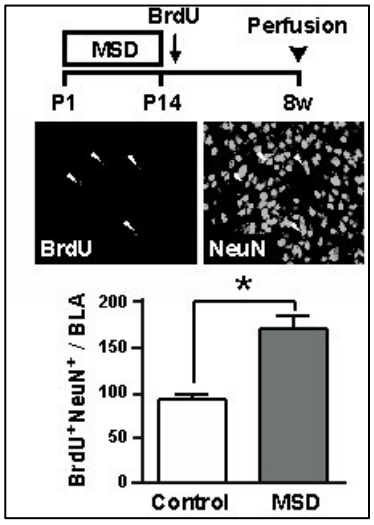

Fig 1: Amygdalar neurogenesis after MSD

White arrowheads point $BrdU^{+}NeuN^{+}$ Cells

This study elucidated the origins, cell subtypes, and differentiation potential of newborn neurons at different developmental stages after early-life stress in the amygdala by lineage tracing system. We determine whether the postnatal amygdala harbors neurogenic potential that may underlie its function.

３．研究の方法

The experiment was performed using a transgenic mouse line carrying the inducible Cre recombinase coupled with estrogen receptor ligand-binding domain (ER) under the neural stem cell (NSC)-specific Nestin promoter/enhancer and oligodendrocyte precursor cell (OPC)-specific NG2 promoter then cross with the Z/EG reporter mouse line to generate NestinCreER; Z/EG, and NG2CreER; Z/EG, respectively. The maternal and social deprivation (MSD) were performed throughout the study, followed by serological analysis using immunohistochemistry staining of neuron-specific markers, oligodendrocyte-specific markers and astrocyte-specific markers, as well as proliferation markers such as BrdU incorporated in the short term and long term labelling in the amygdala. We also performed behavioral analysis to elucidate the functional changes in newborn cells after MSD.

To trace the alteration in newborn neurons, we injected GFP-expressing lentivirus to the right lateral ventricle of postnatal day 0 (P0) neonates. Later, at P30 or 8 weeks, GFP+ NSCs were isolated by the neurosphere assay, and GFP+ NSCs were picked up and amplified by serial passaging. Then, DNA was extracted from the neurospheres, and integration-site-specific nested PCR was performed to estimate the fate of progeny from the lentivirus-infected cells in the amygdala regions. Subsequently, we constructed GFP and tetracycline-inducible diphtheria toxin A-expressing lentivirus to ablate postnatal neurogenesis. We also added single-cell RNA sequencing analysis to elucidate the origin of newborn cells and identify their specific cell lineage in the postnatal amygdala.

We utilized the MSD paradigms as previously described (Daun et al. 2020) in all experiments by separating each pup from its dam and littermates for 3 hours daily from P1 to 14. The non-separated control pups will remain in the home cage with the mother (**Fig. 2**)

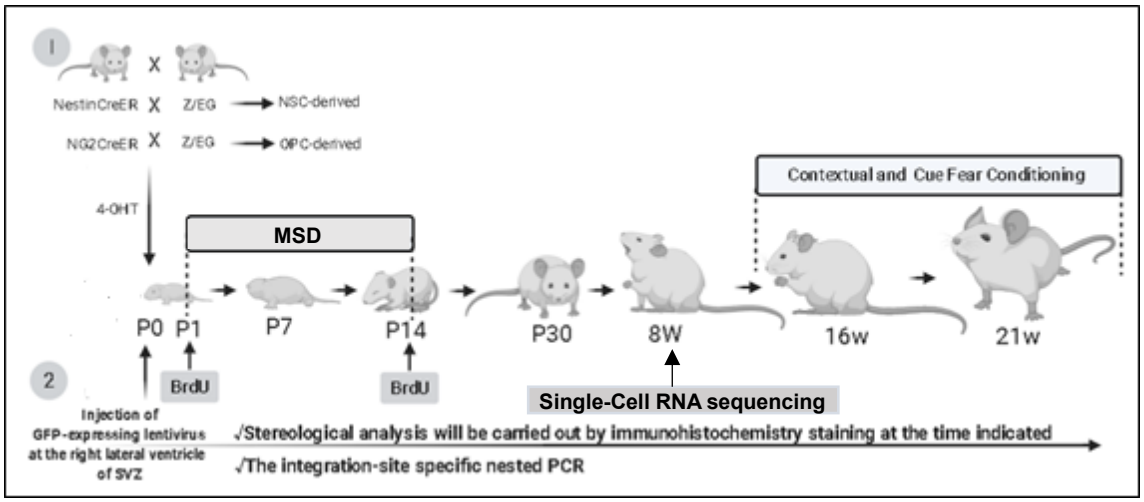

Fig 2: Summary of the experimental flow

４．研究成果

Identification of cell-specific lineage in the postnatal amygdala after MSD.

Using integration-site nested PCR and serological analysis, we analyzed to estimate the fate of progeny in cells infected with lentivirus. Following MSD, maternal and social deprivation paradigms, we identified the specific lineage of these cells. Given that the amygdala is comprised of multiple anatomical regions, each defined by distinct functionality. Our findings revealed that the impact of MSD, particularly within the first two weeks of life, where the stress-induced excessive release of corticosterone does not affect the postnatal brain due to the inactivation of the hypothalamus-pituitary-adrenal axis (HPA) also known as the stress-hyporesponsive period (SHRP), led to diversify in the composition of cell types within the amygdala. In addition, these cell-type compositions, as observed through our detailed behavioral analysis which included fear conditioning test, affect the behavior. This suggests that MSD has a substantial influence on the cell-type composition in the postnatal amygdala and provides clarity and direction of newborn cells to the structural, survival, and functional identities.

Single-cell RNA sequencing revealed functional identities of individual cells in the postnatal amygdala

To directly examine the molecular signatures of newborn neurons and identification of the origin, we performed single-cell RNA sequencing. This transcriptomics analysis achieved detailed cell identification and distributions within the distinct regions in the amygdala, including the basolateral, central, and medial sub-regions.

In addition, the molecular signatures of neuronal activation after MSD and differentiation trajectories of newborn cells could be encoded in specific amygdalar cell types that link to their neurogenic potential underlying its physiological function in adulthood. We concluded that there is a sensitive developmental period during which environmental factors could alter specific aspects of the amygdala functionality and plasticity.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Kenny Daun, Naoko Morimura, Tadateru Fukami, Yoshitaka Hayashi, Natsu Koyama, Kenji Tanigaki, Akihiko Shiino, Kazuhiko Nozaki, Seiji Hitoshi
２．発表標題 RNF20 as an epigenetic regulator for GBM stem-like cells maintenance and regulation
３．学会等名 第１８回 成体脳ニューロン新生懇談
４．発表年 2022年

１．発表者名 Takuma Yoshizawa, Kenny Daun, Tadateru Fukami, Naoko Morimura, Seiji Hitoshi
２．発表標題 Functional roles of synaptic adhesion-like molecule, LRFN2 in gliomagenesis.
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１．発表者名 Naoko Morimura, Kenny Daun, Yui Igarashi, Tadateru Fukami, Seiji Hitoshi
２．発表標題 Expression and malignant role of an extracellular matrix integrin α8β1 ligand, POEM/NPNT in glioblastoma
３．学会等名 The International Society of Neurochemistry (ISN) and the European Society of Neurochemistry (ESN)（国際学会）
４．発表年 2023年

１．発表者名 Yui Igarashi, Kenny Daun, Tadateru Fukami, Atsushi Yamada, Ryutaro Kamijo, Seiji Hitoshi, Naoko Morimura
２．発表標題 POEM/NPNT is a new malignant factor for glioblastoma
３．学会等名 The 46th Annual Meeting of the Japan Neuroscience Society
４．発表年 2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

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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