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海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人 日本学術振興会 理事長　殿

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受 付 番 号　767
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（氏名は必ず自署すること）

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：　マクデブルク　（国名：　ドイツ　）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
アミノ酸報酬の学習を支える神経回路網の行動遺伝学的解析

3. 派遣期間：平成　29　年　4　月　5　日　～　平成　31　年　4　月　4　日

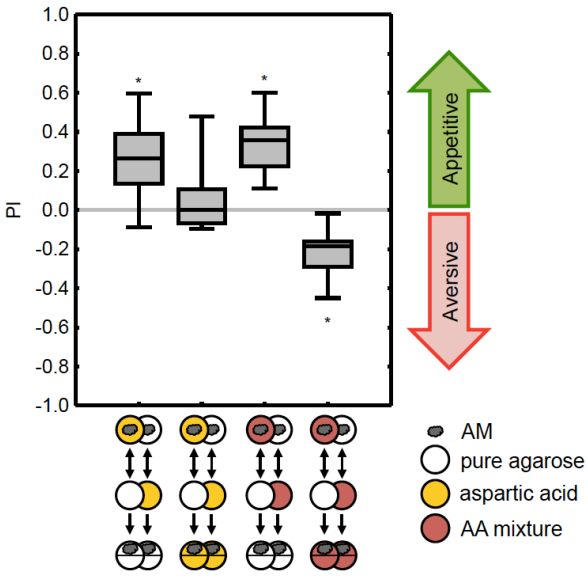
4. 受入機関名及び部局名
ライプニッツ神経生物学研究所、遺伝学部門

5. 所期の目的の遂行状況及び成果…書式任意　**書式任意（A4 判相当 3 ページ以上、英語で記入も可）**
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

Although amino acids are important nutrients for *Drosophila melanogaster*, how flies detect amino acids and how behaviour towards amino acids is regulated is largely unknown. Previously Toshima and Tanimura (2012) found that adult *Drosophila* enhance their feeding preference to amino acids, but not to sugar, when deprived of amino acids. Different from the adult flies, which can survive without obtaining amino acids, larvae continuously require ingesting protein source for growth. Larval brain consists of relatively small number of neurons, which are ten times fewer than adult brain. Nevertheless, larvae are intelligent enough to exhibit associative learning of odours and taste stimuli. Given that learning about tastants should be related to feeding motivation, it is intriguing to ask whether larvae show reward learning to amino acids. The mushroom body (MB) is the memory centre of insect brain. It receives input from various sensory modalities, integrates information and forms associative memory. The aim of this study is to reveal the neuronal circuits for amino acid processing from the sensory periphery towards the central brain.

What can larvae memorize about amino acids?

Figure 1



In collaboration between my previous lab and current host lab, we performed learning experiments for 20 individual amino acids, and found that larvae learn all individual amino acids as reward (Kudow et al. 2017). This result is interesting because not all amino acids are attractive when innate taste preferences are tested (Croset et al. 2016; Kudow et al. 2017). These results raise a question whether all 20 individual amino acids are detected by a single amino acid-reward sensor.

First I asked whether also a mixture of 20 amino acids works as a reinforcer, and if so whether such mixture is rewarding or punishing. I used standard associative learning paradigm that was established by the host lab (Gerber and Hendel 2006). Groups of larvae were trained either paired or unpaired association of n-amylacetate (AM) odour and tastant, and then tested the preferences for AM either in the presence of or the absence of the tastant. Performance index (PI) was calculated from preferences of reciprocally trained groups. Positive value indicates that appetitive memory is expressed, and negative value indicates that aversive memory is expressed.

Schleyer et al. (2015) previously showed that 10 mM aspartic acid induced appetitive memory when larvae were tested on a pure agarose dish, and that this appetitive memory expression was blocked when larvae were tested in the presence of aspartic acid. An amino acid-mixture, which contains 20 amino acids at 10 mM total concentration, also induced appetitive memory. However, larvae showed aversive memory when they were tested in the presence of amino acid-mixture, indicating that an amino acid-mixture works not only as reward, but also as punishment (Figure 1). These results are surprising because individually all amino acids induce appetitive memory (Kudow et al. 2017). I performed several parametric experiments, such as dose dependency, different number of training trials and retention intervals of different length. For a further investigation towards identifying whether any one individual amino acid would be responsible for the punishing effect, I used an essential amino acid and a non-essential amino acid-mixture as reinforcer. The result suggests that the essential amino acids are largely responsible for both appetitive and aversive memory of amino acids. I then started screening of individual essential amino acids to find a single punishing amino acid, and found that tryptophan can induce both appetitive and aversive memory.

These results are now prepared for publication. As next step, I am planning to use a genetic tools to silence different subsets of dopaminergic or octopaminergic neurons that are known to be necessary for either reward or punishment learning, in order to identify the central neuronal circuit for appetitive and aversive memory induced by single amino acid.

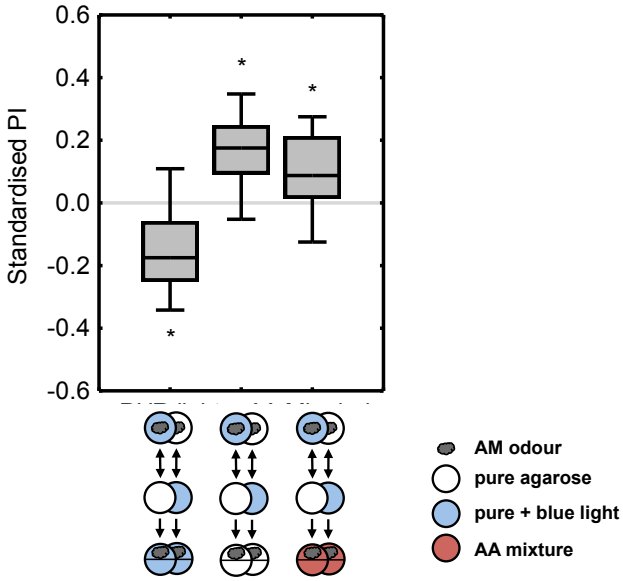
Searching for peripheral amino acid-sensing neurons through an optogenetic approach

Croset et al. (2016) reported that an ionotropic receptor, IR76b, is necessary for innate taste preference for amino acids, and IR76b neurons respond to amino acid stimuli. To investigate the necessity of IR76b neurons for amino acid learning, I tested larvae in which IR76b-expressing neurons were silenced by *kir* transgene expression, using the Gal4/UAS system. Since aspartic acid is one of the amino acids that activates IR76b neurons and also IR76b mutant flies show reduced

aspartic acid preference (Croset et al. 2016), I first tested innate aspartic acid preference. Indeed the larvae showed reduced aspartic acid preference. This result confirms that IR76b neurons are necessary for innate aspartic acid preference. I next tested the necessity of IR76b neurons for aspartic acid learning. Appetitive learning of aspartic acid was intact in the flies, suggesting that distinct sensory neurons might contribute to preference for and appetitive learning about aspartic acid.

In contrast to aspartic acid, innate taste preference for an amino acid-mixture was intact in the experimental animals, whereas appetitive learning was impaired. These results likewise suggest that distinct sensory pathways mediate innate preference and appetitive learning, this time of the amino acid mixture. Of note, aversive learning of an amino acid-mixture was intact, indicating that IR76b neurons might mainly detect appetitive amino acids.

Figure 2



Optogenetic tools allow us to see whether artificial activation of particular neurons can induce memories. I used larvae in which IR76b neurons are expressing Channel Rhodopsin (ChR2-XXL), so that I am able to temporarily activate IR76b neurons by presenting blue light. Larvae received paired or unpaired presentation of odour and light, and then tested the odour preferences either under dark, light or amino acid-mixture conditions (Figure 2). Figure 2 shows normalized memory scores of experimental genotype by subtracting the scores of genetic controls. Relative to genetic controls, experimental larvae showed aversive memory when they were tested under light and showed appetitive memory when they were tested in darkness, meaning that activation of IR76b neurons can provide both appetitive and aversive memory. This is also a novel discovery that activation of sensory gustatory neurons substitutes reward and punishment in larvae. To test whether appetitive memory expression is abolished and/ or aversive memory facilitated in the presence of real taste, larvae were also tested on the amino acid-mixture dish. The larvae still showed appetitive memory, meaning that optogenetic activation of IR76b neurons is not equivalent to amino acid-mixture taste for larvae at least under this condition.

In conclusion IR76b neurons are involved in amino acid sensing and learning. Particular combinations of IRs might be important for amino acid sensitivity, since IR76b is broadly expressing among a variety of gustatory receptor neurons. Further study will be necessary to further narrow down the specific sensory inputs for specific amino acids and their mixtures.

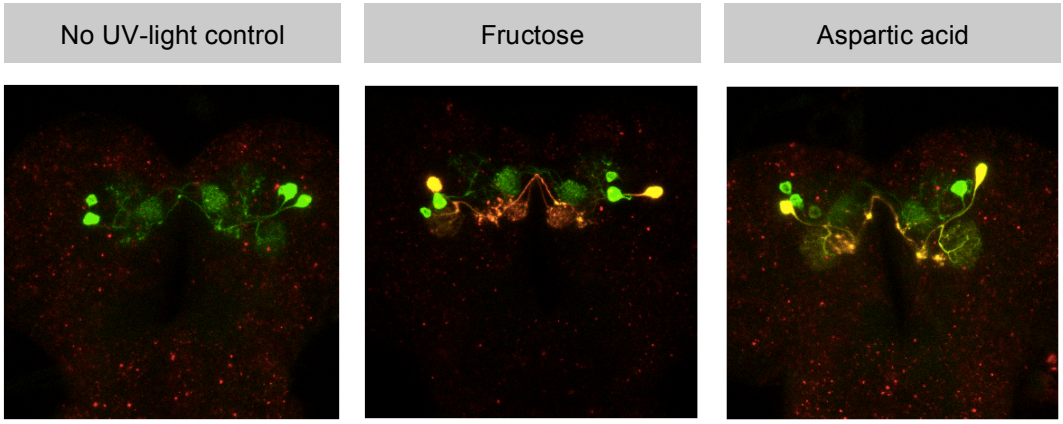
These results were presented at ANN Spring Meeting in March 2018, the Maggot Meeting in October 2019, and the Göttingen meeting of the German Neuroscience Society in March 2019.

in vivo imaging of reward neuron activity

CaMPARI is a recently established tool to monitor calcium activity in intact animals (Fosque et al. 2015). I visited Prof. Pankratz's lab (University of Bonn), who established the method to apply CaMPARI in larval *Drosophila*, to learn the method and to test if a reward-mediating dopaminergic neuron, DAN-i1, is activated by different tastants. DAN-i1 is one of the MB input neurons and is required for full sugar reward memory (Saumweber et al. 2018). A split-Gal4 strain for DAN-i1 is available, which allows us to label a small subset of neurons. An intact larva was introduced into test solution and UV-light was applied. When the influx of calcium and the illumination of UV-light happen coincidently in the neurons expressing CaMPARI, the green fluorescent signal converts to red. After the treatment, the larvae were immediately dissected and the brains were observed under the confocal microscope. The left panel of Figure 3 shows an example of a control brain. Without illuminating UV-light, DAN-i1 neurons exhibit green fluorescence. I measured the green/red ratio of DAN-i1 neurons and compared the larvae that received different treatment (Figure 3). There was no significant difference in green/red ratio between tested solutions (water, fructose and aspartic acid). DAN-i1 does not receive direct input from peripheral gustatory neurons, which might make it difficult to detect the neuronal activity by this method. For further study, we therefore want to modify the current approach.

I established the CaMPARI method in the host lab; I will apply this method to now search sensory and central neurons that are involved in amino acid sensing.

Figure 3



Summary

In the past two years, I found that an amino acid mixture can work both as reward and punishment, and that these memories can be formed in parallel. Parametric experiments, such as tests for temporal stability and concentration dependency, were performed to investigate the nature of these memories. These results are in preparation for a publication. By means of genetic tools, I started to search for the neurons involved in amino acid learning. I found that sensory IR76b neurons are necessary for appetitive learning of an amino acid-mixture. It will be interesting to know the synaptic partners of IR76b neurons. Since the EM reconstruction project is on going by collaboration of several laboratories (Eicher et al. 2017), I expect that information about the circuitry between sensory IR neurons and the MB will be available soon. I am therefore currently performing experiments to identify the MB input neurons for amino acid learning. Once completed, I will submit a paper to report the sensory and the central circuit of amino acid learning.

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