Insights into the role of dopamine in olfactory learning behavior of honeybee

Muhammad Fahad Raza[1, 2], Zhiguo Li[1], Hongyi Nie[1], Songkun Su [1,*]

[1] College of Animal Sciences (College of Bee Science)
Fujian Agriculture and Forestry University, Fuzhou, China.
[2] College of Life Sciences, Fujian Agriculture and Forestry University,
Fuzhou, China

Corossponding author: susongkun@zju.edu.cn

Abstract

One of the brain's primary functions is remembering and learning information related to food and odour. Since biogenic amines were discovered in invertebrates and vertebrate, dopamine is considered a key modulator and neurotransmitter in honeybees' olfactory learning. Dopamine (DA) is significant in rewarding prediction, learning, invigorating social behavior, and motivation. Here we examined the effect of dopamine in the olfactory learning behavior of honeybees. We used the same age (14-days old) honeybees, *Apis mellifera* and *Apis cerana* to evaluate the proboscis extension response and characterized brain dopamine's effect on olfactory learning behavior. Both species were individually trained by performing three learning trials with sucrose solution and odor 1-Hexanol. High-performance liquid chromatography (HPLC) determined the brain dopamine level using electrochemical detection. Our findings showed that *Apis mellifera* learned better and had higher brain dopamine levels than *Apis cerana*. Thus, we show that dopamine acts as an essential neurotransmitter and modulator of motivation and influences honeybee cognition.

Keywords: Biogenic amine; dopamine; olfactory conditioning; proboscis extension response; social insect.

1. Introduction

In animals' central nervous system, associative learning is essential for predicting the ecosystem process rules(Benca *et al*., 2009). Several types of learning behaviour have been studied, but classical learning has been acknowledged due to its basic principles in different species. In associative learning behavior, insects learn by associating conditioned stimulus (Odor) with the unconditioned stimulus (Sucrose solution). Initially, the odor (a neutral stimulus) does not exhibit the conditioned response, while the latter exhibits the instinctive response due to a biologically relevant stimulus(Webb, 2012). The pairing of odor (CS) with the sucrose solution(US) develops an associative relationship between the conditioned stimulus (CS) and the unconditioned stimulus (US), thus exhibiting the prominent response to the odor that predicts the sugar solution(Chabaud *et al*., 2006). Insects are important model organisms for the Pavlovian conditioning study(Menzel

& Müller, 1996). Numerous studies on the *Apis mellifera* have been carried out using Pavlovian conditioning protocol to describe the behavioral, molecular and neural studies (Giurfa 2007, Palottini *et al*., 2018, Schleyer *et al*., 2018). In proboscis extension response protocol, odorant (CS) was presented to retrained bees with a paired association of sucrose solution (US), which is touched with both antenna and next to the proboscis. The sugar solution is offered to the antenna for eliciting the (PER) (proboscis extension response), which is established by odor following the successful conditioning(Smith & Burden, 2014, Van Nest, 2018).

The most important principle is that behavioral plasticity and odorant stimuli association have been shown in the brain of numerous species of insects. Odorant receptors detect different odors by the antenna and the sensory information is processed in sequential steps to sensory pathways, including mushroom bodies' antennal lobes and the lateral horns (Fiala 2007, Sandoz 2011, Rössler & Brill, 2013). Changes in neural plasticity activity have been identified at these different levels due to classical or Pavlovian conditioning(Gerber *et al*., 2009, Busto *et al*., 2010, Rössler & Brill, 2013). However, neural differences exist concerning unconditioned stimulus activity in insects' brains; even conditioned stimulus (sugar solution) is rewarded with appetitive learning. While biogenic amines have a crucial effect on sucrose reward learning, the unique biogenic amine needed for this function varies between species of insects. In this instance, dopamine (DA) plays an essential signaling role in fruit flies (*Drosophila melanogaster*) trained with the pairing of odor and sucrose solution as a reward. In mammals, dopamine has been associated with motivation, reward and pleasure. Also mediate in aversion learning (Van Swinderen & Andretic 2011, Ichinose *et al*., 2017); several studies reported that dopamine neurons are also directly involved in appetitive reinforcement (reward stimuli) and aversive learning (aversive stimuli)(Roussel *et al*., 2010).

Our findings are directed at both species' appetitive olfactory learning and analysing the relationship between learning success and dopamine (DA) levels. We hypothesised that dopamine levels might be linked with the predisposition to learn the olfactory learning association between odor and sucrose and vary between learners and non-learners. This study aims to investigate olfaction learning success and evaluate the role of dopamine in the learning performance of *Apis mellifera* and *Apis cerana*. We used high-performance liquid chromatography (HPLC) with an electrochemical detection (ECD) system to quantify dopamine levels in both species' brains following appetitive olfactory learning. We revealed that olfactory learning's success was directly related to dopamine (DA) levels. The DA could act as a motivational neurotransmitter and improve olfactory learning success.

2. Materials and methods

2.1 Experimental Bees

The bees of *A. mellifera* and *A cerana* were obtained from the experimental apiary of the College of Animal Sciences, Fujian Agriculture and Forestry University. Capped combs of both species were obtained from six different healthy colonies (Three colonies of *A. mellifera* and three colonies of *A.cerana*) and placed in an incubator. The newly emerged bees were collected every day and

kept in cages. Plastic cages were kept in an incubator temperature of 30ºC (±1, 30ºC), and relative humidity 70% (±1 70%) to get new emerging bees. More or less 50 newly emerged bees were in s plastic cages. The total number of *A. cerana* (n=350) and *A. mellifera* (n=250) were used to evaluate the learning behavior. Every day, mortality was recorded and dead bees were removed from cages. The food and sugar solution was replaced after about three days. The honeybees were maintained following the standard procedure(Williams *et al*., 2013). When the bees were 14 days old, they were brought to the laboratory for olfactory PER conditioning.

2.2   Sucrose Sensitivity

Honeybees were brought for a sucrose sensitivity test and transferred to a glass vial. The glass vials were kept in the icebox for 3-4 minutes to immobilize the bees and harnessed following a standard procedure(Matsumoto *et al*., 2012) and kept in an incubator at 30ºC and relative humidity of 70% (±1, 70%) for one hour. Before conditioning, a drop of 30% (w/v) sucrose solution was delivered to the antennae to check for intact PER. Bees not responding with PER to this stimulation were discarded from the experiment. Bees were trained using odorant 1- hexanol (Sigma Aldrich, France) was always paired with 30% sucrose solution. Each CS+ trial lasted 39 sec. First, the harnessed bee was placed in front of the olfactometer and clean air was delivered to the antennae for 15 s. Then an odorant was then delivered during 4 s. Two seconds after odor onset, Sucrose sucrose solution was delivered for 2 sec, 2 sec after odor onset. Therefore, the interstimulus interval was 2 sec and the CS and US finished ended simultaneously. Finally, clean air was delivered without other stimulations for 20 sec to complete the 39-sec trial. The intertrial interval was 10 min. The PER (proboscis extension response) to each odorant (conditioned response) was recorded during training. Responses were noted as "1" or "0" (no PER).

2.3   Quantification of dopamine level

At the end of conditioning, bees were transferred to death in nitrogen liquid and stored at -80°C for subsequent brain dissection and dopamine quantifications. Brains dissection was performed into the frozen dish in dry ice under a cold-light source and kept frozen throughout all dissection. Compound eyes, hypopharyngeal glands, celli, trachea, and glandular tissues were removed during dissection. Brains in which pieces of tissue were lost and discarded so that only intact brains were used for HPLC analyses. Each brain was kept at -80° C in a 1.5 mL centrifuge tube until it was analyzed. To compare dopamine levels, we randomly chose twenty brains of 'learners' and twenty brains of 'non-learners' to detect accentuated differences, if any. We used high-performance liquid chromatography (HPLC) with electrochemical detection to measure the concentration of the biogenic amine dopamine, according to (Harris & Woodring 1992, Li *et al*., 2009). Details of the HPLC procedure are provided in the Supplementary Information.

3. Results

The in vitro rearing of emerging honeybees (*A. cerana*, *A. mellifera)* has become a gradually essential honeybee research method to get the same-age bees for our experiment specifically. In this experiment, I compared the survival percentage of *A. cerana* versus *A. mellifera* based on 14 days' intervals. Survival percentage is expressed in survival bees during the monitoring period of 14 days (Figure 1). Using Kaplan-Meier survival analysis, the Log-Rank test was used to determine the survival percentage. Both species, *A. cerana* (survivorship, 81.42%) and *A. mellifera* (survivorship, 67.6%), showed a significant difference in survival percentage during 14 days. (***$p \leq 0.001$, IBM SPSS Statistics 21,) (Figure 1). During the development, the bees of *A. cerana* showed a significantly higher survival range than bees of *A. mellifera*. Survival percentage is expressed of survival bees during the monitoring period of 14 days (Figure 1).
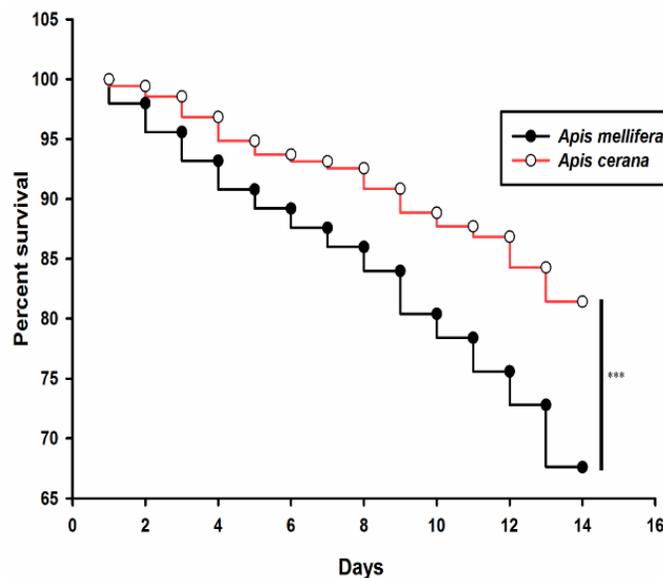

Fig. 1. Kaplan–Meier survival analysis curves showing the percentage survival among *Apis cerana* (n=350) and *Apis mellifera* (n=250) at different day's intervals. Both species *A. cerana* (survivorship, 81.42%) and *A. mellifera* (survivorship, 67.6%) showed significant differences during 14 day's intervals (Figure 1) (***p ≤ 0.001, IBM SPSS Statistics 21, ANOVA).

After 14 days, population responses of bees *A. mellifera* (n=150) versus *A. cerana* (n-=115) were trained to discriminate between the learner and non-learner during three conditioning trials (T1, T2, T3). Both species showed PER to an odor in each of the three conditioning trials; one-way ANOVA was used to analyse the learning trials (Figure 2). *A.mellifera* exhibited significance difference among three condition trials represented with small letters a,b,c (F (2, 6) = 501.1, p-value P<0.0001) and *A. cerana* showed significant difference with capital letters A,B, C (F (2, 6) = 468.9, p-value P<0.0001) (Figure 2).
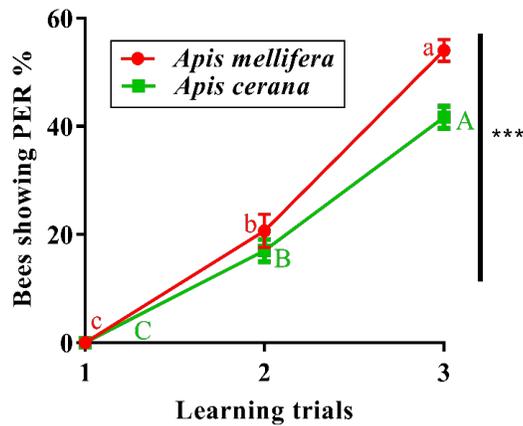
Fig. 2. Learning trials comparison in the same age bees of *A. cerana* and *A. mellifera*.

Our results indicated that *A. mellifera* has a significantly higher proboscis extension response than *A. cerana*. The bees learning ability of *A. mellifera* learner versus *A. cerana* learner was significant. Bees showing Proboscis extension reflex (Mean ± SE) of 14 days' adult honeybees from both species. The percentage proboscis extension response of *A. mellifera* was significantly higher than *A. cerana* using t-test. Proboscis extension response of leaner bees was the significant difference (Assigned letter a, b) found in *A. mellifera* and *A. cerana* (t=9.175, df=4, p-value .001***p ≤ 0.001). No significant difference (A, A) was observed between non-learner in *A. cerana* and *A. mellifera* (t=-1.091, df=4, p-value .336). The of *A. mellifera* (t=12 df=4, p-value 0.0003, ***p ≤ 0.001) and *A. cerana* (t=2.885 df=4, p-value 0.0448, *p ≤ 0.05) showed significant differences (Figure 3).
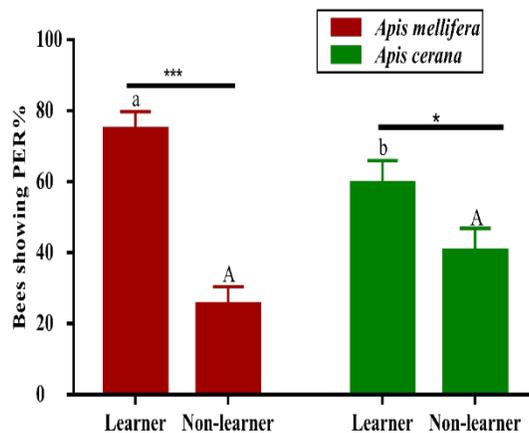

Fig. 3. Olfactory learning performance in the same age bees of *A. cerana* and *A. mellifera*. The learning trials of *A. mellifera* versus *A. cerana*. Bees showing Proboscis extension reflex (Mean ± SE) of 14 days' adult honeybees from *A. mellifera* (n=150) versus *A. cerana* (n-=115). The PER percentage of *A. mellifera* was significantly higher than *A. cerana* by t-test.
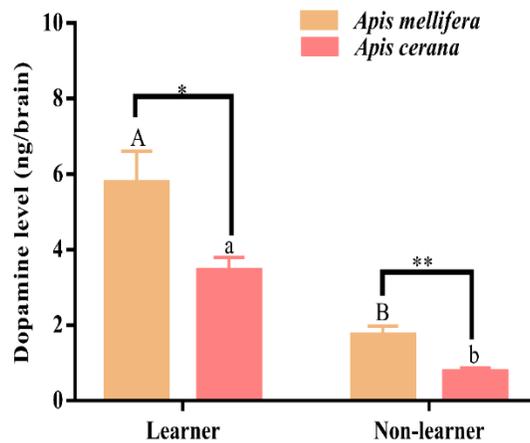
Fig. 4. Dopamine (DA) levels (ng/brain; mean ± S.E.) measured in individual brains of learners (n=40) and non-learner (n=40) of both species. DA level was higher in the learner of *A. mellifera* than *A. cerana*.

The experiment was performed to measure the dopamine level of learner and non-learner groups of both species *A. mellifera* versus *A. cerana* (Figure 4). The data of brain dopamine levels in 14 days old bees are expressed as Mean ± SE. Comparison of brain dopamine level between learner and non-learner in *A. mellifera* and *A. cerana* analysed by t-test. Dopamine levels of the learner and non-learner bees were significantly found in both species. *A. cerana (*a, b) (t=7.243 df=18, p-value 0.0001, ***p ≤ 0.001) and *A. mellifera (*A, B) (t=4.574, df=18, p-value 0.0002, ***p ≤ 0.001) showed significant difference between learners and non-learners bees. Statistically, learner bees of *A. cerana* versus *A. mellifera* showed significant results (t=2.53 df=18, p-value 0.0209, (*p ≤ 0.05). The dopamine level varies significantly in the non-learner group of *A. mellifera* versus *A. cerana* (t=3.573 df=18, p-value 0.0022 **p ≤ 0.01).

4. Discussion

For crops' pollination, Olfactory learning behavior was beneficial for ecological survival (Wright & Schiestl 2009, Iqbal *et al*., 2019). Due to honeybee's impressive learning ability, the olfactory foraging behavior is closely related to memorizing and learning the routes and local features, directly associated with the nectar source's reliability and quality (Dyer *et al*., 2008). The learning and memory mechanisms in honeybees are highly conserved. Nevertheless, several species of honeybees' learning performance variation cannot be ignored due to several factors such as geographical area, diversified foraging behavior, genetic variation, local climate, bee size, and evolutionary lability(Al-Ghamdi *et al*., 2017).

Several experiments have been conducted with free-flying honeybee visiting differently scented feeders(Reinhard *et al*., 2004). These studies have provided significant advantages in the ecosystem's context, but several experiment variables, such as bee or inter-trial intervals' physiological status, cannot be precisely controlled. Moreover, investigating olfactory learning for the neural basis needs neurophysiological measures to monitor the bees' brain. Simultaneously, it

learns odor and learning processes (Laska *et al*., 1999, Giurfa 2007). For these considerations, the experimental conditional protocol was developed to study the olfactory learning behavior of individual restrained bees (Hammer 1997). Three conditioning trials were conducted to evaluate the learning performance of both species. Our results revealed that learner bees of *A. mellifera* showed increased PER than *A,cerana*. The learning performance of A.mellifera is better than A.cerana bees revealed in our previous study(Raza *et al*., 2019). (Zhengwei WANG and Ken TAN., 2013) also reported that *A.cerana* is a slow learner and bees of *A.cerana* showed more PER than *A. mellifera* after three learning trials (Wang & Tan, 2014). Our results suggested that bees of *A. cerana* can be used as a remarkable organism for olfactory learning, neurobiological and physiological research. Here we cannot conclude that some unspecific parameters may affect olfactory learning behavior. For example, both species need different amounts of food, the difference in harnessing reactions in the metal tube before olfactory learn, and sucrose concentration level as a reward during learning trials. We predicted that *A. mellifera* bees showed more response and learned better with less concentrated sucrose solution compared to that of *A. cerana*. We've come to the conclusion that bees of *A. cerana* have a distinctive pattern in olfactory learning behavior in response to odor and sucrose solution from *A. mellifera* bees.

Our results showed that the level of biogenic amine DA is significantly elevated in learners' brains after appetitive olfactory learning trials involving an odor and sucrose solution reward. These results are innovative as the traditional view of honey bee learning has related learning success to DA signaling, which facilitates the reinforcing efficacy of sucrose solution in appetitive odor conditioning (Mizunami *et al*., 2009). On the contrary, DA is also associated with aversive learning reinforcement signalling in the bee brain (Liu *et al*., 2012). So that the higher levels found in our work after successful appetitive learning was unexpected. Yet, the question remains of why DA levels were also elevated compared to non-learners. To reconcile the opposite views referred to the role of DA in the bee brain provided by prior works (Vergoz *et al*., 2007, Guiraud *et al*., 2018, Marchal *et al*., 2019) and the present one, we suggest that besides dopaminergic neurons conveying aversive signaling in the bee brain, an additional class of such neurons exist that mediate attentional processes, and thus facilitate learning (Tedjakumala *et al*., 2014). This would explain why learners consistently have higher DA levels with greater attention to the discrimination problem. This hypothesis is reinforced by the demonstration of the mechanism of attention, similar to those described in vertebrates (Dyer & Chittka, 2004, Giurfa 2004, Miller *et al*., 2011, van Swinderen 2011, Van Swinderen & Andretic, 2011). In the fruit fly, a neural correlate of such processes is a transient increase in a 20-30 Hz local field potential recorded in a brain region called the medial protocerebrum (van Swinderen & Greenspan, 2003). Transient attenuation of DA release in fly mutants attenuates the 20-30 Hz responsiveness to the object to be attended, and oral delivery of methamphetamine, which increases DA release, rescues this responsiveness (Andretic *et al*., 2005). Thus, the higher levels of DA in learners' brains may reveal that their attentional processes were more efficient, thus leading to better discrimination learning.

The scenario emerging from our study indicates that depressing DA levels before conditioning should lead to deficits in learning performance, particularly for differential appetitive

conditioning, which requires higher levels of attention to achieve the discrimination between a rewarded stimulus (Giurfa 2004).

5. Conclusion

This study determined dopamine's effect on olfactory learning behaviour on both species' learners and non-learner bees. Both species, *A. cerana* (survivorship, 81.42%) and *A. mellifera* (survivorship, 67.6%) showed a significant difference in survival percentage during 14-days. *A. mellifera* has a significantly higher proboscis extension response than *A. cerana* . The learning ability of *A. mellifera* learner versus *A. cerana* learner bees was significant. Dopamine levels of the learner and non-learner bees were significant that found in *A. mellifera*. *A. cerana* and *A. mellifera* showed significant differences between learner and non-learner bees.

ACKNOWLEDGEMENTS

Conflict of interest

The authors declare that there is no conflict of interest.

References

Al-Ghamdi, A., N. Adgaba, Y. Tadesse, A. Getachew and A. Al-Maktary (2017). Comparative study on the dynamics and performances of *Apis mellifera jemenitica* and imported hybrid honeybee colonies in southwestern Saudi Arabia. Saudi Journal of Biological Sciences. **24**(5): 1086-1093.

Andretic, R., B. van Swinderen and R. J. Greenspan(2005). Dopaminergic modulation of arousal in *Drosophila*. Current Biology, **15**(13): 1165-1175.

Benca, R., M. J. Duncan, E. Frank, C. McClung, R. J. Nelson and A. J. Vicentic (2009). Biological rhythms, higher brain function, and behavior: Gaps, opportunities, and challenges.Brain research reviews, **62**(1): 57-70.

Busto, U., I. Cervantes-Sandoval and R. L. J. P. Davis. (2010). Olfactory learning in Drosophila. Physiology, **25**(6): 338-346.

Chabaud, A., J.M. Devaud, M.Pham-Delègue, T. Preat and L.A. Kaiser (2006). Olfactory conditioning of proboscis activity in *Drosophila melanogaster*. Journal of Comparative Physiology A. **192**(12): 1335-1348.

Dyer, A. G. and L. Chittka (2004). Fine colour discrimination requires differential conditioninginbumblebees.Naturwissenschaften. **91**: 224-227.

Dyer, A. G., M. G. Rosa and D.B. Reser. (2008). Honeybees can recognise images of complex natural scenes for use as potential landmarks.Journal of experimental biology. **211**(8): 1180-1186.

Fiala, A. J. Coin. (2007). Olfaction and olfactory learning in Drosophila: recent progress. Current opinion in neurobiology. **17**(6): 720-726.

Gerber, B., R. F. Stocker, T. Tanimura and A. S. Thum (2009). Smelling, tasting, learning: Drosophila as a study case. Chemosensory systems in mammals, fishes, and insects, Springer**:** 187-202.

Giurfa, M. (2004). "Conditioning procedure and color discrimination in the honeybee *Apis mellifera*." Naturwissenschaften **91**(5): 228-231.

Giurfa, M. (2007). "Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well." Journal of Comparative Physiology A **193**(8): 801-824.

Guiraud, M., L. Hotier, M. Giurfa and M. G. de Brito Sanchez (2018). Aversive gustatory learning and perception in honey bees.Science Report. **8**(1): 1343.

Hammer, M. J. T. i. n. (1997). The neural basis of associative reward learning in honeybees. **20**(6): 245-252.

Harris, J. W. and J. Woodring (1992). "Effects of stress, age, season, and source colony on levels of octopamine, dopamine and serotonin in the honey bee (*Apis mellifera* L.) brain.Journal of Insect Physiol **38**: 29-35.

Ichinose, T., H. Tanimoto and N. J. Yamagata (2017). Behavioral modulation by spontaneous activity of dopamine neurons. Frontiers in systems neuroscience. **11**: 88.

Iqbal, J., H. Ali, A. A. Owayss, H. S. Raweh, M. S. Engel, A. S. Alqarni and B. H.Smith (2019).Olfactory associative behavioral differences in three honey bee *Apis mellifera* L. races under the arid zone ecosystem of central Saudi Arabia.**26** (3): 563-568.

Laska, M., C. G. Galizia, M. Giurfa and R. Menzel (1999).Olfactory discrimination ability and odor structure–activity relationships in honeybees. Chem senses **24**(4): 429-438.

Li, N., J. Guo, B. Liu, Y. Yu, H. Cui, L. Mao and Y. Lin (2009). Determination of monoamine neurotransmitters and their metabolites in a mouse brain microdialysate by coupling high-performance liquid chromatography with gold nanoparticle-initiated chemiluminescence. Anal Chim Acta. **645**(1-2): 48-55.

Liu, C., P. Plaçais, N. Yamagata, B. Pfeiffer, Y. Aso, A. B. Friedrich, I. Siwanowicz, G. M. Rubin, T. Preat and H. J. N. Tanimoto (2012). A subset of dopamine neurons signals reward for odour memory in Drosophila.Nature. **488**(7412): 512-516.

Marchal, P., M. E. Villar, H. Geng, P. Arrufat, M. Combe, H. Viola, I. Massou and M. Giurfa. (2019). Inhibitory learning of phototaxis by honeybees in a passive-avoidance task.Learn Memory. **26**(10): 412-423.

Matsumoto, Y., R. Menzel, J.-C. Sandoz and M. Giurfa. (2012). Revisiting olfactory classical conditioning of the proboscis extension response in honey bees: a step toward standardized procedures. **211**(1): 159-167.

Menzel, R. and U. Müller (1996). Learning and memory in honeybees: from behavior to neural substrates. Annual review of neuroscience **19**(1): 379-404.

Miller, S. M., T. Ngo and B. van Swinderen (2011). "Attentional switching in humans and flies: rivalry in large and miniature brains." Frontier Human Neuroscience. **5**: 188.

Mizunami, M., S. Unoki, Y. Mori, D. Hirashima, A. Hatano and Y. Matsumoto(2009).Roles of octopaminergic and dopaminergic neurons in appetitive and aversive memory recall in an insect. **7**(1): 46.

Palottini, F., M. C. Estravis Barcala and W. M. J. F. i. p. Farina (2018). "Odor Learning and Its Experience-Dependent Modulation in the South American Native Bumblebee Bombus atratus (Hymenoptera: Apidae)." Frontiers in psychology. **9**: 603.

Raza, M., Z. Li, M. Rizwan, H. A. Kalan and S.Su., (2019). Comparison of learning and memory of eastern (*Apis cerana cerana*) and western honeybees (*Apis mellifera* L.). Applied ecology and environemntal research. **17**(2): 4971-4984.

Reinhard, J., M. V. Srinivasan, D. Guez and S. Zhang (2004). "Floral scents induce recall of navigational and visual memories in honeybees." **207**(25): 4371-4381.

Rössler, W. and M. F. J. J. o. C. P. A. Brill (2013). Parallel processing in the honeybee olfactory pathway: structure, function, and evolution. Journal of Comparative Physiology A. **199**(11): 981-996.

Roussel, E., J.-C. Sandoz and M. J. F. i. b. n. Giurfa (2010). Searching for learning-dependent changes in the antennal lobe: simultaneous recording of neural activity and aversive olfactory learning in honeybees." Frontiers in behavioral neuroscience. **4**: 155.

Sandoz, J.C. (2011). "Behavioral and neurophysiological study of olfactory perception and learning in honeybees." Frontiers in systems neuroscience **5**: 98.

Schleyer, M., M. Fendt, S. Schuller and B. J. Gerber (2018). "Associative learning of stimuli paired and unpaired with reinforcement: evaluating evidence from maggots, flies, bees, and rats." Frontiers in psychology **9**: 1494.

Smith, B. H. and C. M. J. J. Burden (2014). A proboscis extension response protocol for investigating behavioral plasticity in insects: application to basic, biomedical, and agricultural research. JoVE.(91): e51057.

Tedjakumala, S. R., M. Aimable and M. Giurfa (2014). "Pharmacological modulation of aversive responsiveness in honey bees." Frontiers Behavior Neuroscience. **7**.

Van Nest, B., (2018). The olfactory proboscis extension response in the honey bee: a laboratory exercise in classical conditioning.Journal of Undergraduate Neuroscience Education. **16**(2): A168.

Van Swinderen, B. and Andretic, R., (2011). Dpamine in Drosophila: setting arousal thresholds in a miniature brain. Proceedings of the Royal Society B: Biological Sciences, 278(1707): 906-913.

Van Swinderen, B. and Greenspan, R.J. (2003). Salience modulates 20-30 Hz brain activity in Drosophila. Nature Neuroscience, 6(6): 579-586.

Vergoz, V., E. Roussel, J. C. Sandoz and M. Giurfa (2007). Aversive learning in honeybees revealed by the olfactory conditioning of the sting extension reflex. PLoS One. **2**(3): e288.

Wang, Z. and K. Tan (2014). Comparative analysis of olfactory learning of *Apis cerana* and *Apis mellifera*. **45**(1): 45-52.

Webb, B., (2012). Cognition in insects." Philosophical Transactions of the Royal Society B: Biological Sciences. **367**(1603): 2715-2722.

Williams, G., C. Alaux, C. Costa, T. Csáki, V. Doublet, D. Eisenhardt, I. Fries, R. Kuhn, D. McMahon and P. Medrzycki (2013).Standard methods for maintaining adult Apis mellifera in cages under in vitro laboratory condition." **52**(1): 52-04.

Wright, G. A. and F. Schiestl (2009). The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. **23**(5): 841-851.