氏　　名　Banerjee, Piu

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学 位 論 文 題 目　Study of Spontaneous Activity in the Peripheral Structure of Developing Mouse Somatosensory System

論 文 審 査 委 員　主　査　平田 たつみ
遺伝学専攻　教授
木村 暁
遺伝学専攻　教授
小出 剛
遺伝学専攻　准教授
久保 郁
遺伝学専攻　准教授
吉村 由美子
生理科学専攻　教授

(Form 3)

Summary of Doctoral Thesis

Banerjee, Piu

Title Study of Spontaneous Activity in the Peripheral Structure of Developing Mouse Somatosensory System

Spontaneous activity is the activity that occurs without any external sensory input. During the early postnatal period, the nascent sensory cortices exhibit correlated spontaneous activity, which is believed to be crucial for the establishment of precise and mature neural circuits following the Hebbian principles of plasticity. In the mouse somatosensory system, our lab's previous study revealed that during the first postnatal week, the layer 4 excitatory neurons in the somatosensory cortex (barrel cortex) show spontaneous activity where neurons within the same barrel fire together, thereby giving a patchwork-like appearance. Little is known about where this spontaneous activity originates from. Based on studies by our lab, I hypothesized that the trigeminal ganglion (TG), which harbors the cell bodies of the sensory neurons innervating the whisker-pad, is the source of the patchwork-type spontaneous activity observed in postnatal day 5 (P5) mouse barrel cortex. Alongside the developing barrel cortex, the mouse visual and auditory cortices also exhibit spontaneous activity during the early postnatal period, which is known to originate from their respective peripheral structures- the retina and cochlea, which exhibit spontaneous activity themselves. However, to my knowledge, no study had uncovered whether the peripheral neurons of the developing somatosensory system fire spontaneously.

To elucidate this, I established an *ex vivo* system to perform calcium

imaging in neonatal TG using the *Avil*-Cre:GCaMP6s mouse, which expresses the calcium indicator GCaMP6s in the peripheral sensory neurons. Additionally, I generated and characterized a bacterial artificial chromosome transgenic mouse line that expresses the red fluorescent protein (RFP) in the nuclei of TG neurons (*Avil*-nlsRFP mouse). Using this mouse, I saw the distribution of sensory neurons in the TG. To identify the region in TG that has neurons innervating the whisker pad, I placed DiI crystals in different whisker rows and saw subsequent dye localization. Consistent with previous findings, I observed there to be a whisker-row dependent topography in the TG, and could thereby identify the whisker-innervating region. I then performed *ex vivo* calcium imaging in the whisker-innervating region of TG isolated from P5 *Avil*-Cre:GCaMP6s mouse and discovered clear spontaneous activity.

Majority of neurons fired infrequently and did not exhibit obvious oscillatory calcium transients. There was no noticeable pattern among most active neurons. However, a small percentage (1.9%) of neuronal pairs showed high correlation (>0.5), and the median distance between the correlated neuronal pairs was significantly less than that between the non-correlated neuronal pairs. To compare spontaneous activity in TG across different stages of development, alongside the P4-P6 stage, I also performed calcium imaging in P0-P1, P14-P16, and adult (>P60) TG. I observed clear spontaneous activity during the first two postnatal weeks, however, it was mostly diminished in adult TG. This indicated that spontaneous activity in TG is likely a hallmark of the early postnatal period. The peripheral sensory neurons are classified based on their soma-diameter into small-diameter (SD) (<20 um), medium-diameter (MD) (20-25 um), and large-diameter (LD) neurons (>25 um). In the neonatal TG, >90% neurons had SD-MD, and <10%

neurons had LD. To decipher if the spontaneously firing neurons in TG have a sub-type specificity, I measured the diameter of the spontaneously firing cells and found that >80% of these have MD-LD, which is a characteristic of the mechanosensory neurons. Therefore, spontaneously firing neurons in P4-P6 TG seem to exhibit sub-type specificity.

Lastly, to elucidate the mechanism of spontaneous activity in P4-P6 TG, I performed RNA-Sequencing of P5 and P15 TGs. Based on high absolute expression in these two stages, and literature survey, I selected *P2RX3* as a candidate gene. I generated its global knockout mouse, which however, showed clear spontaneous activity in the P4-P6 TG. Parallelly, I tested the broad pharmacological inhibitors of glutamatergic, purinergic, GABAergic, cholinergic, and glycinergic receptors *ex vivo*. None of these had any obvious effects in perturbing the spontaneous activity in TG, suggesting that the activity might be generated in a cell-intrinsic manner. Next, I chelated the extracellular calcium using EGTA and found that it almost completely blocked the spontaneous activity *ex vivo,* indicating that extracellular calcium is crucial for the generation of spontaneous activity in P4-P6 TG.

In conclusion, this study provides the first evidence of the occurrence of spontaneous activity in the peripheral structures of the developing somatosensory system. Spontaneous activity in TG is observed during the first two postnatal weeks, and majorly diminishes by adulthood. Spontaneous activity in P4-P6 TG displays a neuronal sub-type specificity, and is majorly blocked by chelation of extracellular calcium. These findings will help further our understanding of the role of spontaneous activity in the development and establishment of precise and mature sensory circuits.

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Results of the doctoral thesis defense

博士論文審査結果

Name in Full
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生後間もない動物において、一部の脳領域では外界からの刺激がなくとも神経細胞が自発活動しており、この活動が将来の正確な神経回路の構築に重要であると考えられている。Banerjee さんの研究室では、生後１週目のマウス体性感覚皮質のバレル野４層において、パッチワーク状の神経細胞の自発活動が起きることを見出している。バレルは洞毛１本 1 本からの入力を処理する単位であり、感覚入力なしに個々のバレル神経細胞が同期して活動するということは、何らかの発信源が動物個体内に存在することを意味している。

Banerjee さんは末梢の三叉神経節がバレル野の自発活動の発信源ではないかと考えた。その仮説を確かめるために、三叉神経節でカルシウムインジケーターGCaMP6s 遺伝子を発現するマウスを作出し、そこから摘出した三叉神経節細胞の神経活動を生体外で光学的に観察する系を確立した。そしてこの系を用いて、洞毛を支配する領域の三叉神経節細胞が実際に自発的神経活動を示すことを明らかにした。この自発活動は生後 1 日目から２週間目までのマウスで顕著に観察されるが、成体マウスの三叉神経節ではほとんど観察されず、生後発達期特異的にバレル野で観察されるパッチワーク状の自発活動への関与が示唆される。

さらに Banerjee さんはこの系を用いて三叉神経節細胞の自発活動について解析を進め、1）三叉神経節内に存在する神経細胞同士には、多くの場合、自発活動の時空間的パターンに相関が認められないこと、2）ごく一部の神経細胞のペアのみが同期活動を示すが、それらにおいて細胞体の距離が近い傾向が認められること、３）中―大の細胞体の大きさをもつ機械受容性の神経細胞タイプが主に自発活動を示すこと、を明らかにした。

自発活動の分子機構の解析として、自分で行った RNA 解析の結果から生後発達期の三叉神経節で特に強い発現が認められた *P2RX3* プリン受容体に注目してノックアウトマウスを作成したが、残念ながら三叉神経節の自発活動に変化は認められなかった。薬理学的解析からも自発活動に対する各種神経伝達物質の寄与は認められず、唯一細胞外カルシウムが自発活動には不可欠であることがわかった。以上の結果から、Banerjee さんは、三叉神経節細胞の自発活動は細胞自律的な機構により引き起こされる可能性

を指摘している。

以上のように、Banerjee さんは興味深い生命現象をみつけ、独自のアプローチにより数々の新規発見を得ている。今後の神経科学研究に貢献する成果であり、博士号授与の要件を満たすと委員会で判定した。