様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

# 科学研究費助成事業　　研究成果報告書

令和　４ 年　 ９ 月　 ８ 日現在

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研究種目：若手研究
研究期間：2019～2021
課題番号：１９Ｋ１６２６１

研究課題名（和文）Structural-Functional Development of the Olfactory System

研究課題名（英文）Structural-Functional Development of the Olfactory System

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,100,000円

研究成果の概要（和文）：新生児鼠に匂いを届けることができるアウェイクイメージングセットアップをセットアップすることができました。 子犬の呼吸も測定できました
イメージング期間中も。
私たちが発見したのは、P2はP6よりも広く調整されているということでした。つまり、P6鼠はより区別できるはずです。さまざまな匂い。 さらに、dF/F信号は、P2の信号と比較した場合、P6の方がはるかに強力でした。 これは私たちがチューニングの変更ではなく、より小さなイベントを検出できる（またはチューニングが開発によってマスクされている）。同僚と今、この年齢で僧帽細胞の電気物理的特性を調査し始めました。

研究成果の学術的意義や社会的意義
私たちは、生体内カルシウムイメージングで糸球体層の匂い反応を記録した最初の研究室でした。 私たちの調査結果は、僧帽細胞の匂い応答の発達に関与する未知数がたくさんあることを明らかにしました。 特に糸球体層のシナプス接触と
社会的意義
私たちは、出生時に匂いがどのように知覚されるかを調べることに興味がありました。 生後1週間で匂いの感度に大きな変化があることがわかりました。 次に、この最初の週に何が変化しているかを調べたいと思います。

研究成果の概要（英文）：We were able to set up an awake imaging set up that could deliver odour to neonatal mice. We were also able to measure the breathing of thepup
during the imaging period too. What we discovered was that P2 mice were broadly more tuned than P6 mice meaning that P6 mice should be more able to discriminate between different odours.
Additionally, the deltaF/F signals were much stronger at P6 when compared to those at P2. This may have meant that we were able to detect smaller events rather than a change in tuning (or that the tuning is masked by development). fter sharing my findings with a colleague we have now begun to explore the electrohpysical properties of mitral cells at this age. Without this grant such a study would not have been made.

研究分野：神経科学

キーワード：Neurosciece　Olfaction　Development

## １．研究開始当初の背景

Our previous work showed the importance of spontaneous activity for mitral cell pruning. During normal development mitral cells change from having multiple primary dendrites going multiple glomeruli, to just having a single primary dendrite innervating a single dendrite (figure 1).

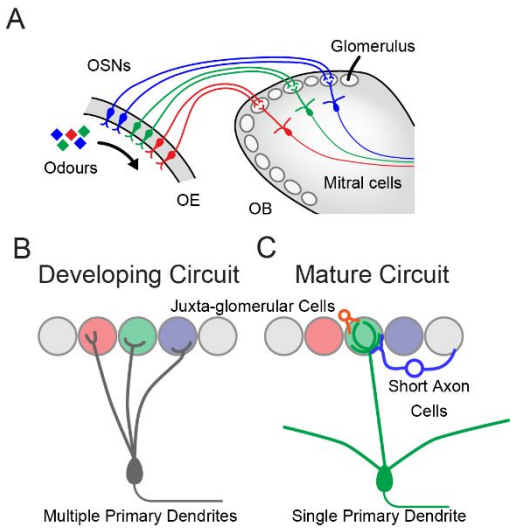


**Figure 1** – Anatomical development of mitral cells in the OB.

## ２．研究の目的

Our aim was to investigate the consequences of circuit changes and find out what changes during development. Specifically we asked the following questions...

1. Do changes in odour perception occur in development?
2. Do they reflect the changes in circuity taking place?
3. Can we link neural structure and function?

To do this we aimed to...

1. Create the first developmental profile of odour perception
2. Identify the purpose of circuit changes, by altering circuit development, and observing the effects.

## ３．研究の方法

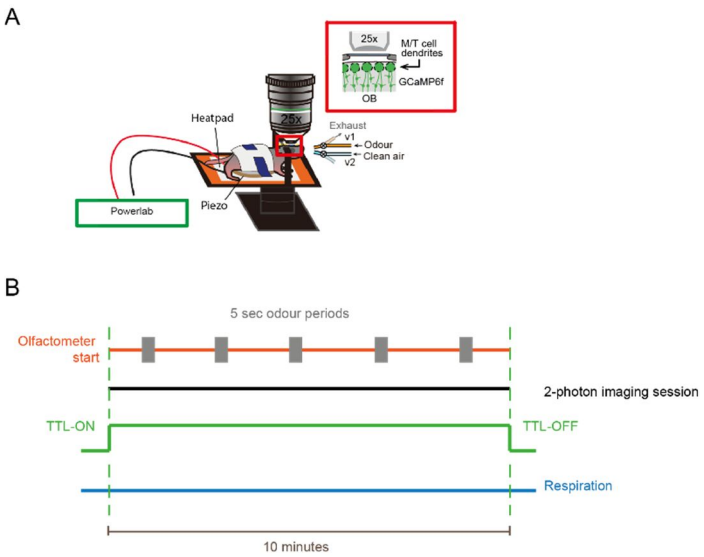


**Figure 2** – Our *in-vivo* imaging set up and experimental schema to record from mitral cells

As we have developed an awake in-vivo imaging set up and we already had the Thy1-GCaMP6f mice which express GcAMP6f exclusively in mitral cells in the olfactory bulb (OB), for our initial explorations we used Calcium imaging. However, to also record the respiration of neonates we developed a piezo electric transducer to record the respiration of neonatal mice (figure 2). We also built an olfactometer to deliver odours to the mouse.

To discover the importance of anatomy and function we also tried to alter the spontaneous activity patterns by using channel rhodopsin that was expressed in all mitral and tufted cells (figure 3). We then devised a method to hand-rear pups, which allowed us to stimulate pups for a full 5 days (the whole pruning process, P1 to P6). This would serve as the basis for turning to observe what happens

with our altered dendritic pruning mitral cells. In short we hope to alter the anatomy, and then uncover differences in the responses of mitral cells after their altered activity. Naturally, this will be don if our prediction that the differences in altered activity patterns does alter the pruning process of mitral cell dendrites.

## 4．研究成果

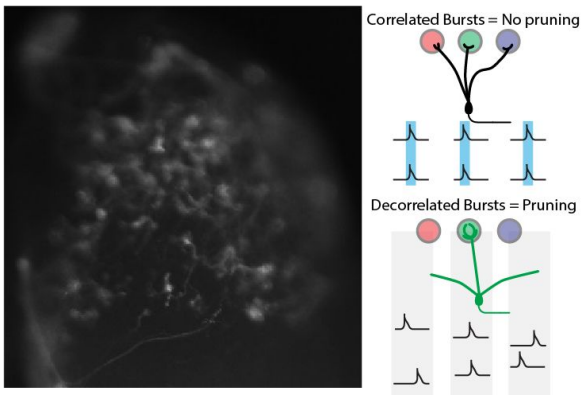


**Figure 3** – Channel Rhodopsin used to change activity patterns and so alter anatomy

Our first set was to create a developmental profile of odour responses (figure 4). We delivered 6 chemicals at a high concentration to see if we could notice any change in amplitude of the responses to odour. However, in large we found that the amplitude of responses was highly variable depending on the quality of the cranial window, the level of GCamP6f expression as well as any possible developmental changes. In short our proposed methodology was not adequate to accurately determine changes in mitral cell odour responses during development. In the future, understanding what is changing synaptically may be of more use.

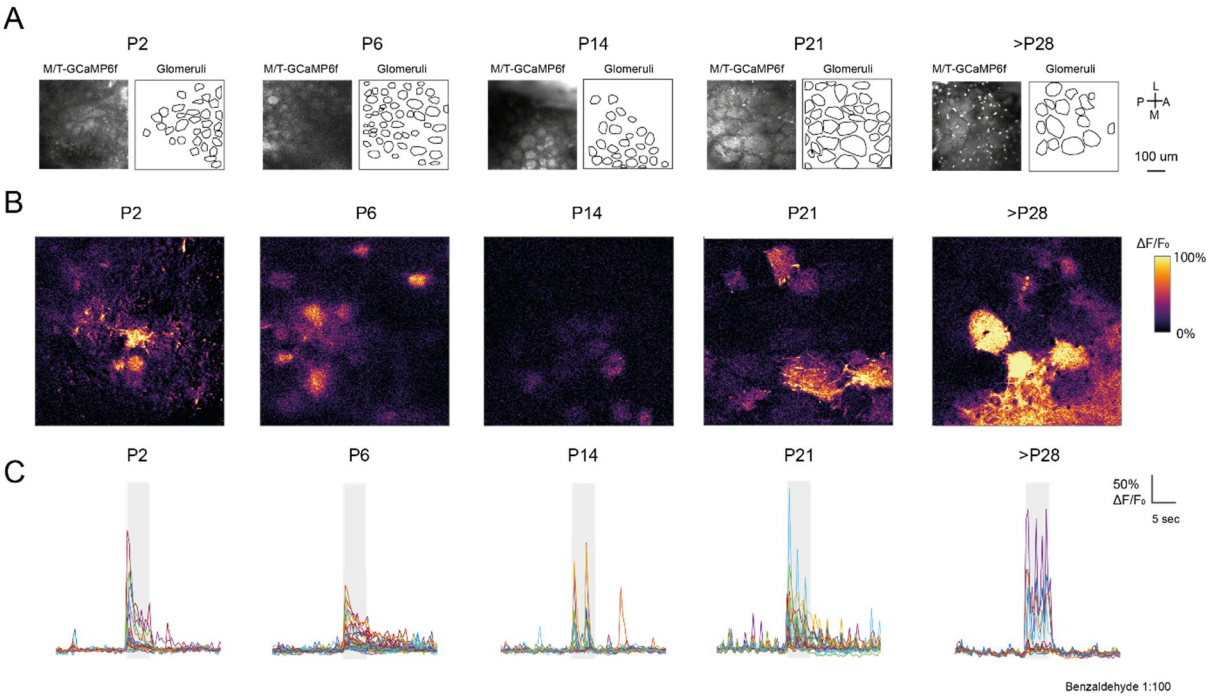


**Figure 4** – Odour response profiles of mice throughout development. In this example, a 1:100 dilution of benzaldehyde was delivered, and we can see the emergence of stronger responses as mice get older.

We also tried to create tuning profiles for ages P2 and P6, this essentially asks how many odourants are glomeruli responsive to (figure 5). We assessed both tuning in response to 3 different odourants (top row) and responses to 6 different odourants (bottom row). To try to compensate for the variations in signal to noise we evaluated tuning based on any response (mean + 3sd, left column), strong responses (mean +10sd, middle column) and weak responses (>/= mean +3sd and < mean+10 sd, right column). Although we could see significant differences for the weak responses where P2 glomeruli were more likely to respond weakly to our initial investigation with 3 odourants. Once we

extended it to 6 different odourants there differences in tuning were not seen. Likewise when analysing strong responses, we could really only see these emerge at P6, with significantly fewer P2 glomeruli existing or responding (this is likely due to synaptic strengthening rather than the dendritic puring that happens between P2 and P6). These experiments highlight the need for key electrophysiological investigations into the synaptic properties of mitral cells during development.

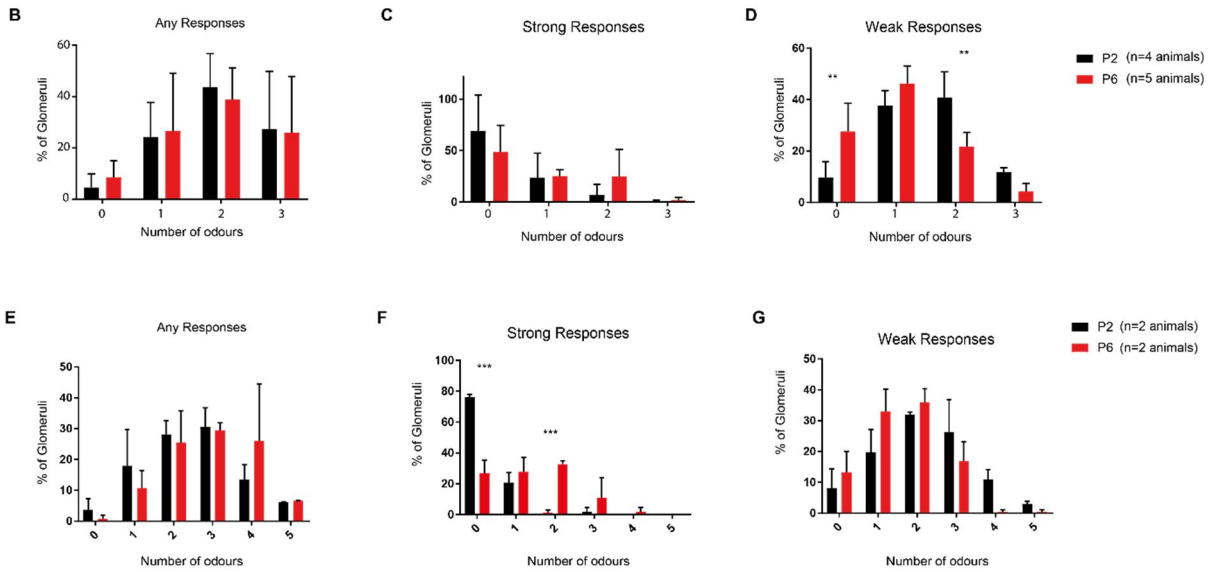


**Figure 5 -** Odour tuning differences between P2 and P6

Perhaps if we could prevent dendritic pruning we could investigate the differences between mitral cells of the same age but only with differences in dendritic architecture? To do this we controlled the electrical activity of mitral cells, using channel rhodopsin 2. Specifically we cuase mitral cells to fire at the same rate as stage I spontaneous activity (Fujimoto, Leiwe, et al 2019). To do this we developed a method of hand-rearing pups. However, we did not find any significantly different changes in the pruning of mitral cells despite the pattern of electrical activity changing (figure 6).

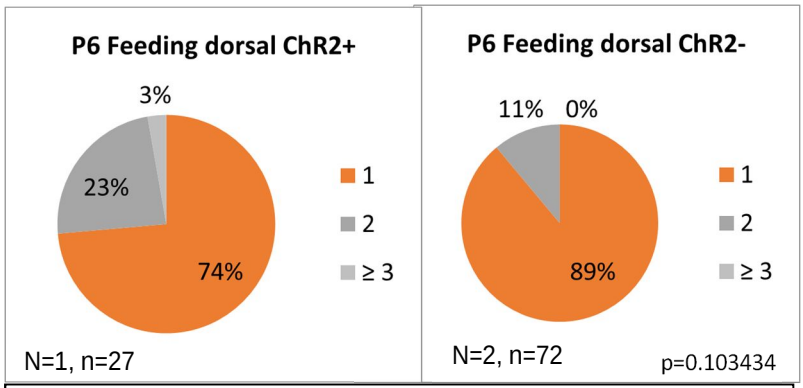


**Figure 6** – Spontaneous activity patterns are not necessary to control mitral cell dendrite pruning

While disappointing, this provided additional information of the exact role of spontaneous activity. This forced us to look at what downstream cascades are triggered by neuronal activity. Something that will form the basis of a future research project.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　0件／うち国際共著　0件／うちオープンアクセス　0件）

| | |
|---|---|
| １．著者名<br>Leiwe Marcus N.、Fujimoto Satoshi、Imai Takeshi | ４．巻<br>15 |
| ２．論文標題<br>Post hoc Correction of Chromatic Aberrations in Large-Scale Volumetric Images in Confocal Microscopy | ５．発行年<br>2021年 |
| ３．雑誌名<br>Frontiers in Neuroanatomy | ６．最初と最後の頁<br>1-10 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.3389/fnana.2021.760063 | 査読の有無<br>無 |
| オープンアクセス<br>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<br>－ |

〔学会発表〕　計7件（うち招待講演　3件／うち国際学会　1件）

| |
|---|
| １．発表者名<br>Marcus Leiwe, Satoshi Fujimoto, Shuhei Aihara, Takeshi Imai |
| ２．発表標題<br>Spontaneous Activity Generated within the Olfactory Bulb Establishes the Discrete Wiring of Mitral Cell Dendrites |
| ３．学会等名<br>International Symposium On Olfaction and Taste（招待講演）（国際学会） |
| ４．発表年<br>2020年 |

| |
|---|
| １．発表者名<br>Marcus Leiwe, Satoshi Fujimoto, Takeshi Imai |
| ２．発表標題<br>Spontaneous activity generated within the olfactory bulb establishes the discrete wiring of mitral cell dendrites |
| ３．学会等名<br>Current Trends and Future Directions of Synapse-Circuit Plasticity Research |
| ４．発表年<br>2019年 |

| |
|---|
| １．発表者名<br>Marcus Leiwe, Satoshi Fujimoto, Marlieke Van Erp, Takeshi Imai |
| ２．発表標題<br>Spontaneous and Evoked Activity in the Awake Neonatal Mouse Olfactory Bulb |
| ３．学会等名<br>International Symposium on Molecular and Neural Mechanisms of Taste and Olfactory Perception |
| ４．発表年<br>2019年 |

１．発表者名
Marcus Leiwe, Satoshi Fujimoto, Takeshi Imai

２．発表標題
Spontaneous and Evoked Activity in the Developmental Olfactory Bulb

３．学会等名
Japan-UK Neuroscience Symposium

４．発表年
2019年

１．発表者名
Marcus Leiwe, Satoshi Fujimoto, Takeshi Imai

２．発表標題
Super-multicolour labelling with automated neuronal reconstruction without tracing

３．学会等名
CREST Risning Star Ceremony（招待講演）

４．発表年
2021年

１．発表者名
Marcus Leiwe, Satoshi Fujimoto, Takeshi Imai

２．発表標題
Super-multicolour labelling with automated neuronal reconstruction without tracing

３．学会等名
Hong Kong City University（招待講演）

４．発表年
2021年

１．発表者名
Marcus Leiwe, Satoshi Fujimoto, Takeshi Imai

２．発表標題
Lateral inhibition signals for synaptic competition

３．学会等名
Spont 2022（招待講演）

４．発表年
2022年

〔図書〕　計0件

〔出願〕　計1件

| 産業財産権の名称<br>解析方法、解析装置およびプログラム | 発明者<br>Leiwe, Fujimoto, Imai | 権利者<br>同左 |
|---|---|---|
| 産業財産権の種類、番号<br>特許、2021-122029 | 出願年<br>2021年 | 国内・外国の別<br>国内 |

〔取得〕　計0件

〔その他〕

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６．研究組織

| | 氏名<br>（ローマ字氏名）<br>（研究者番号） | 所属研究機関・部局・職<br>（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 |
|---|---|