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Abdominal contact of fluvalinate induces olfactory deficit in *Apis mellifera*

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ABSTRACT

τ–Fluvalinate (fluvalinate) is a highly selective pyrethroid insecticide compound used for controlling ectoparasitic mites that cause major damages in honey bee colonies. Although honey bees have resistance and low toxicity to this xenobiotic chemical, little is known about the effects of this chemical on sensory modulation and behaviors in honey bees. Here we addressed the effect on olfactory cognition at the behavioral, molecular, and neurophysiological levels. First, we found that topical application of fluvalinate to honeybee abdomen elicited somewhat severe toxicity to honey bees. Furthermore, honeybees treated with sublethal doses of fluvalinate showed a significant decrease in olfactory responses. At the molecular level, there was no change in gene expression levels of odorant receptor co-receptor (Orco), which is important for electrical conductivity induced by odorant binding in insects. Rather, small neuropeptide F (sNPF) signaling pathway was involved in olfactory fluctuation after treatment of fluvalinate. This indicates that olfactory deficits by abdominal contact of fluvalinate may stem from various internal molecular pathways in honey bees.

1. Introduction

The Western honey bee, *Apis mellifera* L., is an essential pollinator that provides ecological services and economic values (Klein et al., 2007; Southwick and Southwick Jr., 1992). While a number of different factors have been discussed for colony collapse disorder (CCD), the ectoparasitic mites, *Varroa* spp., are major suspect for colony loss of *A. mellifera* (Cox-Foster et al., 2007; Le Conte et al., 2010; Vanengelsdorp et al., 2009). The reason is that miscellaneous syndromes and viral epidemics are driven by *Varroa* mites (Boecking and Genersch, 2008; Zemene et al., 2015). To prevent the damages from *Varroa* mite, beekeepers commonly use the Apistan® and Wang's strip® miticides, which contain one of the type II pyrethroids, τ–fluvalinate (hereafter, fluvalinate). The pyrethroids as neurotoxic pesticides target voltage-gated sodium channels and induce repetitious discharging (type I without α-cyano) or sustained membrane depolarization (type II with α-cyano) (Gupta and Crissman, 2013a, 2013b; Soderlund et al., 2002). Although toxicity for voltage-gated sodium channels of both honey bees and *Varroa* mites (Gosselin-Badaroudine and Chahine, 2017), honey bees are more resistant to miticides due to the efficient xenobiotic detoxification by cytochrome P450 monooxygenases (CYP450s) (Johnson et al., 2006; Mao et al., 2011).

Despite the relative tolerance to fluvalinate in the honey bee, various side effects of fluvalinate on mortality, reproductive and neurophysiological activities have been reported (Berry et al., 2013; Dai et al., 2017; Frost et al., 2013; Pettis et al., 2004; Rangel and Tarpy, 2015; Rinderer et al., 1999). However, the effect of fluvalinate on the olfactory cognitive system still remains unexplored. In nature, the olfaction highly contributes to colony maintenance in honey bees since various social behaviors such as hygienic and foraging behaviors and learning and memory are medicated with odorants (Dötterl and Vereecken, 2010a, 2010b; Fleischer et al., 2018; Slessor et al., 2005). Thus, olfactory impairment can cause many problems in honey bee colonies, which may lead to the CCD.

Previous studies on fluvalinate treatment in honey bees were performed by feeding, thorax-injection, and natural exposure, even though external contact by the abdomen has commonly occurred in bee hives. Here we employed an important situation of fluvalinate treatment in beekeeping. First, fluvalinate strips are designed to treat honey bees by external contact. Second, the abdomen contains important organs. In particular, fat bodies are the center for nutrient metabolism and very important internal organ to modulate honey bee physiology (Arrese and Soulages, 2010). Lastly, the abdomen is a favorable site for *Varroa* mites which impale the abdomen to reach fat bodies and take nutrients (Ramsey et al., 2019). Therefore, this logical assumption led us to investigate the relationships between fluvalinate abdominal exposure and

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olfactory modulation effects. The aim of the current study was to examine the effect of topical application of fluvalinate onto bee abdomen on the olfactory modulation in honey bees at neurophysiological and molecular levels. This was the first attempt to demonstrate the effects of fluvalinate on olfactory modulation in honey bees.

2. Materials and methods

2.1. Insects

The honey bees were raised in the Incheon National University Apiary, Republic of Korea (37°22′25″, 126°37′40″). For all experiments, we used the pollen-foragers captured directly at the entrance of three colonies in April 2019.

2.2. Preparation of fluvalinate

The τ-fluvalinate (Sigma–Aldrich, St Louis, MO) solutions were prepared at concentrations of 0.5, 1, 2, 4, 8, 16 and 32 μg/μl. The appropriate concentration range was selected based on the average LD_{50} (Median of lethal dose = 5.49 ± 1.38 μg/bee) of previous studies that injected or fed fluvalinate to adult bees (Dahlgren et al., 2012; Johnson et al., 2006, 2009, 2012, 2013; Mullin et al., 2010; Santiago et al., 2000). Acetone was chosen as the solvent according to the Organization Européenne et méditerranéenne Pourla Protection de Plantes/European and Mediterranean Plant Protection Organization (OEPP/EPPO) guidelines because it is a rather generalist solvent (European and Organization, 1992). The experimental foragers were given anesthesia with ice. Each bee was received 1 μl of prepared fluvalinate solution topically on the ventral side of the abdomen before awoke from the anesthesia.

2.3. Determining sublethal dose

Each bee after the topical application of fluvalinate was kept in an incubator (darkness/30 ± 2 °C / RH 60 ± 5%) and fed sugar candy *ad libitum*. The mortality was recorded at 3, 8 and 24 h of post-treatment (hpt). The bees were divided into seven dose groups as mentioned in 2.2 and a control group administered only with solvent. 20–28 bees per group were performed at one time and a total of 1520 bees were used. The sublethal dose was measured depending on the statistical significance of the 24 hpt mortality compared to the control group. Immediately after 24 hpt, some of the survivors were used in the electrophysiological experiment and the others were stored in deep-freezer (−80 °C) for molecular experiments.

2.4. Electrophysiological recordings

Electroantennography (EAG) is an electrophysiological technique for recording odorant-evoked changes in antenna's transepithelial potential (Schneider, 1957). EAG responses were amplified and recorded from the left antennae of honey bee using a data acquisition controller (IDAC-4) and EAGPro software (Syntech, Hilversum, Netherlands). Foragers were immobilized by ice for 30 min and then be harnessed in an acrylic holder, holding head and appendages in place for electrophysiological measurements (Fig. 1-1). Both of the antennal sulci and the pedicel of the left antennae were fixed with eicosane (Sigma–Aldrich, St Louis, MO; Fig. 1-2), to prevent antennal moving. The glass recording electrode was placed over the 10th antennal segment and the glass reference electrode was inserted near the antennal sulcus (Fig. 1-3). We used glass capillary electrodes (ID = 1.5 mm, World Precision Instruments, Sarasota, FL) filled with 0.01 M KCl. For the experiment, we used some of the volatile organic compounds (VOCs) derived from the major floral scent (Knudsen et al., 2006), honey bee's alarm pheromone (Blum et al., 1978) and a disease odor inducing hygienic behavior (Swanson et al., 2009). All VOCs used in this experiment were purchased from Sigma-Aldrich (St Louis, MO). For this experiment, 20 μl of each 1 M VOCs diluted in mineral oil was absorbed onto a filter paper, which was then inserted into individual Pasteur pipettes. Each odorant was delivered by 1-s air pulse through constant airflow (40 cc/s) to the antennae, which was automatically controlled by a stimulus controller (CS-55, Syntech, Hilversum, Netherlands). Stimulated odorants were removed from the recording area through a ventilation system. The intervals between the stimuli were more than 60 s to prevent the test animal from adapting to a certain odorant. To exclude physical response, control (mineral oil) stimulation was given 10 to 15 s before the odorant delivery (Fig. 1-4), and the normalized EAG response was calculated as follows:

$$Normalized\ EAG\ response = (Response\ to\ Odorant) - (Response\ to\ control)$$

2.5. Analysis of candidate gene expression

For each quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) analysis, 15 pairs of antennae were isolated. Then, immediately, total RNAs were extracted from the antennae using a Qiagen RNeasy Mini Kit according to the manufacturer's instructions (Qiagen, Valencia, CA). Using 500 ng of total RNA, cDNA was synthesized with oligo-dT with Superscript III enzyme (Invitrogen, Carlsbad, CA). qRT-PCR was performed on the AriaMx Real-Time PCR System (Agilent Technologies, Santa Clara, CA) using Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent Technologies, Santa Clara, CA). All primer sets (Table 1) were synthesized in Macrogen Inc. (Seoul, Korea). For all performed analysis, ribosomal protein 49 (RP49) was used as a housekeeping gene for normalization. Thermal cycling was set at 95 °C for 1 min, followed by 40 cycles of 95 °C for 5 s and 55–60 °C for 10 s. Three technical replicates were performed for each biological replicate and relative mRNA expression levels were calculated using the $2^{-\triangle\triangle CT}$ method as described by Livak and Schmittgen Livak and Schmittgen (2001).

2.6. Analysis of Orco protein expression

For each western blot analysis, 15 pairs of antennas were lysed with 300 μl of RIPA buffer (Biosesang, Seongnam Korea) with Halt™ protease inhibitor (Thermo Scientific, Waltham, MA). Tissue lysates were harvested and vortexed at room temperature for 10 min. The lysates were cleared by centrifugation at 15,000*g* for 10 min at 4 °C. After quantitation of protein, 20 μg of each sample was loaded onto a 4–12% Bis-Tris Protein gel (Novex, San Diego, CA) and transferred to nitrocellulose membrane. The membrane was blocked in PBS with 0.1% Tween-20 and 5% skim milk for 1 h at room temperature and incubated for overnight with primary antibodies. Subsequently, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies. Primary antibodies used in this study included rabbit anti-Am/AcOr2 (Orco) used in our previous study (1:500 dilution; (Jung et al., 2014), and rabbit anti-actin (1:1000 dilution; Merck Millipore, Burlington, MA). The secondary antibody used in this study was HRP-conjugated anti-rabbit IgG (1:1000 dilution; GeneTex, CA, USA) and HRP-conjugated anti-mouse IgG (1:1000 dilution; Thermo Scientific). The ratio of Orco to actin was quantitated using ImageJ as described by Collins Collins (2007).

2.7. Statistical analysis

LD_{50} was calculated using non-linear regression analysis with GraphPad® Prism 7 (GraphPad Software, Inc., La Jolla, CA). Two-way analysis of variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) test was used to determine the sublethal dose based on the mortality over time. To compare olfactory sensitivity, the one-way ANOVA with Tukey's HSD test was performed. Differences in mRNA and protein expression levels were examined by Student's *t*-test. All statistical analysis, excluding non-linear regression, were performed

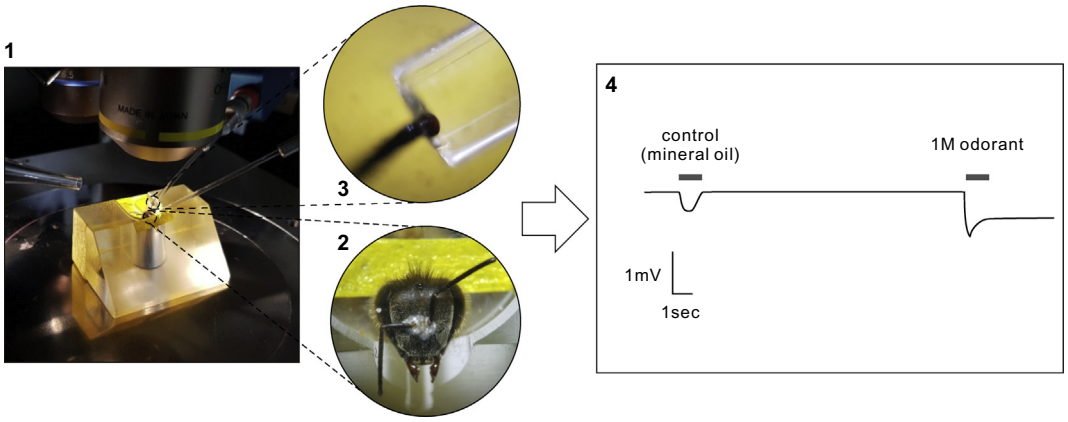

Fig. 1. The overall process of electroantennography.
(1) Harnessing bee in an acrylic holder. (2) Antennas fixed by Eicosan. (3) 10_{th} antennal segment located on a glass recording electrode. (4) 1 M odorants were given 10–15 s after the control (mineral oil) was delivered.

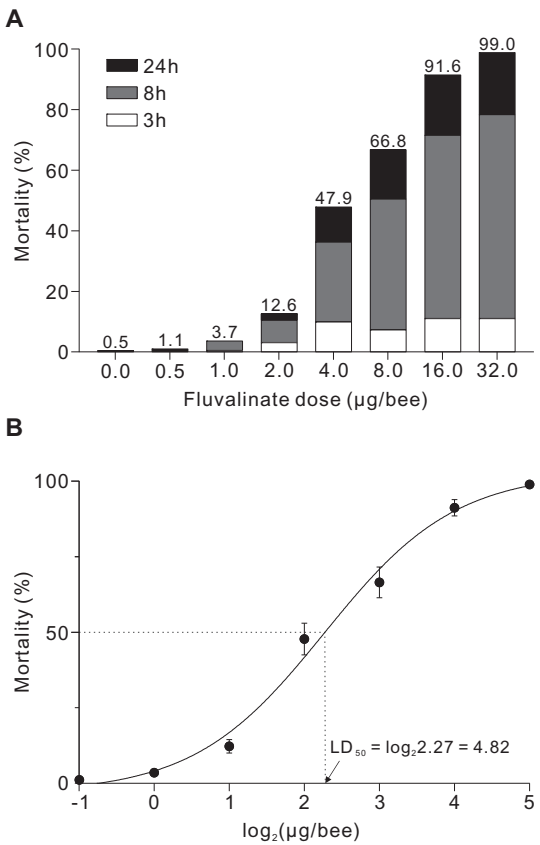

Fig. 2. Toxicity of fluvalinate abdominal contact.
(A) Cumulative mortality 3, 8, and 24 h after treatment. The number above bars represent the final mortality. (B) Dose-mortality 24 h after treatment. The dotted line indicates the median of a lethal dose. Each value represents the mean ± SE.

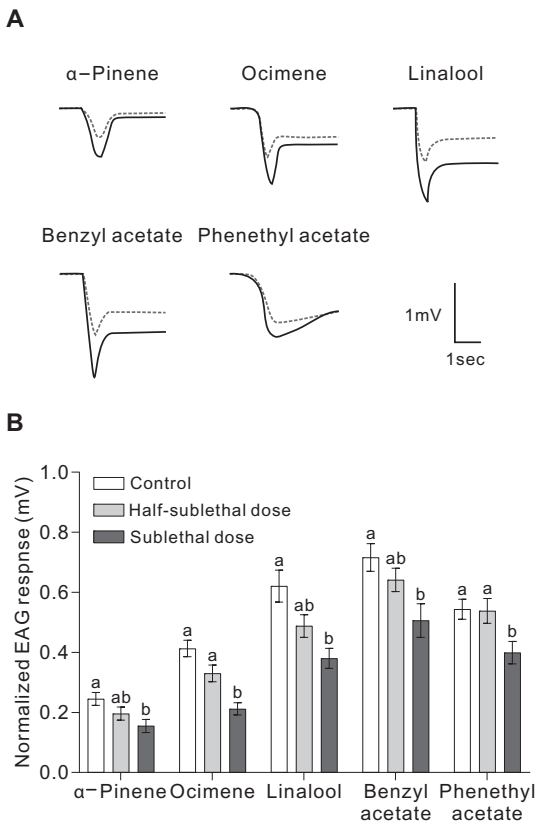

Fig. 3. The olfactory responses 24 h after fluvalinate abdominal contact.
(A) Illustrative EAG traces of control (black) and sublethal dose-treated (grey) honey bee. (B) Comparison of the olfactory response among the three groups (n = 30). Each bar represents the mean ± SE. Statistical significance was determined by one-way ANOVA with Tukey's HSD test (different letter: P < .05).

on SPSS® Statics 25 (IBM, Armonk, NY). All Graphs were prepared in GraphPad® Prism 7 and final arrangement and labeling were carried out using CorelDRAW® Graphics Suite 2019 (Corel Corporation, Canada).

3. Results

3.1. Determination of sublethal dose

Observation of 1520 bees confirmed that the dose of fluvalinate abdominal contact correlated with mortality (Fig. 2A). The two-way ANOVA showed a significant effect of fluvalinate dose (F = 175.408, P < .001), elapsed time (F = 219.715, P < .001) and their interaction (F = 30.893, P < .001; Table S1). According to Tukey's HSD test, doses greater than 2 μg showed statistically significant mortality compared to control ($P_{0.5\mu g}$ = 1.000; $P_{1\mu g}$ = 0.990; $P_{2\mu g}$ = 0.064; $P_{4\mu g}$ < 0.001; $P_{8\mu g}$ < 0.001; $P_{16\mu g}$ < 0.001; $P_{32\mu g}$ < 0.001), consequently 2 μg was considered as sublethal dose. The deviation between the groups became severe as elapsed time and the significance was also

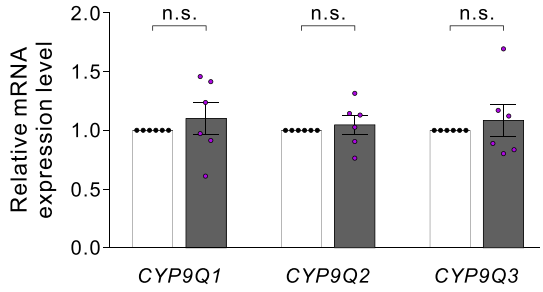

Fig. 4. The expression patterns of CYP450s.
Relative mRNA expression levels of CYP450s in antenna from control (white bar) and sublethal dose treated (grey bar) foragers. Each bar represents the mean ± SE. Data points represent values from biological replicates. Statistical significance was determined by student's *t*-test (n.s. = non-significant, $P > .05$).

increased (Fig. S1). The non-linear regression curve shows that the LD_{50} of abdominal contact was 4.82 μg/bee (95% confidence interval = 4.02–5.95, Hill slope = 0.4980 ± 0.0795, R^2 = 0.9473, df = 52; Fig. 2B).

3.2. Sublethal effect on olfactory sensitivity

We asked whether the abdominal contact of fluvalinate at a sublethal dose would affect olfactory sensitivity in honey bees., In order to test this hypothesis, we employed EAG techniques using VOCs that are considered crucial for honey bee's food-seeking, defense, and hygienic behaviors. EAG showed that the abdominal contact of fluvalinate decrease the olfactory responses to all tested VOCs (Fig. 3). The three groups (control, half of sublethal and sublethal dose) showed significant differences in olfactory responses to flower scents (α-pinene: $F_{2,87} = 3.664$, $P = .030$; ocimene: $F_{2,87} = 15.830$, $P < .001$; linalool: $F_{2,87} = 8.237$, $P = .001$), an alarm pheromone (benzyl acetate: $F_{2,87} = 5.508$, $P = .006$), and a disease odor (phenethyl acetate: $F_{2,87} = 4.755$, $P = .011$; Fig. 3B). Significant reduction of olfactory responses to all tested VOCs was observed in a sublethal dose (Tukey's HSD test, $P_{\alpha\text{-pinene}} = 0.022$, $P_{\text{ocimene}} < 0.001$, $P_{\text{linalool}} < 0.001$, $P_{\text{benzyl acetate}} = 0.005$, $P_{\text{phenethyl acetaet}} = 0.021$), but not in half of sublethal dose ($P_{\alpha\text{-pinene}} = 0.358$, $P_{\text{ocimene}} = 0.060$, $P_{\text{linalool}} = 0.071$, $P_{\text{benzyl acetate}} = 0.603$, $P_{\text{phenethyl acetate}} = 0.992$).

3.3. Effects of the sublethal dose of fluvalinate on olfactory-related genes and proteins

First of all, we observed the detoxification against the antennae.

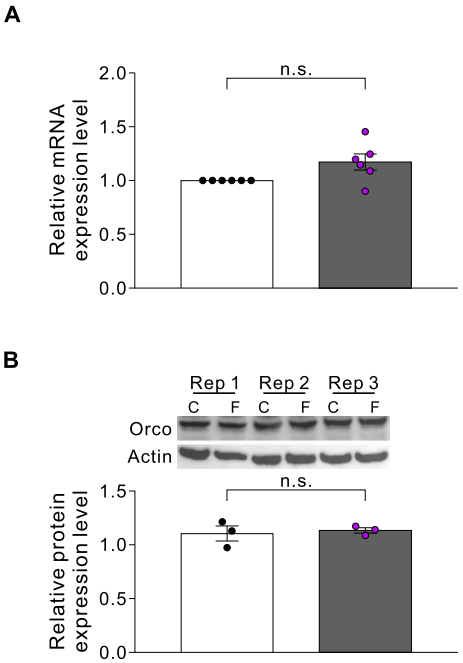

Fig. 5. The expression patterns of Orco.
Relative (A) mRNA and (B) protein expression levels of Orco in antenna from control (white bar) and sublethal dose-treated (grey bar) foragers. The bands show three repetitions (Rep) of immunoblotting (C: control, F: sublethal dose-treated foragers). Each bar represents the mean ± SE. Data points represent values from biological replicates. Statistical significance was determined by student's t-test (n.s. = non-significant, $P > .05$).

qRT-PCR showed that sublethal dose didn't affect the mRNA expression pattern of three CYP450s ($t_{CYP9Q1} = 0.756$, $P_{CYP9Q1} = 0.484$; $t_{CYP9Q2} = 0.606$, $P_{CYP9Q2} = 0.571$; $t_{CYP9Q3} = 0.626$, $P_{CYP9Q3} = 0.559$; $n_{control} = 6$, $n_{fluvalinate} = 6$; Fig. 4). Next, we considered odorant receptor co-receptor (Orco), one of the core olfactory-related molecules. qRT-PCR revealed no significant change in Orco mRNA expression pattern ($t = 2.312$, $P = .069$, $n_{control} = 6$, $n_{fluvalinate} = 6$; Fig. 5A). Western blot also showed no change in Orco protein expression ($t = 0.371$, $P = .730$, $n_{control} = 3$, $n_{fluvalinate} = 3$, Fig. 5B). Finally, Tachykinin (TK) and short Neuropeptide F (sNPF) were examined, because previous studies demonstrated that they involve in olfactory-related neuromodulation (Ignell et al., 2009; Jung et al., 2013; Ko et al., 2015; Root et al., 2011). According to qRT-PCR, sublethal dose of fluvalinate significantly increases sNPF mRNA expression, but not tachykinin ($t_{sNPF} = 4.407$, $P_{sNPF} = 0.007$; $t_{TK} = 0.804$, $P_{TK} = 0.458$;

Table 1
The primer sets for qRT-PCR. Primers without a reference were designed by S. Lim. (Mao et al., 2011).

Gene	Accession No.		Sequences (5′ - 3′)	Size (bp)	TM (°C)
Ribosomal protein 49	AF441189	F	GGGACAATATTTGATGCCCAAT	100	55–60
		R	CTTGACATTATGTACCAAAACTTTTCT		
CYP9Q1*	XM_006562301	F	TCGAGAAGTTTTTCCACCG	116	55
		R	CTCTTTCCTCCTCGATTG		
CYP9Q2*	XM_392000	F	GATTATCGCCTATTATTACTG	127	55
		R	GTTCTCCTTCCCTCTGAT		
CYP9Q3*	XM_006562300	F	GTTCCGGGAAAATGACTAC	107	55
		R	GGTCAAAATGGTGGTGAC		
Tachykinin	XM_026441578	F	GGCGGGGATTTACGGATCAA	166	60
		R	CCCTCGAAATTCCCATCGTG		
short Neuropeptide F	XM_003250107	F	ATAGATTACTCAGATGAAATACCAG	218	60
		R	GCACTCATTGGTTTTGATAGAATAG		
Receptor of short Neuropeptide F	XM_006561685	F	GCATTTTGTTACATCTGCGTC	112	55
		R	TCGTTCGCTTCTTCCTCTC		
Olfactory receptor co-recpetor	NM_001134943	F	CGTCCACCTGGTCCTGATAC	154	60
		R	ACTCCTGACCGCGAAGTAAA		

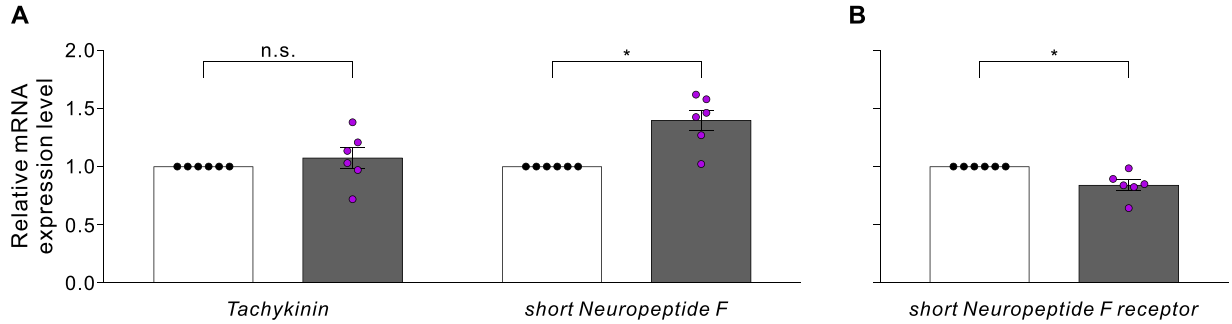

Fig. 6. The expression patterns of TK, sNPF, and sNPFR.
Relative mRNA expression levels of (A) TK, sNPF and sNPFR in antenna from control (white bar) and sublethal dose-treated (grey bar) foragers. Each bar represents the mean ± SE. Data points represent values from biological replicates. Statistical significance was determined by student's t-test (n.s. = non-significant, P > .05; * = $p < .05$).

$n_{control} = 6$, $n_{fluvalinate} = 6$; Fig. 6A). It is well known that sNPF acts on target cells, usually through interaction with specific membrane receptor, known as the sNPF receptor (sNPFR). Therefore, we examined whether sNPFR expression pattern change after fluvalinate exposure. qRT-PCR showed that the expression pattern of sNPFR was significantly decreased in fluvalinate exposed honey bees ($t = 3.487$, $p = .006$, $n_{control} = 6$, $n_{fluvalinate} = 6$; Fig. 6B).

4. Discussion

It is assumed that approximately 10% of the active substance in fluvalinate strip is diffused to contaminate about 0.125 μg of individual bees per day (Tremolada et al., 2004). In the real bee hive, however, the actual exposure dosage varies due to individual differences in the degree of direct contact with the strip. Concentrations of coumaphos, similar to fluvalinate, was detected about 10 times higher than estimated in the same manner as fluvalinate strip (Haarmann et al., 2002). In the case of fluvalinate, Mullin et al. (2010) showed that fluvalinate was detected up to 5.86 ppm in real-apiary. Moreover, the lipid-soluble properties of fluvalinate allow accumulate in hive products. In particular, previous studies reported that fluvalinate was detected in wax of honey bee comb at 0.0015 to 204 ppm levels (Bonzini et al., 2011; Chauzat and Faucon, 2007; Mullin et al., 2010). Therefore, honey bees in the field might have sufficient risk to be exposed to the sublethal dose identified in the current study.

In nature, the olfactory sense is essential in animals to find foods, to protect from dangers, and to communicate intra- and interspecifically (Ache and Young, 2005; Fleischer et al., 2018). Several studies showed that insects, fish, mice, and humans might suffer from olfactory disorders such as anosmia, hyposmia, and dysosmia induced by various xenobiotics (Coughlan et al., 2015; Gobba and Abbacchini, 2012; Ngamdu et al., 2012; Tierney et al., 2010; Tricoire-Leignel et al., 2012). Notably, these neurological disorders in human beings are regarded as an initial step of neurodegenerative diseases such as schizophrenia, Alzheimer's and Parkinson's disease (Hawkes et al., 1997; Morgan et al., 1995; Turetsky et al., 2009). Nonetheless, the mechanism of olfactory disorder induced by pesticides still remains elusive. Our findings provide strong evidence that honey bees, one of the powerful model organisms, also undergo olfactory deficit induced by the miticide that is thought to be less toxic to honey bees (Johnson et al., 2006; Mao et al., 2011). Therefore, it can be assumable that this olfactory deficit induced by xenobiotics present in the bee hives would be able to suppress colony maintenance and ultimately lead to the CCD (Farooqui, 2013). Furthermore, since the olfactory deficit shown by this study was the result of only external contact, there is a risk to beekeepers handling the miticide as well as honey bees.

While previous *in vitro* assay illustrated the direct intracellular effects of pyrethroids on olfactory-related neurons (Kadala et al., 2011, 2014), the olfactory deficit induced by miticide abdominal contact may not be caused by damage to the cation channel on the neurons, nor the olfactory-related genes such as Orco genes. Since CYP9Q1–3 in forager's antennae are expressed enough to carry out detoxification (Mao et al., 2015), fluvalinate transmitted from the abdomen to the antenna would be little and almost detoxified within 24 h. Indeed, a previous study showed that fluvalinate conveyed between body parts was little and quickly detoxified (Hillier et al., 2013), indicating that other possible mechanisms would elicit olfactory deficit in honey bees.

Our findings suggest that the olfactory modulation under the fluvalinate exposure of honey bees is dependent on sNPF signaling pathways, not on TK. The neuropeptides TK and sNPF are peptidergic modulators in the olfactory systems of insects. In *Drosophila* and *Periplaneta americana*, it has been shown that olfactory sensitivity in ORN can be modulated by TK secreted in local interneurons in antenna by increased amounts of octopamine influxed into the antenna (Ignell et al., 2009; Jung et al., 2013). In contrast, sNPFR in ORNs of *Drosophila* and *Bactrocera dorsalis*, which is regulated by insulin-like peptides (ILPs), facilitates the olfactory sensitivity (Jiang et al., 2017; Ko et al., 2015; Root et al., 2011). Surprisingly, sNPFR was decreased with the olfactory deficit which is consistent with previous studies (Jiang et al., 2017; Ko et al., 2015; Root et al., 2011), but the mechanism of increased sNPF was enigmatic. One of the candidates for this issue is to interact with insulin signaling. In *Drosophila,* sNPF stimulates extracellular-activation receptor kinases (ERKs) in insulin-producing cells (IPCs) to regulate hemolymph glucose and cell growth by expressing ILP (Lee et al., 2008). Interestingly, du Rand and her colleagues demonstrated that the carbohydrate catabolism was up-regulated for detoxification when the honey bees were exposed to xenobiotics (du Rand et al., 2015a, 2015b), which would be accompanied by an increase in the amount of glucose influx to the cells from the hemolymph. Therefore, it is possible that increased energy demand due to detoxification of fluvalinate induces the expression of sNPF but increases ILP levels, which in turn the expression of sNPFR in ORNs was suppressed by high expression of ILP. By this pathway, decreased expression of sNPFR would lead to olfactory deficit. To clarify this hypothesis, it will be interesting to investigate how sNPF and ILP modulate olfactory sensitivity after fluvalinate exposure. Taken together, the current study is significant to show that non-target miticides treated in honey bee hives affect the sensory perception ability of honey bees. Therefore, a better understanding of molecular and neural mechanisms underlying the effect of these xenobiotics on honey bee behaviors and physiology will be important to solve the current problems of honeybee colony disorders.

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Author contributions

S.L. and H.W.K. designed the experiment. S.L. performed overall experiments, analysis, and artworks. H.L. carried out the western blot assay. S.L., U.Y., R.I., and H.W.K wrote the main manuscript text. All authors reviewed the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pestbp.2020.02.005.

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