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研究課題名(和文) Dynamics and roles of aminergic neurons in pheromone processing.

研究課題名(英文) Dynamics and roles of aminergic neurons in pheromone processing

研究代表者

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研究成果の概要(和文)：動物はフェロモンを介して社会行動や性行動を営むが、個体から自然な形で提示されたフェロモンがどのように神経活動や行動に影響を与えるのかは未知な部分が多い。この課題に取り組むために、ショウジョウバエがマーキング行動で放出する分泌物を通じたコミュニケーションに着目して研究を行った。まず、広い空間で自由行動する動物の特定の神経細胞から活動を記録する、発光に基づくシステムを構築した。続いてクロマトグラフィー・質量分析法を用いて、分泌物に個体の性別と年齢の情報が含まれていることを突き止めた。最後に、動物はこの性別と年齢の情報を読み出して行動できることを見出した。

研究成果の学術的意義や社会的意義

フェロモンは動物間のコミュニケーションを支える重要な物質であるが、実験者が人工的に提示するのではなく、動物が自然な行動を通して、性差や発生段階に応じて適切なタイミングと量で提示したフェロモンが脳内でどのように処理され、そしてどのように行動に影響を与えるのかについては未知な部分が多い。本研究はマーキング行動を通して提示されるフェロモンの正確な化学的成分と行動への影響を性や発生過程ごとに明らかにし、そして自由行動下における神経活動解析への道筋を立てたという学術的意義を持つ。

研究成果の概要(英文)：Animals communicate with their conspecifics using pheromones, signals mediating social and sexual behaviors. However, we still know little about the neural and behavioral impact of pheromones naturally presented by animals. To address this issue, we used fruit fly *Drosophila melanogaster* and studied the communication mediated by pheromones contained in olfactory landmarks that are deposited naturally by individual animals. We developed a bioluminescence-based system where the activity of specific neurons in the brain can be recorded from freely behaving *Drosophila melanogaster* over a large area. We then conducted a gas chromatography/mass spectrometry analysis to quantify the amount of cuticular hydrocarbons contained in olfactory landmarks, and found that the content carries information about the identity of both sex and age of the landmark depositor. Finally, we found that the interacting flies can extract the information about the age from these landmarks.

研究分野：神経科学

キーワード：嗅覚情報処理 フェロモン ショウジョウバエ

1 . 研究開始当初の背景

Animals communicate with their conspecifics using pheromones, signals mediating social and sexual behaviors. These chemicals act as profound modulators of the animal's internal state and motivation eliciting arousal, sexual behaviors and aggression. The effect of pheromones is heavily studied in neuroscience and ethology by assessing how the behavior of animals is influenced by the application of a given chemical presented at an arbitrary concentration. However, we still know little about the neural basis of behavioral modulation triggered by pheromones naturally presented by the animals. To address this issue, we would ideally like to record neural activity of aminergic neurons (including dopaminergic, octopaminergic, and serotonergic neurons) that reflect the internal state and motivation while the animal is freely sampling pheromones deposited by conspecifics.

Typical neural recording techniques suffer from limitations when applied to freely interacting animals. Multi-electrode arrays can be mounted only to organisms capable of carrying the devices and it is often difficult to target specific neurons. Functional imaging can monitor the activity of specific types of neurons when combined with genetics, but it usually restricts the experiment to restrained animals. Recently, using a bioluminescence probe, we succeeded in recording the activity of specific types of neurons in freely interacting *Drosophila melanogaster*, opening a way to examine the dynamics of aminergic neurons in an environment decorated with pheromones that are naturally deposited by animals.

2 . 研究の目的

Here, we aim to examine the impact of pheromones on neural activity and behavior. We will study this using fruit fly *Drosophila melanogaster* and focus on the communication mediated by pheromones contained in olfactory landmarks that are deposited naturally by individual animals. We will characterize the chemical content of landmarks using chromatography/mass spectrometry analysis. Furthermore, we aim to characterize the context-dependent effects of olfactory landmarks on neural activity and behavior as the content of fecal materials deposited by the flies is reported to change depending on the age and social environment.

3 . 研究の方法

(1) Development of a multi-channel recording system

A genetically-encoded bioluminescence calcium indicator will be used to probe the activity of a small number of specific aminergic neurons. We will develop a system comprising an array of photo multiplier tubes (PMTs) to simultaneously record the neural activity and the location of a fly freely navigating in a large area.

(2) Examination of the content of olfactory landmarks naturally deposited by the animals

We will perform a gas chromatography/mass spectrometry (GC/MS) analysis to quantify the amount of cuticular hydrocarbons contained in olfactory landmarks naturally deposited by male flies.

(3) Characterization of context-dependent, behavioral effects of olfactory landmarks

It has been reported that males produce different amount of pheromones depending on the age and social context. We will therefore test the hypothesis that olfactory landmarks exert context-dependent effects on aminergic neural activity and behavior.

4 . 研究成果

(1) Development of a multi-channel recording system

We developed a bioluminescence-based system where the activity of specific neurons in the brain can be recorded from freely behaving *Drosophila melanogaster* over a large area. The system comprises an array of photomultiplier tubes that function independently of each other. This allows us to expand the size of the recording arena as well as to estimate the position of the luminescent fly without using a camera by calculating the centroid of bioluminescence signal.

As we faced difficulties in using the currently available bioluminescence probe to measure the activity of small number of aminergic neurons deep in the brain, in the meantime, we developed a protocol to image the activity of all the dopamine neurons in the secondary olfactory processing center, using a two-photon microscope, while continuing to develop the bioluminescence recording method.

(2) Examination of the content of olfactory landmarks naturally deposited by the animals

We conducted a gas chromatography/mass spectrometry (GC/MS) analysis to quantify the amount of cuticular hydrocarbons contained in olfactory landmarks naturally deposited by male flies. By optimizing the sample collection procedure and the parameters for GC/MS analysis, we were able to detect multiple major cuticular hydrocarbons in single olfactory landmarks. Using a classification approach, we found that information about the identity of both sex and age of the landmark depositor can be decoded from the content of landmarks.

(3) Characterization of context-dependent, behavioral effects of olfactory landmarks

As we found that the landmarks deposited by flies of different sex and age contained different amount of cuticular hydrocarbons, we examined if the flies can read out these differences and behave accordingly. To this end, we prepared landmarks deposited by males of different ages and measured the time the male testers spend time close to these landmarks. As a result, we found that the tester flies spend different amount of time depending on the age of the depositors, indicating that flies can extract the information about the age from landmarks.

5. 主な発表論文等

〔雑誌論文〕 計0件

〔学会発表〕 計0件

〔図書〕 計1件

1. 著者名 Damien Mercier、高木（槌本）佳子、太田和美、風間北斗	4. 発行年 2020年
2. 出版社 羊土社	5. 総ページ数 222
3. 書名 実験医学別冊 発光イメージング実験ガイド（永井健治、小澤岳昌編集）	

〔産業財産権〕

〔その他〕

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6. 研究組織

氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
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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
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