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　５ 年　 ６ 月 １２ 日現在

機関番号：８２４０１
研究種目：若手研究
研究期間：2020～2022
課題番号：２０Ｋ１５９０９

研究課題名（和文）Measurement of Chromatin Architecture, and its Function in Regulating Neuronal Activity

研究課題名（英文）Measurement of Chromatin Architecture, and its Function in Regulating Neuronal Activity

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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）：この研究の目的はショウジョウバエ幼虫の体感覚神経細胞であるC4da細胞を利用して、神経細胞の活性化時におけるクロマチン組織化により誘発される遺伝子発現の変化、およびそれによる動物の長期的な行動への影響を調査することである。これまでにC4daがTrpA1を通じて常に活性化されている一方で、信号伝達関連遺伝子の発現が低下していることが示唆された。発現が上昇していた遺伝子群の中にはクロマチンリモデリング複合体の１つが含まれており、遺伝子の発現低下はクロマチン構造レベルより制御させている可能性があることが示唆された。 今後C4daを用いてATACまたはChIP-seqによりこれらの変化を確認する。

研究成果の学術的意義や社会的意義
Our research will shed light on deciphering how gene expression will change upon activation of somatic sensory neurons. The results will also contribute to the knowledge of longer-lasting behavior effects in the whole animal caused by sensory neuron activation and related gene expression changes.

研究成果の概要（英文）：Activation of somatic sensory neurons commonly leads to fast behavioral responses are relatively well studied by far. However, what longer-lasting influences could be left on the overall fitness of the animals is not yet clear. We are interested in investigating the gene expression changes caused by chromatin organization upon neuronal activations, and also the longer-term behavioral consequences caused by the gene expression changes.
To address this question, we utilize a sub-type of somatic sensory neurons (C4da) in the fly larvae. Our current data suggested that while C4das are constantly activated through TrpA1, many other signaling-related genes are down-regulated. Furthermore, one significantly up-regulated gene has been found in one of the chromatin remodeling complexes, suggesting that the gene expression reductions could be controlled from the chromatin structure level. We are set to further confirm and investigate those changes with ATAC-seq or ChIP-seq using C4da neurons.

研究分野：Neuroscience

キーワード：Neuronal Activity　Gene Expression　Chromatin Organization　Transcription Factor

１．研究開始当初の背景

Animals respond to stimulations from the surrounding environment. Somatic sensory neurons are the frontline sensor for the stimuli. Neuronal activations commonly lead to fast behavioral responses, while whether there will be longer-lasting influences on the overall fitness of the animals is not yet clear. We are interested in investigating the gene expression and chromatin organization changes when neurons are activated. Among the genes that show expression changes through chromatin organization control, we intend to look for the ones that can influence the longer-term behavior. Thus, we could identify the role sensory neurons play in conducting the stimuli sensed to effects on the whole animal at both the molecular and behavioral levels.

２．研究の目的

Our initial data has shown that besides the fast behavioral responses caused by the activity changes of a subtype of somatic sensory neurons, larvae also react to nutrition differently while sensory neurons are silenced or activated. Therefore, firstly we are interested in looking for the gene expression and chromatin organization changes when sensory neurons are activated. For the genes showing expression changes, they would be good candidates for linking sensory neuron activity changes to how larvae change their responses to nutrition.

３．研究の方法

To investigate this question, we mainly utilize a sub-type of the somatic sensory neurons in the fly larvae (C4da neurons), which can react to diverse stimuli such as short wave-length light, heat, chemicals, and mechanical forces [1]. They are able to be activated by overexpression of TrpA1 channels and high-temperature treatment (30C). They can also be silenced by overexpression of a temperature-sensitive form of vesicle transportation protein (Shi^{ts}). Larvae are usually raised on a standard fly culture medium. When they intake food differently on the regular medium, their sensitivity to higher nutritious food might change at the same time [2]. We planned to either activate or silence the C4da neurons for a certain period (from the late 2nd to early 3rd stage, aiming to not affect their development), then test their preference for 20% glucose liquid food. If larvae intake regular food differently, their hungry level would be different, thus, showing some difference in preference for higher nutrition food. Similar experiments can be done with other genotypes of larvae, in which their C4da neuron activity might be altered.

To further investigate the gene expression changes when C4da neuron activity changes, we planned to perform RNA-seq on FACS-sorted C4da isolated from larvae. Potential chromatin organization changes can be assessed by ATAC-seq, which could

map out the open region in the genome. They can also be detected by ChIP-seq using antibodies specific to markers that label chromatin at a specific status (e.g. antibody for acetylated histone). Genes that show expression changes and that can be found in chromatin regions show different accessibilities upon C4da activation would be candidates of our interest. We can further test whether they play any role in affecting the sensitivity to food as described in the previous section.

４．研究成果

When we tested how C4da activities affect the nutrition intake we found that the larvae were less willing to uptake the higher-concentration-glucose liquid food when the neurons were activated. At the same time, more larvae in the population will take the higher-concentration-glucose liquid food when the neurons were silenced. Moreover, knocking down and overexpression of a transcriptional factor, which is critical for C4da development and its ability for sensing stimulation, also led to the difference in preference for the higher-concentration-glucose liquid food. Having taken all the data so far, alterations of C4da neuron activity seem to affect the feeding state of the larvae.

After confirming the behavioral phenotype, we are interested in looking for the genes downstream of activity change in C4da. One possibility is that those genes of interest would be C4da specifically enriched since sensing the different types of stimuli is one feature for C4da neurons. Therefore, before I finalized the protocol for isolating C4da neurons and during the work-from-home time during COVID, I re-analyzed some old microarray data from past members in the lab, in an attempt to narrow down to some potential interesting candidates. A group of genes in charge of cell-cell interactions and some GPCRs for nutrition-controlled peptides appeared from the dataset. Knocking down many of them also showed some difference in the higher concentration of glucose intake, which suggests that they could be linking the activity-induced transcriptional changes and the long-term effect caused by C4da activation.

Our recently finished RNA-seq data showed similar and different candidate genes from my previous analysis. For this set of experiments, C4da neurons are activated by TrpA1 and 30C treatment temporarily. Differential expression analysis showed that many other ion channels and receptors (including some GPCRs found in the previous analysis) were down-regulated upon C4da activation. This result may indicate that a certain type of activation may lead to signal-receiving reductions in other pathways. On the other hand, one gene that is significantly upregulated has been found in the nucleosome remodeling and deacetylase (NuRD) complex, suggesting that

neuronal activation could possibly cause gene expression regulation controlled from the chromatin structure level.

To continue our investigation on not only individual genes that show expression changes but also on whether those individual changes are organized at the chromatin structure level, we have planned to perform ACTA-seq and/or ChIP-seq against different chromatin state markers. It has been a technical challenge to perform ATAC-seq or ChIP-seq for samples below 5000 cells. But a protocol adjusted for ~1000 FACS-sorted da neurons for ChIP-seq and ~500 neurons for ATAC-seq have been developed. We are looking forward to discovering more data regarding the chromatin level changes in C4da under different activity states or even other conditions. Those experiments can allow us to verify whether the targets showing expression changes are in the open chromatin region pre- and post-activation.

For the candidate genes of interest (possibly one or more genes for GPCRs), we are also willing to test their roles in affecting C4da neuron activity in return. With the living imaging method I set up during this granted period, the activity of C4da can be measured with GCamP upon treatment of peptides, chemicals, or some stimuli. This data would be helpful to answer whether there is bi-directional regulation between gene expression control and neuronal activity state.

Reference

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2.Ugrankar, R., Theodoropoulos, P., Akdemir, F. et al. Circulating glucose levels inversely correlate with Drosophila larval feeding through insulin signaling and SLC5A11. Commun Biol. 2018 1, Article number: 110

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 van Alphen Bart、Stewart Samuel、Iwanaszko Marta、Xu Fangke、Li Keyin、Rozenfeld Sydney、Ramakrishnan Anujaianthi、Itoh Taichi Q.、Sisobhan Shiju、Qin Zuoheng、Lear Bridget C.、Allada Ravi	４．巻 20
２．論文標題 Glial immune-related pathways mediate effects of closed head traumatic brain injury on behavior and lethality in Drosophila	５．発行年 2022年
３．雑誌名 PLOS Biology	６．最初と最後の頁 e3001456
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pbio.3001456	査読の有無 無
オープンアクセス 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 －

〔学会発表〕　計0件

〔図書〕　計0件

〔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

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