# 学 位 論 文 の 要 旨

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学 位 論 文 名　Morphological Features of Microglial Cells in the Hippocampal Dentate Gyrus of Gunn Rat: a Possible Schizophrenia Animal Model

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## 論 文 内 容 の 要 旨

### INTRODUCTION

Schizophrenia is a complex and debilitating mental disorder with a prevalence of approximately 1% worldwide. Schizophrenia is still a major challenge in psychiatry, in part because the exact etiology remains unknown.

An accumulating body of evidence point to the significance of neuroinflammation and immunogenetics in schizophrenia, characterized by an increased serum concentration of several pro-inflammatory cytokines. Interestingly, microglial activation or increased microglial cellular density has also been suggested by postmortem studies, at least in subpopulations of individuals with schizophrenia. Moreover, a pro-inflammatory immune state influences the glutamatergic neurotransmission indirectly by bits effects on the tryptophan/kynurenine metabolism.

Previous studies showed a link between hyperbilirubinemia and schizophrenia. Schizophrenia patients have a significantly higher frequency of hyperbilirubinemia relative to patients with other psychiatric disorders and the general healthy population. Based on these facts, we propose that hyperbilirubinemia may play a role in the pathophysiology of schizophrenia.

Our previous study showed behavioral abnormalities, deficits in prepulse inhibition (PPI), and neuropathological changes in Gunn rats that are similar to the characteristics of schizophrenia. The Gunn rat, a mutant of the Wistar strain, has a genetic deficiency in glucuronyl transferase and has been used as an animal model of bilirubin encephalopathy. We found that a high serum

UCB concentration has a pathogenic effect on development of the brain and concluded that the Gunn rat may be used as an animal model of schizophrenia.

UCB in the CNS is toxic to neurons and associated microglia, the resident immune cells in the CNS. However, the effects of UCB on microglia in Gunn rats have never been investigated. Therefore, in the present study, we sought to examine how microglial cells respond to UCB toxicity in Gunn rats. We hypothesized that UCB toxicity induces microglia activation and that prolonged microglial activation plays a role that makes the Gunn rat suitable as an animal model of schizophrenia. We observed the morphological features, distribution, and ultrastructural characteristics of microglial cells in adult Gunn rats. We also determined the ratio of resting/ramified cells to activated cells and examined the neuron–microglia interactions. These studies were performed on the hippocampal dentate gyrus (DG), and the results were compared to those to Wistar rats as a normal control.

## MATERIALS AND METHODS

The animals were male homozygous (j/j) Gunn rats and male Wistar rats (N = 10 each, Japan SLC, Inc.) that were 8 weeks old at the time of the experiments. Under deep intraperitoneal anesthesia, the rats were perfused transcardially. After perfusion, the brain was quickly removed, post-fixed in a solution of 4% paraformaldehyde at room temperature for 4h. Later, the brains were cut in at 50 μm thick in the frontal plane using a freezing mictrotome. Using immunohistochemical techniques, we compared the distribution, morphology, and ultrastructural features of microglial cells in Gunn rats with Wistar rats as a normal control. We used the antibody rabbit anti-Iba1 (1:4000) to determine the microglial cells, and mouse anti-CD11b (1:500) to determine the activated microglial cells. Later, using the Stereo Investigator system software, we measured the number of microglial cells, determined the ratio of activated and resting microglia and observed microglia-neuron interactions. We characterized the microglial cells in the hippocampal dentate gyrus. Later we performed the statistical analysis with the SPSS software. Differences between Gunn rats and controls were compared using the two-tailed Student's t-test with p of $<0.005$ considered to be a significant difference.

## RESULTS AND DISCUSSION

First, we revealed that Iba1-labelled microglial cells showed activated morphology in the DG

of Gunn rats. Second, during ultrastructural observation, we found that these activated cells contained enlarged areas of cytoplasm rich in organelles, and that some of them formed phagocytic pouches or engulfed large phagocytic vacuoles. Third, there was significant difference in CD11b expression areas in the DG of Gunn rats compared to controls.

When the CNS is injured, microglia rapidly shifts into an activated state and migrate to the damaged sites. Activated microglia are marked by a number of characteristic events, affecting cellular morphology, cell size, cell number, and at the molecular level, the pattern of cell surface molecules expressed (immunophenotype) as well as the pattern of cytokines and growth factors produced, which distinguish them from the resting/ramified phenotype.

An additional highly characteristic feature of microglia activation is the remarkable capacity of the microglial cell population to expand, especially in response to acute injury. Our result showed no significant difference between Iba1-labeled microglial cell numbers in Gunn rats and in controls. However, we found a significant difference in the area of CD11b expression as marker of microglial activation. These results suggest that microglial cells in adult Gunn rats showed a feature of microglial activation without expansion of the cell population.

In homozygous (j/j) Gunn rats, few signs of bilirubin toxicity are present during the first postnatal weeks. Our previous study found that blood bilirubin levels in adult Gunn rats were still high and the presence of microglia activation suggested the possibility of chronic neuronal inflammation. When acute microglial activation becomes a chronic condition following injury, the microglial cells is potentially maladaptive or neuroprotective. We suggest that chronic microglial activation in adult Gunn rats is potentially more maladaptive than neuroprotective.

Prolonged microglia activation of microglia may lead to neuronal degeneration, white matter abnormalities, decreased neurogenesis, apoptosis, and brain damage, and may thus be one of the important factors in the pathophysiology of schizophrenia

## CONCLUSION

We propose that activation of microglia could be an important causal factor of the behavioral abnormalities and neuropathological changes in Gunn rats. These findings may provide basic information for further assessment of the Gunn rat as an animal model of schizophrenia.

論文審査及び最終試験又は学力の確認の結果の要旨

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論文審査の結果の要旨

統合失調症は、複雑な病態をとり、未だ病因の不明な精神疾患である。近年、統合失調症の脳内でミクログリアを介した軽微な炎症が生じていることが注目され、「神経炎症仮説」が提唱された。申請者は既に統合失調症モデルラットと報告された、UDP-グロクロニルトランスフェラーゼ遺伝子欠失により高間接ビリルビン血症を呈するGunn ratを用いて、神経炎症の有無を精査した。Wistar ratをコントロール群とし、Gunn rat（j/j）の海馬歯状回ミクログリアの形態学的特徴を比較検討した。通常ミクログリアおよび活性型ミクログリアのマーカーとして、それぞれ抗ionized calcium binding adaptor molecule-1（Iba-1）抗体および抗CD11b抗体を用いた。その結果、統合失調症モデルラットにおいて、抗Iba-1陽性を示す通常ミクログリアの細胞数の増加は認めず、アメーバ状の活性型の形態を呈するものを多く認め、神経細胞に接着した像が高頻度に確認された。Iba-1を用いた免疫電子顕微鏡下で、貪食胞や細胞内オルガネラの増加を認め、傷害細胞を包み込む像が確認された。さらに抗CD11b陽性を示す活性型ミクログリアは統合失調症モデルラットにおいて有意な細胞数の増加を認めた。以上より、統合失調症モデルラットの海馬歯状回において慢性的な神経炎症が生じていることが明らかにされた。

本論文は非抱合型ビリルビン濃度が増加するGunn ratで、海馬ミクログリアが活性化し形態変化を生じて細胞障害性に働いていることを確認した第一報であり、統合失調症の病因への「ミクログリアを介した神経炎症」の関与を強く示唆し、発症機序を考察するうえで学術的価値の高い貴重な報告である。

最終試験又は学力の確認の結果の要旨

申請者は、統合失調症モデルとされる非抱合型ビリルビン血症ラット海馬においてミクログリアの形態変化と貪食像を詳細に分析し、活性化ミクログリアと統合失調症発症との関連を考察した。学位審査において関連分野の知識も豊富で、さらに研究を発展させており、学位授与に値する見識を備えていると判断した。（主査：長井　篤）

申請者は、統合失調症と高ビリルビン血症に関連があることに着目し、高ビリルビン血症ラット（Gunn rat）を用いて、統合失調症の病因解明を目的に海馬におけるミクログリアの形態を検討した。その結果、対照群（Wistar rat）と比較してミクログリアの活性化が生じていることを免疫組織化学的に明らかにした。本研究は、統合失調症がミクログリアを介した神経炎症により惹起されることを示唆するもので、学位授与に相応しいと判断した。（副査：関根浄治）

申請者はGunn ratを用いて、海馬におけるミクログリアの形態変化を詳細に検討し、その活性化が起こっていることを証明した。このことは神経炎症が統合失調症の発症機序の一つで、海馬ミクログリアがそこに関与していることを示唆するものであり、治療法につながる高い学問的価値を有する結果と考えられるため、学位授与に値すると判断した。（副査：丸山理留敬）

（備考）要旨は、それぞれ４００字程度とする。