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

平成 ３０ 年　 ６ 月　 ５ 日現在

機関番号： ３７１１１

研究種目： 挑戦的萌芽研究

研究期間： 2015～2017

課題番号： １５Ｋ１４５６９

研究課題名（和文） ミツバチの尻振りダンスに符号化されたベクトル情報統合の神経機構

研究課題名（英文） Neural mechanism for integration of vector information encoded in honeybee waggle dance

研究代表者

藍　浩之（Ai, Hiroyuki）

福岡大学・理学部・助教

研究者番号：２０３３０８９７

交付決定額（研究期間全体）：（直接経費）　　3,000,000円

研究成果の概要（和文）：尻振りダンスの追随バチは、尻振り相の時間から蜜源への距離を算出している。本研究では、尻振りダンス特有のパルス状空気振動を触角に与えた時の、脳内振動応答性ニューロン応答特性を調べた。DL-Int-1は、自発発火をする性質があり、連続する複数の振動パルス刺激の間、活動電位の持続的な抑制応答が見られる。DL-Int-1は、GABAを伝達物質とする抑制性ニューロンであり、その後シナプスニューロンであるDL-Int-2はこの連続振動パルス刺激の間、持続的な興奮が見られる。これらの結果より、ミツバチの尻振りダンスの距離の符号化には、抑制性ニューロンによる正確な時間計測のメカニズムが存在することがわかった。

研究成果の概要（英文）：The follower of honeybee waggle dance decipher the distance information to the profitable flower from the duration of waggle phase. On this study the morphological and electrophysiological properties of vibration sensitive interneuron in the primary auditory center in the brain have been analyzed in responding to the waggle dance sound mimicked vibration pulse stimuli to the antenna and its possible neural circuit was suggested by experimental and theoretical evidences. DL-Int-1 has spontaneous activities and shows a tonic inhibition during waggle dance sound mimicked vibration pulse stimuli. Anti-GABA immunohistochemical experiment suggested that D-Int-1 is a GABAergic inhibitory neuron. The putative postsynaptic neuron DL-Int-2 shows a tonic excitation during the waggle dance sound mimicked vibration pulse stimuli. These results suggest that disinhibition from the sensory neuron to the DL-Int-2 through DL-Int-1 might be a key neural network to encode the distance information.

研究分野： 神経行動学

キーワード： 脳・神経　行動学　神経科学　昆虫　生理学

１．研究開始当初の背景

Karl von Frisch demonstrated that honeybees use a type of movement called the “waggle dance” to direct their nest mates to a remote food source (von Frisch, 1967). The duration of the phase of the “waggle” movement changes linearly with the distance to the food source, suggesting that this distance information is encoded in a physical parameter of the movement that changes with the duration (von Frisch, 1967). Although it is possible that substrate-borne vibration elicited by the waggle movement conveys spatial information regarding the food source (Michelsen, 2003), air-borne vibration is thought to be the most probable sensory cue delivered during waggle dance communication (Judd, 1995) where wing-beats produce local air-jet flows. During the waggle phase of the dance, the wingbeats of the dancer produce a train of vibration pulses that pass from the tail end of the dancer to a follower bee, which follows behind the dancer. For a fixed target location, the duration and interval of vibration pulses are constant at around 16ms and 33ms respectively, independent of the quality of the food source (Hrncir et al., 2011). However, the precise feature of the train of vibration pulses elicited during the waggle dance that encodes distance information is unclear. Two plausible parameters are (1) the duration of the train of vibration pulses and (2) the number of vibration pulses per waggle phase. These parameters are linearly related because the rate of the pulsed vibration is nearly constant.

Waggle dance followers detect air-borne vibrations via Johnston’s organ (JO), which is located at the second segment (pedicel) of the antenna (Towne and Kirchner, 1989; Kirchner et al., 1991; Dreller and Kirchner, 1993). The structural characteristics of the antenna and the response of JO neurons suggest that mature honeybee antennae and JO neurons are tuned to 250-300 Hz, which matches the frequency of wingbeat generated during the waggle dance (Tsujiuchi et al., 2007). JO afferent fibers are spatially segregated in the medial posterior protocerebral lobe (mPPL) and the dorsal lobe/dorsal subesophageal ganglion (DL-dSEG) (Ai et al., 2007). The dSEG also receives sensory afferents from neck hairs that are thought to be gravity sensors (Brockmann and Robinson, 2007). This suggests that the dSEG integrates vector information about the waggle dance, i.e., distance information coded in air vibrations and direction information coded in the dancer's orientation relative to gravity (Ai and Hagio, 2013).

２．研究の目的

Our goal is to understand how distance and direction information are encoded in the honeybee brain. Accordingly, we are interested in characterizing the distribution and location of neurons that exhibit appropriate response properties for processing distance or direction information. Recently, we identified two interneuron types, DL-Int-1 and DL-Int-2, that receive JO input and are located in the primary auditory center (PAC) of the honeybee. We demonstrated that these interneurons are responsive to antennal stimulation (Ai et al., 2009, Ai, 2010, Ai and Itoh, 2012). In the present report, we describe the above-mentioned interneurons as well as a newly identified neuron type, the Bilateral DL-dSEG-LP. We investigated the morphology, GABA immunoreactivity, and physiology of these three cell types in the PAC, with a particular focus on their responses to trains of vibration pulses with temporal properties similar to those elicited during the waggle dance. This work represents a first step towards understanding the role of these neurons in the encoding of distance information in the honeybee brain.

3．研究の方法

Intracellular recording and staining

To investigate the processing of the sound produced during the waggle dance, we collected auditory interneurons in the PAC, including the previously described interneuron types DL-Int-1 and DL-Int-2 (Ai et al., 2009).

Sensory stimulation

For vibratory stimulation of the JO, the right antenna was inserted into a glass capillary (length, 10 mm; inner diameter of the tip, 200 μm). The opposite tip of the capillary was connected to a piezo-actuator. We used vibrations with frequencies ranging from 100 to 400 Hz and amplitudes (peak to peak) ranging from 0 to 50 μm and 10 or 20 trains of vibration pulses with the interval ranging from 20 to 100 ms and with the duration ranging from 4 to 50 ms.

4．研究成果

During the waggle phase the dancer produces trains of vibration pulses, which are detected by the follower bees via Johnston's organ located on the antennae. To uncover the neural mechanisms underlying the encoding of distance information in the waggle dance follower, we investigated morphology, physiology, and immunohistochemistry of interneurons arborizing in the primary auditory center of the honeybee (*Apis mellifera*). We identified major interneuron types, DL-Int-1, DL-Int-2, and Bilateral DL-dSEG-LP (Fig. 1), that responded with different spiking patterns to vibration pulses applied to the antennae. Experimental (Figs. 2 and 3) and computational analyses (Figs. 4 and 5) suggest that inhibitory connection plays a role in encoding and processing the duration of vibration pulse trains in the primary auditory center of the honeybee. Our results are critical for understanding how the honeybee deciphers information from the sound produced by the waggle dance and provide new insights regarding how common neural mechanisms are used by different species to achieve communication.

Fig. 1 Left: To inform their hivemates about the location of profitable flowers, a honeybee performs the waggle dance with specific vibration patterns.

Right: Composite image of three interneurons in the honeybee brain which show unique responses to such vibrations. Magenta: DL-Int-1; Blue: DL-Int-2; Yellow: Bilateral DL-dSEG-LP.

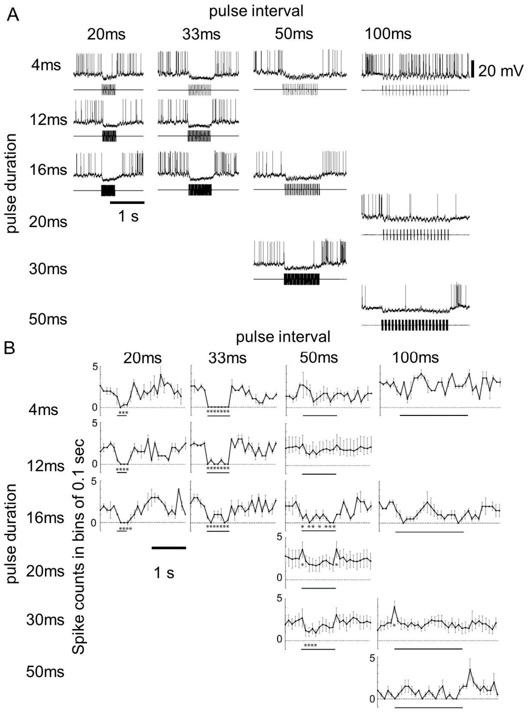

Fig. 2 Responses of DL-Int-1 to trains of vibration pulses. A, Single responses of two example neurons to pulse stimuli applied to the antenna with different temporal patterns. DL-Int-1 neurons showed tonic inhibitory responses for shorter pulse durations (less than

50 ms). B, Instantaneous spike frequencies (Spike counts in bins of 0.1 sec) of DL-Int-1 (N = 7). Asterisks indicate statistical differences with the spontaneous activity in the 1 s interval before each record (*: P < 0.01).

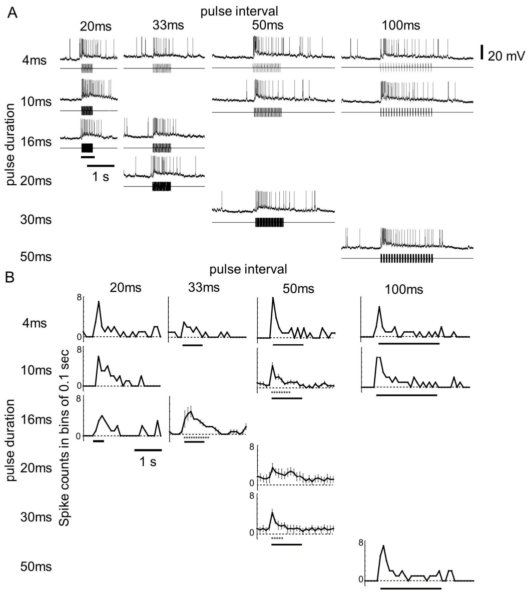

Fig. 3 Responses of DL-Int-2 neurons to trains of vibration pulses. A, Single responses of an example neuron to pulse train stimuli applied to the antenna with different temporal patterns. DL-Int-2 neurons showed tonic excitatory responses during trains of vibratory pulses with different temporal patterns. B, Instantaneous spike frequencies of DL-Int-2 neurons before, during, and after the train of pulses (N = 6).

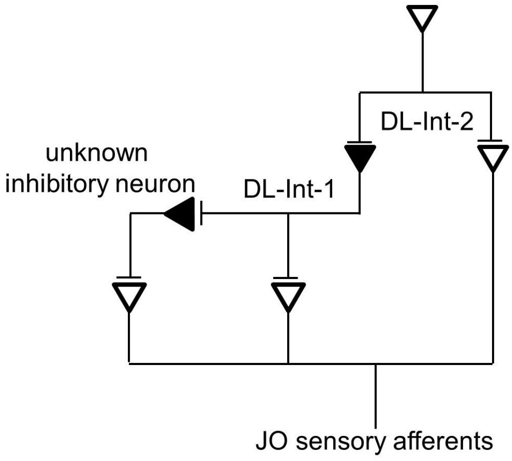

Fig. 4, A putative network model of vibration processing during waggle dance hypothesized by the experimental results.

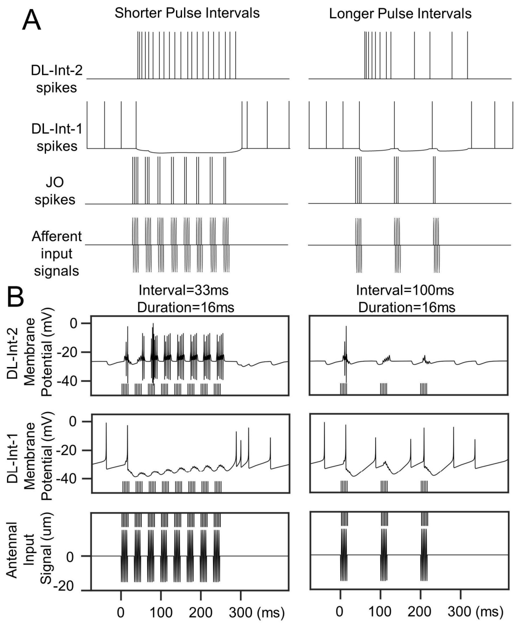

Fig. 5 **A,** Summary of the experimental physiology of JO (bottom), DL-Int-1 (middle), and DL-Int-2 (top) neurons shown using schematic membrane traces for stimuli with shorter (around 30 ms. left column) and longer (around 100 ms, right column) pulse interval values. Note that the shorter intervals correspond to the vibration elicited during the honeybee waggle dance. JO sensory neurons tend to spike at a fixed phase of the input sinusoidal stimulus, showing spike frequency adaptation for later pulses. DL-Int-1 neurons show inhibition that is stronger for stimuli with shorterer pulse intervals than for stimuli with longer pulse intervals, intermittent spikes occur during a train of pulses for long pulse intervals. DL-Int-2 neurons show on-phasic and tonic excitation, with the latter being weaker for stimuli with longer pulse intervals, suggesting it arises from disinhibition due to the tonic inhibition of DL-Int-1. **B,** Simulation results of the network model in Fig. 4 for the same stimuli as in A. JO sensory neurons were assumed to spike regularly at a fixed phase of the sinusoidal stimulus applied to the antenna (bottom row). These spikes are indicated by

vertical lines at the top in the bottom row and at the bottom in the middle and top rows. DL-Int-2 shows subthreshold EPSPs evoked by disinhibition through DL-Int-1. The network model could qualitatively reproduce the different spiking profiles for the two stimulus conditions.

５．主な発表論文等

（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計 6 件）

① Kumaraswamy Ajayrama、Kai Kazuki、Ai Hiroyuki 、Ikeno Hidetoshi 、Wachtler Thomas: Spatial registration of neuron morphologies based on maximization of volume overlap. BMC Bioinformatics, 19, 2018, 1-16, 査読有 doi: 10.1186/s12859-018-2136-z

② 池野 英利, 藍 浩之：ミツバチ脳のリバースエンジニアリング －ダンスコミュニケーションに関わる神経機構－。昆虫と自然、53(6), 2018, 35-40, 査読無 http://hokuryukan-ns.co.jp/cms/books/%E6%98%86%E8%99%AB%E3%81%A8%E8%87%AA%E7%84%B6%E3%80%802018%E5%B9%B4%E3%80%805%E6%9C%88%E5%8F%B7/

③ Ai Hiroyuki、Kai Kazuki、Kumaraswamy Ajayrama 、Ikeno Hidetoshi 、Wachtler Thomas: Interneurons in the honeybee primary auditory center responding to waggle dance-like vibration pulses. *The Journal of Neuroscience*, 37 (44), 2017, 10624-10635, 査読有 doi: https://doi.org/10.1523/JNEUROSCI.0044-17.2017

④ Pham Duy Long、Ito Yasuto、Okada Ryuichi、Ikeno Hidetoshi、Isagi Yuji 、Yamasaki Michimasa: Phototactic behavior of the ambrosia beetle Platypus quercivorus (Murayama) (Coleoptera: Platypodidae) before and after flight. Journal of Insect Behavior, 30, 2017, 318-330. 査読有 Doi: 10.1007/s10905-017-9615-3

⑤ 高橋 伸弥、橋本 浩二、前田 佐嘉志、鶴田 直之、藍 浩之: ミツバチコロニーの巣内行動観察システムの開発。人工知能学会論文誌、32, 2017, B-GC2_1～11, 査読有 doi: https://doi.org/10.1527/tjsai.B-GC2

⑥ Yamashita, T., Haupt, S. S., Ikeno, H., Ai, H.: Walking patterns induced by learned odors in the honeybee, *Apis mellifera* L. *Journal of Experimental Biology* 219 (2016), 12-16, 査読有 doi: 10.1242/jeb.123356

〔学会発表〕（計 20 件）

① 藍浩之：ミツバチのダンス言語の発達の過程 －RFID を用いた羽化後 20 日間の行動観察－。第 40 回ミツバチ科学研究会。2018、東京

② Wachtler, T, Kumaraswamy, A, Kai, K., Ikeno, H., Ai, H.: Interneurons in the primary auditory center of the honeybee brain responsive to air vibration pulses as elicited during waggle dance communication. Society for Neuroscience 2017, Washington DC, USA.

③ 藍浩之：ミツバチの尻振りダンスコミュニケーションにおける蜜源への距離情報の符号化回路。日本動物学会第 88 回富山大会、2017、富山。

④ Hiroyuki Ai, Yuuki Kobayashi, Toshiyuki Matake, Shinya Takahashi, Koji Hashimoto, Sakashi Maeda, Naoyuki Tsuruta: Development of honeybee wagle dance and its differences between recruits and scouts. JSCPB 2017 Fukuoka, 2017, 福岡

⑤ Hidetoshi Ikeno: Virtual computing server for trial use of mathematical model and data analysis software：Sim-P. NIX-odML Global Workshop and Hackathon 2017 in Japan, 2017, Kitakyuushu.

⑥ Hidetoshi Ikeno: Development of a hypertext tutorial for construction of a standard brain. Advances in Neuroinformatics V, 2017, Saitama.

⑦ Ai, H., Matake, T., Hagio, H., Takahashi, S., Hashimoto, K., Maeda, S., Trusuta, N.: Lifetime observation of the honeybee behaviors related with waggle dance by using RFIDs and high resolution camera modules. The 22nd International Congress of Zoology, 2016 年 11 月 14 日～19 日, Okinawa.

⑧ Takahashi, S., Hashimoto, K., Maeda, S., Trusuta, N., Ai, H.: Development of automatic monitoring system of honeybee behaviors using RFID reader and imaging processing. The 22nd International Congress of Zoology, 2016 年 11 月 14 日～19 日、Okinawa.

⑨ Ai, H., Kai, K., Kumaraswamy, A, Rautenberg, P. Wachtler, T, Ikeno, H.: Putative neural circuits of the primary auditory center processing in the pulsed vibration produced by waggle dance of honeybee. The 22nd International Congress of Zoology, 2016 年 11 月 14 日～19 日, Okinawa.

⑩ Hidetoshi Ikeno, Ajayrama Kumaraswamy, Kazuki Kai, Hiroyuki Ai, Philipp Rautenberg, Thomas Wachtler: Morphological comparisons in a vibration sensitive interneuron in the dorsal lobe of the brain among honeybees of different ages. International Conference on Brain Informatics & Health, 2016 年 10 月 13 日～

16日, Omaha, Nebraska, USA

⑪ Hidetoshi Ikeno, Ajayrama Kumaraswamy, Kazuki Kai, Hiroyuki Ai, Philipp Rautenberg, Thomas Wachtler: A method for evaluation of neural structure based on reconstruction and application to an interneuron in the honey bee brain. INCF Congress 2016, 2016年09月03日～04日, Reading, UK

⑫ Ai, H., Kai, K., Kumaraswamy, A, Rautenberg, P. Wachtler, T, Ikeno, H.: Interneurons of the primary auditory center of the honeybee brain responsive to pulsed vibration produced by the waggle dance. JSCPB 2016 (The 38th Annual meeting of the Japanese Society for Comparative Physiology and Biochemistry), 2016年09月02日～04日、東京。

⑬ Hidetoshi Ikeno: Neuron segmentation plugin for Vaa3D and its application to morphometric analysis of an interneuron in the honeybee brain. BigNeuron and Big Neuroscience Workshop, 2016年05月26日～27日, Nanjing, China

⑭ Ai, H., Kai, K., Watanabe, H., Itoh, T., Kumaraswamy, A, Rautenberg, P. Wachtler, T, Ikeno, H.: Honeybee neurons responsive to the pulsed vibration produced by waggle dance. 4th INCF Japan Node International Workshop Advances in Neuroinformatics 2016 and 14th INCF Nodes Workshop, 2016年05月28日～29日、埼玉

⑮ Ai, H., Kai, K., Watanabe, H., Itoh, T., Kumaraswamy, A, Rautenberg, P. Wachtler, T, Ikeno, H.: Honeybee interneurons responsive to the pulsed vibration produced by waggle dance. International congress of neuroethology 2016, 2016年03月30日～04月03日, Montevideo, Uruguay

⑯ Ai, H., Kai, K., Watanabe, H., Kumaraswamy, A, Rautenberg, P. Wachtler, T, Ikeno, H.: Comprehensive analyses of interneurons arborizing in the primary vibration center in honeybee brain. CompBiol 2015, 2015年12月11日～13日, 広島

⑰ H. Ikeno, D. Miyamoto, T. Kazawa, R. Kanzaki：A method for construction and application of standard brain model based on the confocal images. Advances in Neuroinformatics2015 tutorial session. 2015年11月27日。東京

⑱ H. Ikeno, D. Miyamoto, Y. Okamura-Oho, H. Nishibe, A. Sato, T. Furuichi, R. Kanzaki: Development of protocol and software for registration in situ hybridization image into the standard brain. Advances in Neuroinformatics 2015, 2015年11月27日, 東京

⑲ 山下俊也、池野英利、長谷川雄二、伊東綱男、藍　浩之：学習した匂いと尻振りダンス振動によって誘発されるミツバチの歩行の解析。日本動物学会第86回大会、2015年09月17日～19日、新潟

⑳ 山下俊也、Haupt, S.S.、池野英利、伊東綱男、藍　浩之：ミツバチにおける学習した匂いによって誘発される歩行の経時変化と音刺激による影響。日本動物学会九州支部第68回大会（三学会合同福岡大会）2015年05月23日～24日。福岡

〔図書〕（計6 件）

① 池野英利、岩月知香、神崎亮平: 脳地図作成の概要とソフトウェア（昆虫の脳をつくる、第8章），朝倉書店, 2018, 11

② 池野英利、岩月知香、後藤昴彦、宮本大輔、神崎亮平: 標準脳作成の実際（昆虫の脳を作る､第9章), 朝倉書店, 2018, 39.

③ 藍　浩之：虫の鼻はどこ？電気で測る触角の働き－カイコガのフェロモン腺の観察と触角電図による匂い応答解析－、研究者が教える動物実験　第1巻。共立出版。2015.

④ 佐倉　緑、岡田龍一、藍　浩之：パブロフのミツバチ－餌の匂いはどれ？－、研究者が教える動物実験　第3巻。共立出版。2015.

⑤ 森山　徹、弘中満太郎、藍　浩之：ダンゴムシのジグザグ歩行－迷路を用いた交替性転向反応の観察とその仕組みの検討－、研究者が教える動物実験　第3巻。共立出版。2015.

⑥ 池野英利：コンピュータで再現！神経活動　神経細胞の電気的活動シミュレーション－、研究者が教える動物実験　第2巻。共立出版。2015.

〔その他〕

① https://wwwfukuoka-u.ac.jp/research/column/17/11/15135258.html

② http://www.u-hyogo.ac.jp/shse/koho/prev/index.html

③ https://www.japantimes.co.jp/news/2017/11/28/national/science-health/bee-research-may-redefine-understanding-intelligence/#.WwO4WEjRCUk

④ https://www.sciencedaily.com/releases/2017/10/171009135435.htm

⑤ http://neurosciencenews.com/honeybee-waggle-dance-7697/

６．研究組織

(1)研究代表者
藍　浩之（AI, Hiroyuki）
福岡大学・理学部・助教
研究者番号：２０３３０８９７

(2)研究分担者
池野　英利（IKENO. Hidetoshi）
兵庫県立大学・環境人間学部・教授
研究者番号：８０１７６１１４