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研究課題名（和文）The Emergence and Control of Olfactory Circuits in Development

研究課題名（英文）The Emergence and Control of Olfactory Circuits in Development

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研究成果の概要（和文）：開発中に匂いの認識がどのように変化するかを理解したい。 我々はマウスを使い発達中に嗅球回路にいくつかの変化があることを発見した。 まず、この変化はNMDA受容体に依存しており、この受容体をKOすることで活動のパターンを変えるだけでなく、僧帽細胞樹状突起剪定を防ぐことができた。
次に2光子Caイメージングで嗅球を観察しながら赤ちゃんや成体マウスに匂いを与えることによって、発達中の嗅覚がどのように変わるのかを調べている。 現在、取得したデータを解析しているところである。 さらに、我々はオプトジェネティクスを用いて僧帽細胞の発達を乱そうとしたが、その研究は失敗した。

研究成果の学術的意義や社会的意義
These finding will be of significance to the wider scientific community as it begins to explore how a functioning sensory system adapts during its development. We determined that while as previously claimed mice can smell from birth, the nature of what they perceive is different to that of the adult

研究成果の概要（英文）：We wanted to understand how the perception of odour changes during development. By using mice we could see that there were several changes that occurred in the olfactory bulb circuitry during development. Firstly, we found that this activity relies on the NR1 subunit of the NMDA receptor, and by knocking out the sub-unit you not only change the pattern of activity, but you prevent mitral cell dendrite pruning.
We then went on to try to explore what changes in olfactory perception during development by delivering odours from baby-adult mice while imaging the olfactory bulb with 2 photon calcium imaging. We are currently, processing the data in order to determine the effect of the differences and whether the two forms of odour encoding occur seperately or together and when they occur. Additionaly, we tried to perturb the development of mitral cells with optogenetics, however, those studies were unfruitful.

研究分野：Neuroscience

キーワード：Olfaction　Olfactory System　Sensory Systems　Olfactory Bulb　Mitral Cells　Neurodevelopment

１．研究開始当初の背景

For many species early sensory experience is critical for their survival. This is particularly the case for mammals which are often born blind and deaf. In particular, the mouse only begins to hear at post-natal day 6 (P6) and their eyes only open at P12. Therefore, the olfactory system of the mouse is vital in order for the pup to locate its mother, and therefore its source of food.
The olfactory system of the mouse is a highly organised whereby olfactory sensory neurons (OSNs) that detect the same molecule project to the same glomeruli in the olfactory bulb (OB). In each glomerulus OSNs synapse onto mitral and tufted cells (M/T cells) which are the principal output neurons of the OB (Figure 1A). M/T cells are highly specific and in the adult only project to a singular glomerulus.

This means that the olfactory system is a highly specific, finely tuned system. However, the OB is not fully mature at birth. In fact, during development the neonatal OB is a dynamic system that undergoes several critical changes (Figure 1B-C). For example, during the first post-natal week mitral cells prune their primary dendrites from innervating multiple glomeruli to retaining a single primary dendrite that projects to a single glomerulus. Other changes include the emergence of interneurons in the glomerular layer, and the ability of OSNs to respond to airflow stimulation.

We wanted to explore what controls this development, and also begin to explore what changes happen to olfactory perception during this period.

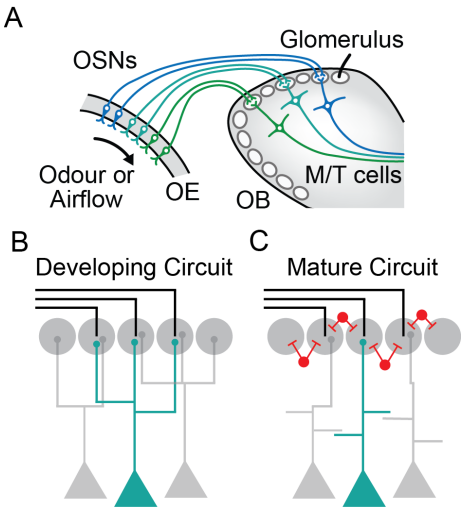

Figure 1 – Schematic of the mouse olfactory system. (A) Odours and airflow travel through the nostril and are detected by olfactory sensory neurons (OSNs) which then project to glomeruli the olfactory bulb (OB). Each glomerulus represents a single molecule and is innervated by 20-25 mitral and tufted cells (M/T cells). **(B-C)** After birth the OB circuits still develop. M/T cells (Triangles) prune their dendrites to connect to a single glomerulus. Additionally, interneurons migrate to the glomerular layer of the OB.

2．研究の目的

In order to retain the focus on our investigation we proposed concentrating on 2 specific questions relating to odour perception

1. How does mitral cell pruning relate to the olfactory acuity?

Our previous research uncovered the mechanisms that underlie the pruning of M/T cell dendrites in the OB. This mechanism is the change from correlated (Stage I) to de-correlated (Stage II) patterns of spontaneous activity (Figure 2A, manuscript in preparation). To demonstrate this, we established an in-vivo imaging set up that allowed us to record neuronal activity through genetically expressed calcium indicators (GCaMP6f) in the neonatal mice (P1-P12). Our hypothesis was that this pruning is vital for improving the odour acuity of M/T cell responses by reducing the number of glomeruli that each M/T cell innervates (Figure 2B). We wanted to demonstrate this effect by preventing the pruning process from taking place (through optogenetics) and then demonstrate the corresponding loss of olfactory acuity.

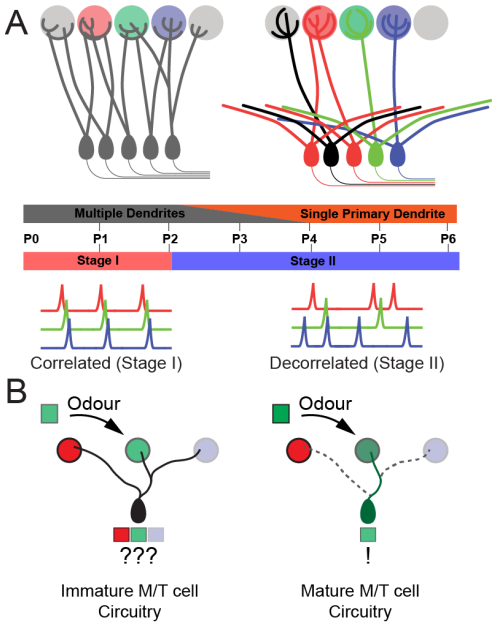

Figure 2 – Mitral Cell Dendrites Prune to Improve Odour Acuity. (A) Schema for normal mitral cell development. **(B)** Our hypothesis that M/T cell pruning improves olfactory acuity.

2. When does the phase code emerge?

Our second area of interest is in the emergence of phase coding. In the adult mouse odour is encoded in two main ways; the rate code, and the phase code. The rate code can be described as an increase in the number of action potentials fired by a M/T cell. Whereas the phase code relies on temporal shifts in oscillatory activity to represent odours. These shifts require a baseline pattern of phasic activity (θ oscillations), which is driven by OSNs that are also sensitive to airflow sensation (Figure 3, Iwata et al 2017). However, our preliminary investigations into the neonate could not find this oscillatory activity. We propose to identify the time point by creating a developmental timeline of odour responses. Furthermore, as our modelling data suggests that interneurons are also necessary for this phasic activity we will alter interneuron activity and see whether we can revert the olfactory system back to an immature state.

Figure 3 – Schematic of Phase Coding. Illustrating how temporal patterns in M/T cells are established by airflow but shifted in the presence of odour

３．研究の方法

This research mostly focussed on 2-photon calcium imaging of the neonatal mouse

We also created a new method to hand-rear pups independently of their mother (Leiwe et al., submitted to Bio-protocols)

Additionally, we did some confirmatory field recordings (*in-vivo electrophysiology)*

４．研究成果

As described in form F-7-2, during the last two years, several achievements have been made.

Firstly, we were able to uncover a key role for the NR1 subunit in mitral cell dendrite pruning. Not only does this retain the multiple dendrites but it also changes the pattern of spontaneous activity. Highlighting the key link between structure and function.

Secondly, we obtained preliminary data for olfactory development. To do this we built an odour delivery machine that was able to sync to our 2 photon microscope, then in conjunction with this we devised a method of recording the respiration rate of neonatal mice down to P2. We are able to analyse these three systems simultaneously and so can measure the responses in relation to actual inhalation time. Then by selecting three key odours we have begun to look at the key changes in the patterns of odour responses at different ages. We can see how maximal responses change as well as the underlying circuit activity. Further analysis is underway in order to assess the link to respiration, in other words the phase code.

Thirdly, using the triple mutant DAT-Cre-TeNT-Thy1 GCaMP6f mice we have analysed the effects of inhibiting dopaminergic activity on odour responses. Unfortunately, as these mice die at P21 our investigations will have to be matched to this juvenile age.

Finally, we have been able to create a way of hand rearing pups to normal development. This allowed us to carry out further manipulations that would have resulted in the maternal rejection of the pups.

５．主な発表論文等

〔雑誌論文〕（計 ２ 件）

① Bright multicolor labeling of neuronal circuits with fluorescent proteins and chemical tags.
Sakaguchi R, Leiwe MN, Imai T
Elife 2018 10.7554/eLife.40350.

② In vivo two-photon imaging of the embryonic cortex reveals spontaneous ketamine-sensitive calcium activity
Yuryev M, Andriichuk L, Leiwe M, Jokinen V, Carabalona A, Rivera C
Scientific Reports 2018 10.1038/s41598-018-34410-x

〔学会発表〕（計　5　件）

① Marcus Leiwe

Bright multicolour labelling of neuronal circuits with fluorescent proteins and chemical tags

International Symposium of Brain/MINDS (ISBM) 2019

② Marcus Leiwe

Spontaneous Activity of the Olfactory Bulb Establishes the Discrete Connectivity of Mitral Cell Dendrites

AMED-MRC Neuroscience Symposium 2019

③ Marcus Leiwe

Spontaneous Network Activity in the Awake Neonatal Mouse Olfactory Bulb

International Symposium on Molecular and Neural Mechanisms of Taste and Olfactory Perception 2018

④ Marcus Leiwe

Spontaneous Activity and Development in the Neonatal Mouse Olfactory Bulb

UK Semiochemistry Network Annual Meeting（招待講演）2018

⑤ Marcus Leiwe

Spontaneous Network Activity in the Neonatal Mouse Olfactory Bulb Regulates Dendrite Pruning of Mitral Cells

International Symposium on Molecular and Neural Mechanisms of Taste and Olfactory Perception, 2017

６．研究組織