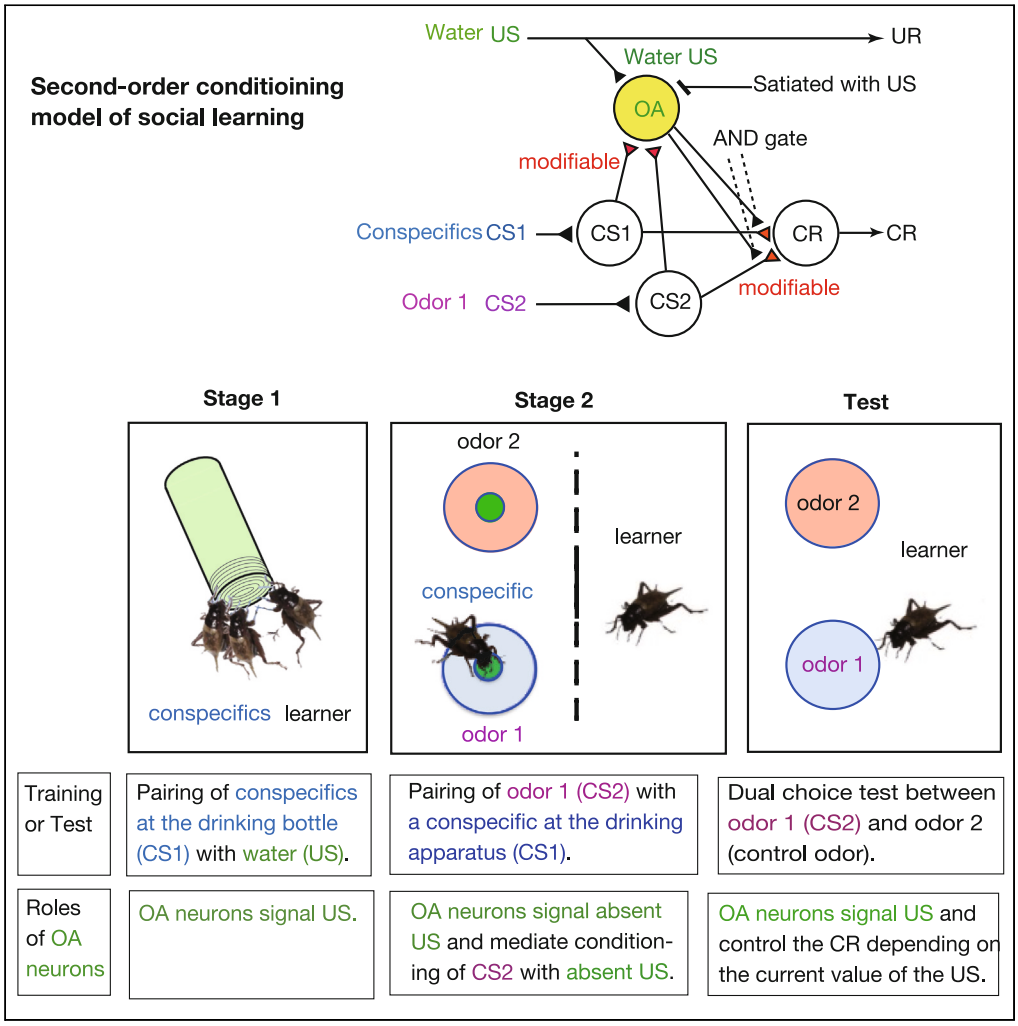
iScience

CellPress
OPEN ACCESS

Article

Octopamine neurons mediate reward signals in social learning in an insect



Yuma Segi, Kohei Hashimoto, Makoto Mizunami

mizunami@es.hokudai.ac.jp

Highlights

We suggest that social learning is based on second-order conditioning in crickets

Octopamine neurons that respond to reward are activated when others receive reward

We propose that mirror-like neural activities mediate social learning

Segi et al., iScience 26, 106612
May 19, 2023
https://doi.org/10.1016/j.isci.2023.106612

iScience

CellPress
OPEN ACCESS

Article

Octopamine neurons mediate reward signals in social learning in an insect

Yuma Segi,[1] Kohei Hashimoto,[1] and Makoto Mizunami[2,3,*]

SUMMARY

Social learning is found in many animals, but its mechanisms are not understood. We previously showed that a cricket that was trained to observe a conspecific staying at a drinking apparatus exhibited an increased preference for the odor of that drinking apparatus. Here we investigated a hypothesis that this learning is achieved by second-order conditioning (SOC), i.e., by associating conspecifics at a drinking bottle with water reward during group drinking in the rearing stage and then associating an odor with a conspecific in training. Injection of an octopamine receptor antagonist before training or testing impaired the learning or response to the learned odor, as we reported for SOC, thereby supporting the hypothesis. Notably, the SOC hypothesis predicts that octopamine neurons that respond to water in the group-rearing stage also respond to a conspecific in training, without the learner itself drinking water, and such mirror-like activities mediate social learning. This awaits future investigation.

INTRODUCTION

Social learning refers to learning that is facilitated by the observation of, or interaction with, another individual or its products.[1] Social learning has been reported in many animals[2,3] including insects.[4–6] Examples of social learning in insects are that bumblebees learn profitable flowers[7,8] or avoid flowers that harbored predators by observing a nestmate's flower choice[9] and that female fruit flies copy mate-choice of other females.[10] Efforts have been made to clarify the neural basis of social learning in mammals[11–14] and insects.[15,16]

We recently reported that crickets exhibit appetitive and aversive forms of social learning.[17] For the appetitive social learning, a demonstrator cricket was allowed to freely visit drinking apparatus that contain water or saltwater and emit apple or banana odors. The demonstrator cricket spent most of the time at the drinking apparatus containing water. Learner crickets were allowed to observe the demonstrator room through a plastic net. After training, the learner preferred the odor of the water-containing apparatus at which the demonstrator spent most of the time. The effect of 8-min training was maintained for at least 24 h. The study showed that crickets have excellent capability for social learning. Since detailed experimental manipulations such as pharmacology, RNAi, and genome editing[18–21] are possible in crickets, crickets may offer useful materials for the study of neural mechanisms of social learning.

In our previous study, we showed that crickets that received social learning training and were then given water until satiation before the post-training test exhibited no increased preference for the learned odor. We reasoned that the response is governed by the odor-water association,[17] since we showed that such water devaluation reduces the response to the odor associated with water reward but not that to the odor associated with sucrose reward.[22] If this reasoning is correct, the learner forms an odor-water association by the observation of a conspecific staying at a drinking apparatus, even though the learner itself does not drink water in the training.

The simplest mechanism to achieve such an association is second-order conditioning (SOC) (Figure 1). The SOC hypothesis assumes that crickets learn to associate conspecifics (conditioned stimulus 1 or CS1) staying at the drinking bottle with water reward (unconditioned stimulus, US) during group drinking in the preceding laboratory rearing stage. Then they learn to associate the odor of the drinking apparatus (CS2) with a conspecific (CS1) staying at the drinking apparatus in training. By combining these experiences, an odor (CS2)-water (US) association is achieved even though the crickets did not experience these two stimuli

[1]Graduate School of Life Science, Hokkaido University, Sapporo 060-0810, Japan

[2]Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan

[3]Lead contact

*Correspondence: mizunami@es.hokudai.ac.jp

https://doi.org/10.1016/j.isci.2023.106612

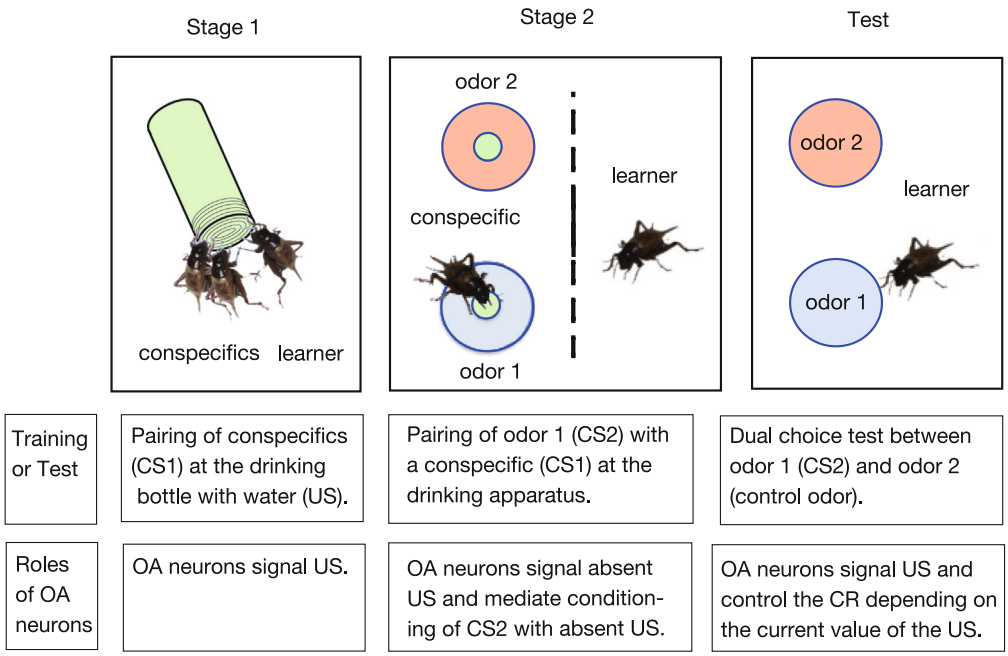


Figure 1. Second-order conditioning hypothesis to account for social learning

In stage 1, a learner cricket associates conspecifics (CS1) staying at the drinking bottle with water (US) during group drinking in laboratory rearing. In stage 2, a learner cricket associates odor 1 (CS2) of a drinking apparatus with a conspecific (CS1) staying at the drinking apparatus. By combining these two experiences, crickets achieve conditioning of odor 1 (CS2) with water (US) and hence explore the odor 1 (CS2) source when thirsty. The hypothetical roles of a class of OA neurons (OA_2 neurons in the model shown in Figure S1A) in the first stage and the second stage of SOC and the test are described at the bottom. The model contains another class of OA neurons (OA_1 neurons), which signal US and mediate conditioning of CS1 with the US in the first stage of SOC, but we do not refer to these neurons since it is assumed that these neurons do not participate in the second stage of SOC and in the execution of the CR in the test.

together in training. Notably, SOC has been proposed to account for social learning in bumblebees, in which foragers learn to select the color of artificial flower at which nestmates stayed.[7,23,24] In that case, it is hypothesized that the nestmate foragers in the flower serve as CS1, the color of the flower serves as CS2 and sucrose solution placed in the flower serves as US. Dawson et al.[7] observed that foragers that luck the experience of observing conspecifics in the artificial flower did not select the color of artificial flower at which nestmate stayed conspecifics, thereby supporting the SOC hypothesis.

The aim of this study was to investigate the SOC hypothesis of social learning by use of pharmacological analysis on the basis of our knowledge of the roles of octopamine (OA) neurons, the invertebrate counterpart of noradrenaline neurons,[25] in the SOC.[26] We previously found that the activation of OA neurons that mediate water (US) signals is needed for the second stage of SOC training and for the execution of the conditioned response (CR), and we proposed a neural circuit model of the roles of OA neurons in SOC training and the test (Figure S1, A neural circuit model for SOC, Related to Figure 1).[26] Notably, the SOC hypothesis of social learning, combined with the neural circuit model, indicates that OA neurons that are activated when a cricket drinks water in group-rearing stage are also activated when a cricket observes a water-associated conspecific staying at the drinking apparatus, just as when the cricket itself drinks water, and such mirror-like activities of OA neurons mediate social learning, as shown in Figure 1. Here we tested the effect of the injection of epinastine, an OA receptor antagonist,[27,28] before the second stage of SOC and before the final test and investigated if the results match the SOC hypothesis of social learning.

RESULTS

Social learning demonstrated with modified experimental procedures

In this study, we used experimental procedures modified from those used in our previous study,[17] the details of which are described in the STAR Methods. In short, crickets were trained in a box consisting of two rooms, a

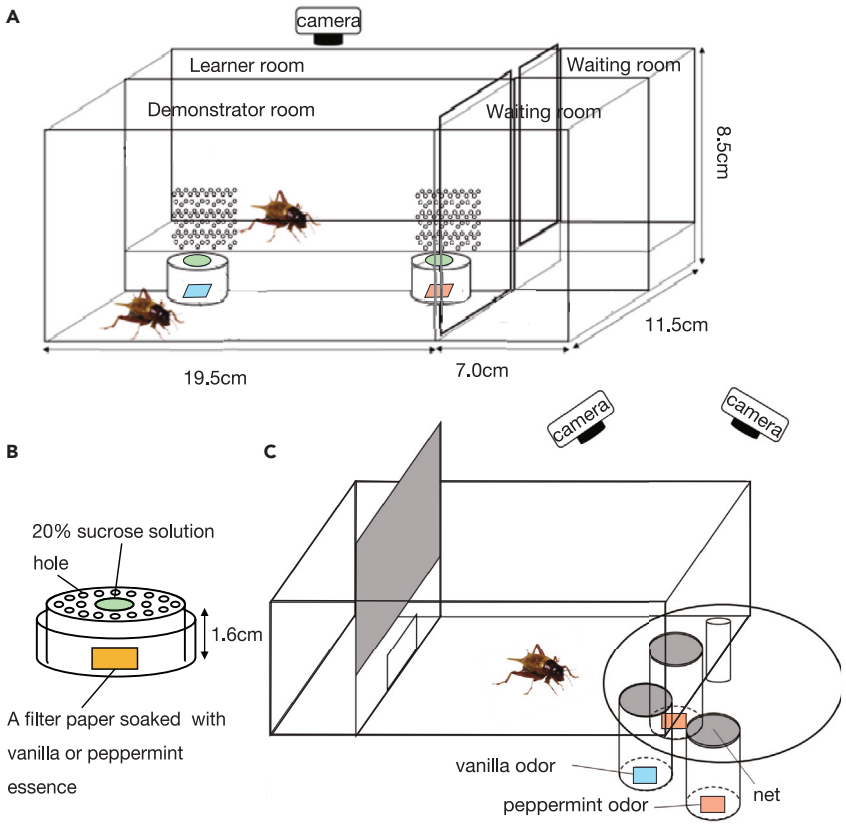


Figure 2. Apparatuses for social learning training and testing

(A) Training arena. The arena consisted of two rooms, a learner room and a demonstrator room, separated by a plastic transparent wall. The wall had many holes in front of the drinking apparatus in the demonstrator room. A waiting room was attached to each room so that the learner and the demonstrator could acclimate to the arena.

(B) Drinking apparatus. At the center of the apparatus, there was a hole in which there was a gauze net soaked with 20% sucrose solution or 20% NaCl solution. A small piece of filter paper soaked with peppermint or vanilla essence, diluted with deionized water, was placed underneath the apparatus. The periphery of the apparatus had many small holes so that odor diffusion was ensured. The floor of the learner room was 1.6 cm higher than that of the demonstrator room, helping the learner to observe the demonstrator's behavior at the drinking apparatus.

(C) Test arena. The arena consisted of a waiting room and a training room separated by a door. There were two holes in the floor of the training room, which connected the floor with the two containers that contained filter papers soaked with peppermint or vanilla essence diluted with deionized water. The top of the container was covered with a gauze net. Three containers are mounted on a rotating holder, so that two of them could be presented at the same time.

demonstrator room and a learner room, separated by a transparent wall (Figure 2A). The wall had many small holes in front of the two drinking apparatus (Figure 2B) in the demonstrator room, so that the learner could sense odor and water vapor being emitted from the drinking apparatus (Figure 2C). Crickets in the two groups (experimental and control groups) received training with different stimulus arrangements. In the experimental group, two drinking apparatus, one containing 20% sucrose solution and emitting peppermint odor and the other containing 20% sodium chloride solution and emitting vanilla odor, were placed in the demonstrator room. In the control group, two apparatus containing 20% saltwater, one emitting peppermint odor and the other emitting vanilla odor, were placed. A demonstrator cricket was released into the demonstrator room and allowed to freely explore the drinking apparatus. For a period of 8 min, we measured the time that the demonstrators in the experimental group and the control group stayed at each drinking apparatus (Figure 3A). The demonstrators in the experimental group spent most of the time at the sucrose-containing apparatus that emitted peppermint odor. The stay time was significantly longer at the peppermint odor source than at the vanilla odor source (Welch t-test, $n = 23$, $p = 2.2e^{-15}$). In contrast, the demonstrators in the control group spent little time at either apparatus. The stay time at the peppermint odor source and that at the vanilla odor source did not significantly differ (Welch t-test, $n = 24$, $p = 0.15$).

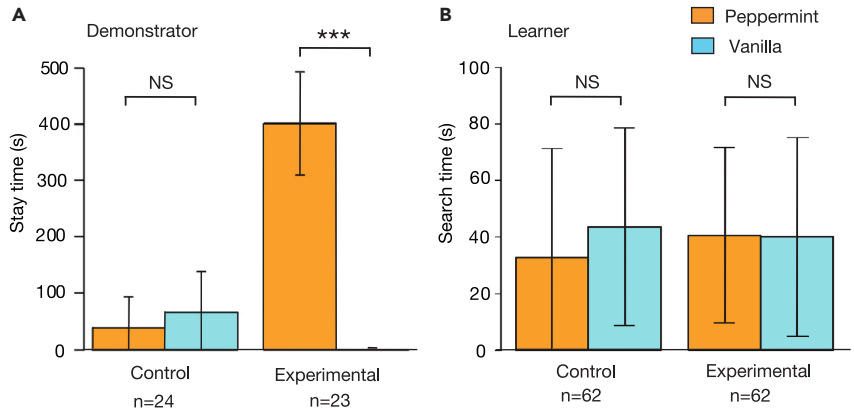


Figure 3. Assessments of the behavior of demonstrators and learners in training

(A) Stay time of demonstrators in the control group (for which drinking apparatus containing NaCl solution and emitting peppermint or vanilla odors were placed) and that of demonstrators in the experimental group (for which a drinking apparatus containing sucrose and emitting peppermint odor and an apparatus containing NaCl and emitting vanilla odor were placed). (B) Search time of learners in front of the drinking apparatus emitting peppermint odor or vanilla odor. The search time is the time that the learner spent touching the wall in front of the apparatus with its antennae, palpi, or mouth (see STAR Methods). The search times are shown as means and standard deviation. Student's *t* test was used for statistical comparison between them (***: $p < 0.001$; NS: $p > 0.05$).

Shortly after a demonstrator was released into the demonstrator room, a learner was released into the learner room. The learner often touched the wall in front of the drinking apparatus of the demonstrator room with its antennae or palpi (Video S1, An example of learner's behavior in the training arena, Related to Figure 2A). In Figure 3B, the time that the learner spent touching the wall in front of the two apparatus, one containing sucrose solution and emitting peppermint odor and the other containing saltwater and emitting apple odor, for a period of 8 min is shown. The stay time of the learners in front of the two apparatus did not significantly differ in the experimental group (Welch t-test, n = 62, p = 0.93) and in the control group (Welch t-test, n = 62, p = 0.11, Figure 3B). Thus, the learners explored the peppermint and vanilla odor sources almost equally in the training.

At 24 h after training, the learners in the experimental and control groups were tested with a relative preference between the peppermint odor and vanilla odor. Relative preference for the peppermint odor was significantly greater in the experimental group than in the control group (M–W test, p = 0.033, Figure 4). This indicates that the learner in the experimental group exhibited an increased preference for the odor of the drinking apparatus at which the demonstrator stayed for a longer period of time and thus social learning was achieved. In subsequent sections, we refer to the experimental group also as peppermint odor-demonstrated group and the control group also as no odor-demonstrated group to clarify the different stimulus arrangements of the two groups.

One of the simplest mechanisms to account for this social learning is sensitization or stimulus enhancement,[29] in which the learner is attracted to a conspecific and is exposed to a stimulus associated with the conspecific and this produces an increased response to that stimulus by the sensitization of that odor. Since the stay time of the learner in front of the peppermint source and that in front of the vanilla source did not significantly differ, increased preference for the peppermint odor is not accounted for by sensitization.

Results of our previous experiments suggested that the association between odor and water reward underlies responses to the learnt odor and we proposed a hypothesis that this association is achieved by SOC.[17] Our studies on SOC in crickets showed that intact synaptic transmission from OA neurons is needed at the second stage of SOC training and for the execution of learned action.[26] We deduced that if social learning is based on SOC, the same results could be found. We investigated this possibility.

Effects of blockade of synaptic transmission from octopamine neurons in training

We first studied the effects of the injection of epinastine (a potent antagonist of the insect OA receptor[27,28]) before training. The dose of epinastine was determined on the basis of our previous

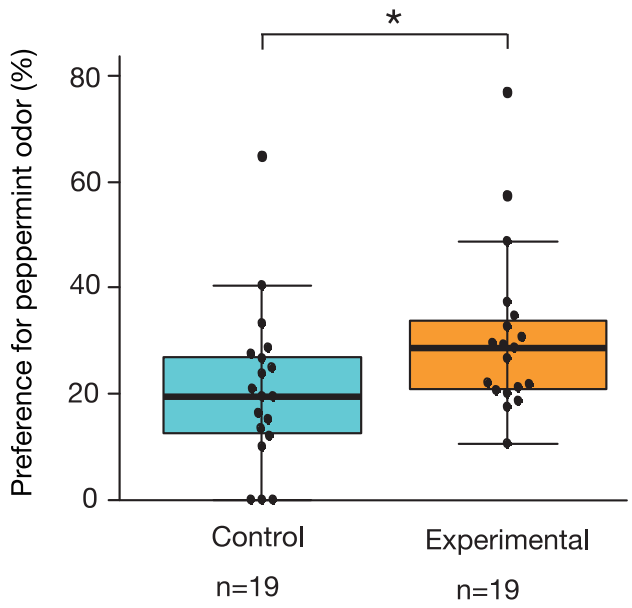


Figure 4. Odor preferences of the control and experimental groups that received training with different stimulus arrangements

Learners in the control group (no odor-demonstrated group) and the experimental group (odor-demonstrated group) were tested for their relative odor preference between the peppermint and vanilla odors 24 h after training. Relative preferences for the peppermint odor are shown as boxplots. The Mann-Whitney U-test was used for statistical comparison (*: $p < 0.05$).

experiments.[18,19,26] Our RNAi studies suggested that the effect of epinastine to impair appetitive learning is mediated by blockade of OA1-like receptors in crickets.[21] At first, learners in the two groups were injected with saline into the head hemolymph. Thirty minutes later, crickets in the experimental group received training in which a demonstrator stayed at the drinking apparatus with peppermint odor and crickets in the control group received training in which a demonstrator stayed at neither of the odor sources. Relative preference of the learners in the experimental group for the peppermint odor was significantly greater than that of the learners in the control group (M–W test, $p = 0.013$, Figure 5A), indicating that the injection of saline did not impair social learning. In the next experiment, on the other hand, learners that were injected with epinastine and then received training to demonstrate peppermint (experimental group) exhibited no significantly different odor preference from that of crickets in the control group that received epinastine injection and then received no odor-demonstrated training, indicating that the injection of epinastine impaired social learning (M–W test, $p = 0.76$, Figure 5B). This impairment was not due to the impairment of sensory functions necessary for achieving social learning, since we observed, at first, that behavioral reactions of epinastine-injected crickets to a conspecific did not differ from those of intact or saline-injected crickets, indicating that their perception of conspecific was intact. In addition, we showed that epinastine does not impair the discrimination of peppermint and vanilla odors[22,30] or motivation to drink water.[18,19,26] We thus conclude that intact synaptic transmission from OA neurons in training is needed for achieving social learning *per se*, in accordance with a prediction from the SOC hypothesis of social learning.

Effects of blockage of synaptic transmission from octopamine neurons in the test

In the final experiment, learners in the two groups received training to demonstrate peppermint odor (experimental group) or to demonstrate no odor (control group) and 24 h after training, they were injected with saline 30 min before the final test. Learners in another two groups received training to demonstrate peppermint odor or to demonstrate no odor and 24 h after training, they were injected with saline containing epinastine. Learners injected with saline in the experimental group exhibited significantly greater preference for the peppermint odor than did learners in the control group (M–W test, $p = 0.012$, Figure 6A), indicating that response to the learned odor is not impaired by the injection of saline. On the other hand, learners injected with epinastine in the experimental group exhibited no significantly different

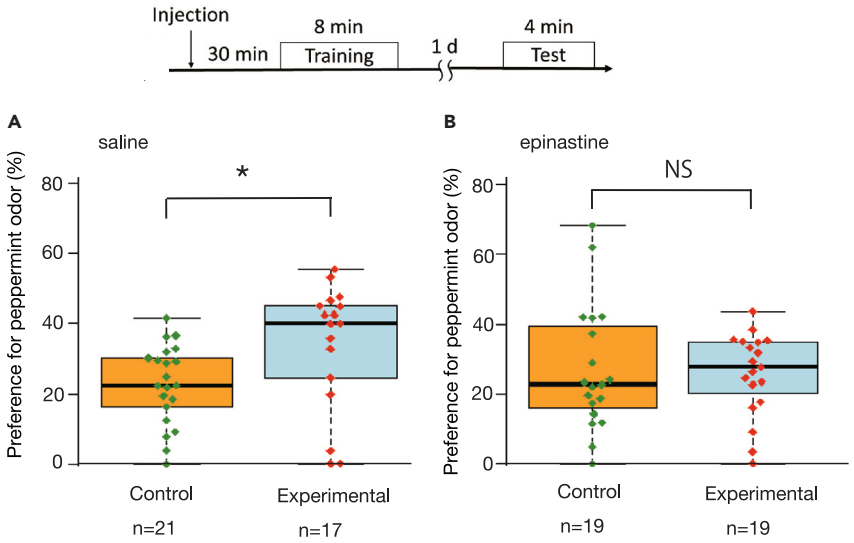


Figure 5. Effects of injection of saline or saline containing epinastine before training in the control and experimental groups

(A) Learners in the two groups were injected with saline before training to demonstrate no odor (control group) or peppermint odor (experimental group).

(B) Learners in the two groups were injected with saline containing epinastine before training to demonstrate no odor (control group) or peppermint odor (experimental group). Relative preferences for the peppermint odor in the test are shown as boxplots. The Mann-Whitney *U*-test was used for statistical comparison (*: $p < 0.05$; N.S.: $p > 0.05$).

preference for the peppermint odor from that of learners in the control group (M−W test, $p = 0.84$, Figure 6B), indicating that learners injected with epinastine exhibit no response to the learned odor. We thus conclude that intact synaptic transmission from OA neurons is needed for the execution of response to the learned odor. We showed that epinastine does not impair locomotory functions necessary for visiting odor sources.[18,19,26] Therefore, we conclude that epinastine impaired execution of the conditioned response to the learned odor, in accordance with the SOC hypothesis of social learning (Figure S1, A neural circuit model for SOC, Related to Figure 1).

DISCUSSION

Results of pharmacological analysis support the second-order conditioning hypothesis for social learning

The results of the present study on the effects of injection of an OA receptor antagonist in training and testing were in accordance with the SOC hypothesis for social learning in crickets, shown in Figure 1.[17] The hypothesis assumes that the presence of a conspecific at the drinking apparatus allowed an odor-water association by associative mechanisms of SOC, namely, crickets learn to associate conspecifics (CS1) at the drinking bottle with water (US) during the laboratory rearing stage and then they learn to associate the odor (CS2) of the drinking apparatus with a conspecific (CS1) that stays at the apparatus in training. The odor (CS2)-water (US) association is achieved by combining experiences in these stages. Injection of epinastine before training or the post-training test impaired learning and execution of response to the learned odor, respectively, in accordance with predictions from the SOC hypothesis.

A remaining issue for the confirmation of the SOC hypotheses is the investigation of whether the formation of an association between conspecifics at the drinking bottle (CS1) and water (US) in the laboratory rearing stage is essential for achieving social learning. Unfortunately, experimental demonstration of this is not easy, since the demonstration of this may require rearing of individual crickets in isolation until they grew into the adult stage, but such long-term isolation is known to impair the development of general capability of social communication in crickets.[31] Hence, experiments need to be designed to prevent group drinking experience but not prevent other forms of social interactions.

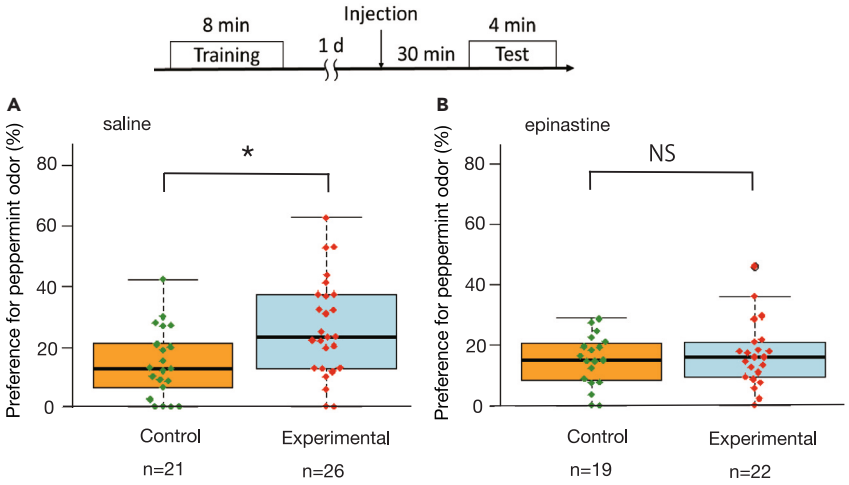


Figure 6. Effects of injection of saline or saline containing epinastine before the test in the control and experimental groups

Learners in the two groups received training to demonstrate no odor (control groups). Learners in another two groups received training to demonstrate peppermint odor (experimental groups). They were injected with saline (A) or saline containing epinastine (B) before testing. Relative preferences for the peppermint odor are shown as boxplots. The Mann-Whitney *U*-test was used for statistical comparison (*: $p < 0.05$; N.S.: $p > 0.05$).

Since the requirement of the first stage of SOC for achieving social learning has not yet been experimentally demonstrated, we would also like to consider the possibility that social learning is achieved without the first stage of SOC, namely, first-order Pavlovian conditioning in which the odor serves as CS and the conspecific at the drinking apparatus serves as the appetitive US. Following this hypothesis, adult male crickets have innate preference to conspecific adult males at the drinking apparatus and this attraction is transferred to the odor of the drinking apparatus. Unfortunately, the present pharmacological analysis using an OA receptor antagonist did not discriminate first-order conditioning and second-order conditioning following the model we proposed (Figure S1, A neural circuit model of SOC, Related to Figure 1).[26] The first-order conditioning hypothesis, however, does not match our previous finding that water devaluation abolishes the response to the learnt odor after social learning training, which suggests that the association between the odor and water, not that between the odor and conspecific, underlies the response to the learned odor.[17] Moreover, the assumption that adult male crickets are innately attracted to conspecific adult males is not in accordance with an observation that adult males, in general, exhibit aggression to adult males when they approached too close. Hence, it is very difficult to speculate that first-order conditioning is involved in this social leaning, but experiments are needed to fully rule out this possibility.

Mirror-like activities of octopamine neurons mediate social learning in crickets

The SOC hypothesis of social learning shown in Figures 1 and S1 indicates that a class of OA neurons that mediate water US signals play critical roles in social learning. Specifically, the SOC hypothesis indicates that (1) a class of OA neurons are activated when a learner drink water in the rearing stage, (2) these neurons are also activated when the learner observes a conspecific (CS1) at the drinking apparatus in training, without itself drinking water, (3) activation of these neurons produces conditioning of odor (CS2) with water (US), and (4) these neurons are also activated in response to the learned odor (CS2) and control execution of the CR in accordance with current value of the US.[22] Most notably, these OA neurons are activated when the learner drinks water (in group-rearing stage) and when it observes a conspecific staying at a drinking apparatus (in training), which are analogous to that mirror neurons in the premotor cortex and the posterior parietal cortex of primates are activated both when an animal makes a certain action and when the animal observes another animal's similar action.[32,33] Following our SOC hypothesis of social leaning, we suggest that mirror-like activities of OA neurons mediate social learning in crickets.

In rodents, it has been reported that neurons in the anterior cingulate cortex (ACC) exhibit mirror-like activities and encode the pain of the self and others,[34] and such mirror-like activities are considered as the

basis of fear learning by the observation of others.[14] In humans, it has been reported that the ACC responds when witnessing pain in others and that it responds when experiencing pain in the self,[35,36] which are likely to be the basis of observational fear learning. Social leaning in crickets and observational fear learning in mammals differ in their complexity and sophistication, i.e., the former is based on simple conditioning mechanisms and the latter involves sharing the emotional experiences of others. Nevertheless, this study suggests that they share common neural principles, i.e., vicarious activation of US signals (water in the case of crickets and pain in the case of mammals) by the observation of others is critical for mediating social leaning.

Limitation of the study

Further physiological studies are needed to determine which OA neurons participate in social learning and whether their neural activities match to the predictions from the SOC hypothesis of social learning. Watanabe et al.[37] established the transgenic reporter system of activities of brain neurons in crickets, which is driven by immediate-early gene promotor, and they successfully visualized OA neurons that are activated in response to the presentation of sucrose solution to the mouth. Our next step is to use that reporter system and investigate which OA neurons are activated by the observation of a conspecific in social learning.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Animals
- METHOD DETAILS
  - Training procedure
  - Procedure for the odor preference test
  - Pharmacology
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106612.

ACKNOWLEDGMENTS

This study was supported by Japan Society for the Promotion of Science (no. 21K19245) to MM.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: Y.S., K.H., and M.M. Performed the experiments: Y.S. and K.H. Analyzed the data: Y.S. and K.H. Wrote the article: Y.S., K.H. and M.M.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in their field of research or within their geographical location. While citing references scientifically relevant for this work, we also actively worked to promote gender balance in our reference list. We avoided "helicopter science" practices by including the participating local contributors from the region where we conducted the research as authors on the paper.

Received: October 22, 2022
Revised: December 14, 2022
Accepted: March 29, 2023
Published: April 8, 2023

REFERENCES

1. Heyes, C.M. (1994). Social learning in animals: categories and mechanisms. Biol. Rev. *69*, 207–231.

2. Heyes, C. (2012). What's social about social learning? J. Comp. Psychol. *126*, 193–202. https://doi.org/10.1037/a0025180.

3. Laland, K.N., Atton, N., and Webster, M.M. (2011). From fish to fashion: experimental and theoretical insights into the evolution of culture. Phil. Trans. R Soc. B *366*, 958–968. https://doi.org/10.1098/rstb.2010.0328.

4. Coolen, I., Dangles, O., and Casas, J. (2005). Social learning in noncolonial insects? Curr. Biol. *15*, 1931–1935. https://doi.org/10.1016/j.cub.2005.09.015.

5. Alem, S., Perry, C.J., Zhu, X., Loukola, O.J., Ingraham, T., Søvik, E., and Chittka, L. (2016). Associative mechanisms allow for social learning and cultural transmission of string pulling in an insect. PLoS Biol. *14*, e1002564. https://doi.org/10.1371/journal.pbio.1002564.

6. Perry, C.J., Barron, A.B., and Chittka, L. (2017). The frontiers of insect cognition. Curr. Opin. Behav. Sci. *16*, 111–118. https://doi.org/10.1016/j.cobeha.2017.05.011.

7. Dawson, E.H., Avargue's-Weber, A., Chittka, L., and Leadbeater, E. (2013). Learning by observation emerges from simple associations in an insect model. Curr. Biol. *23*, 1–4. https://doi.org/10.1016/j.cub.2013.03.035.

8. Dunlap, A.S., Nielsen, M.E., Dornhaus, A., and Papaj, D.R. (2016). Foraging bumble bees weigh the reliability of personal and social information. Curr. Biol. *26*, 1195–1199. https://doi.org/10.1016/j.cub.2016.03.009.

9. Dawson, E.H., and Chittka, L. (2014). Bumblebees (*Bombus terrestris*) use social information as an indicator of safety in dangerous environments. Proc. Biol. Sci. *281*, 20133174. https://doi.org/10.1098/rspb.2013.3174.

10. Mery, F., Varela, S.,A.M., Danchin, E., Blanchet, S., Parejo, D., Coolen, I., and Wagner, R.H. (2009). Public versus personal information for mate copying in an invertebrate. Curr. Biol. *19*, 730–734. https://doi.org/10.1016/j.cub.2009.02.064.

11. Joiner, J., Piva, M., Turrin, C., and Chang, S.W.C. (2017). Social learning through prediction error in the brain. NPJ Sci. Learn. *2*, 8. https://doi.org/10.1038/s41539-017-0009-2.

12. Noritake, A., Ninomiya, T., and Isoda, M. (2018). Social reward monitoring and valuation in the macaque brain. Nat. Neurosci. *21*, 1452–1462. https://doi.org/10.1038/s41593-018-0229-7.

13. Carcea, I., and Froemke, R.C. (2019). Biological mechanisms for observational learning. Curr. Opin. Neurobiol. *54*, 178–185. https://doi.org/10.1016/j.cub.2019.03.024.

14. Keum, S., and Shin, H.-S. (2019). Neural basis of observational fear learning: a potential model of affective empathy. Neuron *104*, 78–86. https://doi.org/10.1016/j.neuron.2019.09.013.

15. Monier, M., Nöbel, S., Danchin, E., and Isabel, G. (2018). Dopamine and serotonin are both required for mate-copying in *Drosophila melanogaster*. Front. Behav. Neurosci. *12*, 334. https://doi.org/10.3389/fnbeh.2018.00334.

16. Kacsoh, B.Z., Bozler, J., Hodge, S., and Bosco, G. (2019). Neural circuitry of social learning in *Drosophila* requires multiple inputs to facilitate inter-species communication. Commun. Biol. *2*, 309. https://doi.org/10.1038/s42003-019-0557-5.

17. Ebina, H., and Mizunami, M. (2020). Appetitive and aversive social learning with living and dead conspecifics in crickets. Sci. Rep. *10*, 9340. https://doi.org/10.1038/s41598-020-66399-7.

18. Unoki, S., Matsumoto, Y., and Mizunami, M. (2005). Participation of octopaminergic reward system and dopaminergic punishment system in insect olfactory learning revealed by pharmacological study. Eur. J. Neurosci. *22*, 1409–1416. https://doi.org/10.1111/j.1460-9568.2005.04318.x.

19. Unoki, S., Matsumoto, Y., and Mizunami, M. (2006). Roles of octopaminergic and dopaminergic neurons in mediating reward and punishment signals in insect visual learning. Eur. J. Neurosci. *24*, 2031–2038. https://doi.org/10.1111/j.1460-9568.2006.05099.x.

20. Awata, H., Watanabe, T., Hamanaka, Y., Mito, T., Noji, S., and Mizunami, M. (2015). Knockout crickets for the study of learning and memory: dopamine receptor Dop1 mediates aversive but not appetitive reinforcement in crickets. Sci. Rep. *5*, 15885. https://doi.org/10.1038/srep15885.

21. Awata, H., Wakuda, R., Ishimaru, Y., Matsuoka, Y., Terao, K., Katata, S., Matsumoto, Y., Hamanaka, Y., Noji, S., Mito, T., and Mizunami, M. (2016). Roles of OA1 octopamine receptor and Dop1 dopamine receptor in mediating appetitive and aversive reinforcement revealed by RNAi studies. Sci. Rep. *6*, 29696. https://doi.org/10.1038/srep29696.

22. Mizunami, M., Hirohata, S., Sato, A., Arai, R., Terao, K., Sato, M., and Matsumoto, Y. (2019). Development of behavioural automaticity by extended Pavlovian training in an insect. Proc. Biol. Sci. *286*, 2018–2132. https://doi.org/10.1098/rspb.2018.2132.

23. Leadbeater, E., and Chittka, L. (2007). Social learning in insects – from miniature brains to consensus building. Curr. Biol. *17*, R703–R713. https://doi.org/10.1016/j.cub.2007.06.012.

24. Giurfa, M. (2012). Social learning in insects: a higher-order capacity? Front. Behav. Neurosci. *6*, 57. https://doi.org/10.3389/fnbeh.2012.00057.

25. Roeder, T. (1999). Octopamine in invertebrates. Prog. Neurobiol. *59*, 533–561. https://doi.org/10.1016/s0301-0082(99)00016-7.

26. Mizunami, M., Unoki, S., Mori, Y., Hirashima, D., Hatano, A., and Matsumoto, Y. (2009). Roles of octopaminergic and dopaminergic neurons in appetitive and aversive memory recall in an insect. BMC Biol. *7*, 46. https://doi.org/10.1186/1741-7007-7-46.

27. Roeder, T., Degen, J., and Gewecke, M. (1998). Epinastine, a highly specific antagonist of insect neuronal octopamine receptors. Eur. J. Pharmacol. *349*, 171–177. https://doi.org/10.1016/s0014-2999(98)00192-7.

28. Degen, J., Gewecke, M., and Roeder, T. (2000). Octopamine receptors in the honeybee and locust nervous system: pharmacological similarities between homologous receptors of distantly related species. Br. J. Pharmacol. *130*, 587–594. https://doi.org/10.1038/sj.bjp.0703338.

29. Hoppitt, W., and Laland, K.N. (2008). Social Processes influencing learning in animals: a review of the evidence. Adv. Stud. Behav. *38*, 105–165. https://doi.org/10.1016/S0065-3454(08)00003-X.

30. Terao, K., Matsumoto, Y., and Mizunami, M. (2015). Critical evidence for the prediction error theory in associative learning. Sci. Rep. *5*, 8929. https://doi.org/10.1038/srep08929.

31. Stevenson, P.A., and Rillich, J. (2013). Isolation associated aggression – a consequence of recovery from defeat in a territorial animal. PLoS One *8*, e74965. https://doi.org/10.1371/journal.pone.0074965.

32. Gallese, V., Fadiga, L., Fogassi, L., and Rizzolatti, G. (1996). Action recognition in the premotor cortex. Brain *119*, 593–609. https://doi.org/10.1093/brain/119.2.593.

33. Fogassi, L., Ferrari, P.F., Gesierich, B., Rozzi, S., Chersi, F., and Rizzolatti, G. (2005). Parietal Lobe: from action organization to intention understanding. Science *308*, 662–667. https://doi.org/10.1126/science.1106138.

34. Carrillo, M., Han, Y., Migliorati, F., Liu, M., Gazzola, V., and Keysers, C. (2019). Emotional mirror neurons in the rat's anterior cingulate cortex. Curr. Biol. *29*, 1301–1312.e6.

35. Lamm, C., Decety, J., and Singer, T. (2011). Meta-analytic evidence for common and distinct neural networks associated with directly experienced pain and empathy for pain. Neuroimage *54*, 2492–2502. https://doi.org/10.1016/j.neuroimage.2010.10.014.

36. Apps, M.A.J., Rushworth, M.F.S., and Chang, S.W.C. (2016). The anterior cingulate gyrus and social cognition: tracking the motivation of others. Neuron *90*, 692–707. https://doi.org/10.1016/j.neuron.2016.04.018.

37. Watanabe, T., Ugajin, A., and Aonuma, H. (2018). Immediate-early promoter-driven transgenic reporter system for neuroethological research in a hemimetabolous insect. eNeuro *5*, 0061–18.2018. https://doi.org/10.1523/ENEURO.0061-18.2018.

38. Matsumoto, Y., and Mizunami, M. (2002). Temporal determinants of long-term retention of olfactory memory in the cricket *Gryllus bimaculatus*. J. Exp. Biol. *205*, 1429–1437. https://doi.org/10.1242/jeb.205.10.1429.

39. Matsumoto, Y., Noji, S., and Mizunami, M. (2003). Time course of protein synthesis-dependent phase of olfactory memory in the cricket *Gryllus bimaculatus*. Zool. Sci. *20*, 409–416. https://doi.org/10.2108/zsj.20.409.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals		
epinastine hydrochloride	Sigma-Aldrich	108929-04-0
peppermint essence	Mikoya-Kosho (Tokyo)	N/A
vanilla essence	Meiji-ya (Tokyo)	N/A
Software and algorithms		
R	The R Foundation	http:/www.r-project.org/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Makoto Mizunami (mizunami@es.hokudai.ac.jp).

Materials availability

Hokudai WT strain of the cricket *Gryllus binmaculatus* is available from Dr. Watanabe Takayuki in Sokendai (Watanabe_takayuki@soken.ac.jp).

Data and code availability

- All raw data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.
- All data reported in this study have been deposited at Zenode and are publicly available as of the data of publication. The URL is: https://doi.org/10.5281/zenodo.7732358.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals

A wild-type strain of two-spotted crickets *(Gryllus bimaculatus*) has been inbred for several decades in our laboratory (Hokudai WT strain). Adult male crickets at 1 week after the imaginal molt were used in this study. They were reared in a 12h:12h light: dark cycle at 27 ± 2 °C and were fed a diet of insect pellets and water *ad libitum*. Water was supplied by a glass bottle (4 cm in diameter x 12 cm in length) tightly plugged with cotton wool. At 3-4 days before the training, the crickets were isolated and deprived of water to enhance their motivation to search for water.

METHOD DETAILS

Training procedure

Social learning training was performed in an arena (Figure 2A). The arena consisted of four rooms. Two of the rooms were a demonstrator room and a learner room separated by a plastic transparent wall with many small holes in front of the drinking apparatuses in the demonstrator room. The other two rooms were waiting rooms for the demonstrator room and the learner room, separated by sliding doors. A demonstrator cricket was placed in a waiting room for acclimation for a few minutes and then gently pushed into the demonstrator room. There were two drinking apparatuses in the demonstrator room. At the center of the drinking apparatus there was a hole that contained a gauze net soaked with 20% sucrose solution or 20% NaCl solution (Figure 2B). Underneath the apparatus, a small piece of filter paper soaked with peppermint or vanilla essence, diluted with deionized water, was placed. The periphery of the apparatus had many

small holes so that odor diffusion is ensured. The demonstrator was allowed to freely visit the apparatuses. A learner cricket was also placed in a waiting room and gently pushed into the learner room. It was allowed to observe the behavior of the demonstrator through a transparent wall for 8 min. For experiments, crickets in two groups (experimental group and control group) received training with different stimulus arrangements. For training of the experimental group (peppermint odor-demonstrated group), a sucrose-containing apparatus emitting peppermint odor and an NaCl-containing apparatus emitting vanilla odor were placed in the demonstrator room. For training of the control group (no odor-demonstrated group), two apparatuses containing NaCl solution, one emitting peppermint odor and the other emitting vanilla odor, were placed in the demonstrator room. Air was continuously sucked out from a duct placed above the training arena. The arena was illuminated by a fluorescent lamp (1000-2000 lux). The behavior of the learner and the demonstrator in training and the behavior of the learner in testing were recorded by web camera.

The demonstrator cricket that visited the sucrose-containing apparatus often touched the gauze net that contained sucrose with its palpi or mouth and then drank it. In contrast, the demonstrator that visited the NaCl-containing apparatus left the apparatus soon after touching the NaCl-containing gauze net with its palpi or mouth. The typical behavior of the learner in front of the drinking apparatus at which the demonstrator stayed was as follows. The learner (1) approaches the wall just in front of the apparatus where the demonstrator stays, (2) touches the wall with its antennae and then (3) touches the wall with its palpi or mouth. Then the learner (4) stops touching the wall and (5) leaves the place or resumes touching the wall.[17] We refer to ''search time'' as the time that the learner spent touching the wall in front of the apparatus with its antennae, palpi or mouth.

The experimental procedure used in this study was modified from that used in a previous social learning experiment.[17] The modifications in this study were: (1) the demonstrator and learner rooms were separated by a plastic transparent wall with many small holes, instead of a plastic net used in the previous study, in order to avoid direct contact between a learner and a demonstrator with their antennae or palpi, (2) waiting rooms were added to the training arena so that the learner and the demonstrator could acclimate to the arena, (3) peppermint and vanilla odors (Crickets prefer vanilla odor over peppermint odor) were used in this study, instead of apple and banana odors (Crickets equally prefer these odors) used in the previous study, (4) the experiment was designed to compare two groups, a peppermint odor-demonstrated group and a no odor-demonstrated group, instead of a comparison among three groups, vanilla odor-demonstrated group, peppermint odor-demonstrated group and no-odor demonstrated group in the previous study, for allowing statistical evaluation of data with a relatively small sample size, and (5) drinking apparatuses containing 20% sucrose solution and 20% NaCl solution were used in this study, instead of apparatuses containing water and 20% NaCl solution used in the previous study, to assure that the demonstrator stays for a longer period of time at the drinking apparatus.

Procedure for the odor preference test

The methods used for the preference test were described previously.[18,38] The test was performed in a test arena (Figure 2C) in which there were two holes in the floor connecting the floor with two containers that contained filter papers soaked with peppermint or vanilla essence diluted with deionized water. The upper part of the container was covered with a gauze net. In the test, a cricket was released in the test arena and allowed to freely visit the odor sources for 4 minutes. The relative positions of the odors were exchanged 2 min after the start of the test by rotating the container holder on which three containers were mounted. The time that the cricket spent touching the gauze net covering each container with its mouth or palpi was measured cumulatively.

Pharmacology

Crickets were injected with 3 μl of physiological saline[39] or saline containing 2 μM epinastine (Sigma, Tokyo, Japan) into the head hemolymph at 30 min before training or the preference test. The timing of injection and the concentrations of drugs used are based on our previous studies.[18,19] RNAi studies suggest that impairment of appetitive conditioning by epinastine is through blockade of OA1-like receptors,[21] although possible involvement of other types of OA receptors has not been ruled out.

QUANTIFICATION AND STATISTICAL ANALYSIS

Stay time of the demonstrator and search time of the learner in front of the drinking apparatuses were statistically evaluated by Welch's t-test. The relative preference between peppermint and vanilla odors was measured by the preference index for peppermint odor, which is the percentage of the search time for peppermint odor divided by the total search time for peppermint and vanilla odors. If the total time to visit the odor sources was less than 10 sec, we considered that the cricket was less motivated to visit odor sources, possibly due to a poor physical condition, and the data were discarded. Such crickets were less than 10% of the tested crickets. The Mann–Whitney *U* test was used for statistical evaluation of the data. The data were considered statistically significant when $P<0.05$.