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Impact of acetamiprid on fatty acid composition of the central nervous system in honey bees

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Honey bee populations have suffered a significant decline globally in recent decades, which presents a serious danger to the ecosystem, as well as to agriculture. Food and feed production needs to rise to meet the demands of the expanding world population, which has made widespread pesticide use necessary. Neonicotinoids are widely utilized agents including acetamiprid. Given that lipids function as membrane constructing molecules, disruptions in the oxidative equilibrium within the bee's central nervous system may cause lipid peroxidation, which can change the fatty acid profile and jeopardize cell viability. Honey bees were treated with acetamiprid-containing sugar syrup for 48 hours, at concentrations of 35 µg/mL, 17.5 µg/mL and 8.75 µg/mL in the 'AcetHigh', 'AcetMedium' and 'AcetLow' groups. After dissecting the brain, the samples were homogenized, and the concentrations of the 15 most abundant fatty acids were analyzed using gas chromatography-mass spectrometry (GC-MS). The concentrations of lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and alpha-linolenic acid (C18:3, n-3) were increased by acetamiprid treatment. Total fatty acid concentrations, as well as those of polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs), also showed elevation in acetamiprid-exposed bees, following a non-monotonic dose response. These findings imply that acetamiprid not only impacts the redox homeostasis as it was previously reported, but also adversely affects the lipid metabolism in the central nervous system of honey bees, and at the same time also triggers adaptive mechanisms.

The western honey bees plays a crucial role in global ecosystems, as their pollination is a cornerstone of food and feed production[1]. The global decline in honey bee populations poses a significant threat to agricultural production and ecosystem functioning, a problem expected to intensify over time[2]. Research suggests that over 90% of the world's 107 top most important agricultural crops rely on bee visitation[3]. While honey bees are not the sole pollinators, they are the most critical species globally due to their abundance[4]. Therefore, safeguarding bee health is critically important, as their decline could severely jeopardize agricultural productivity as well as biodiversity[5]. Unfortunately, the decline in bee populations is a global phenomenon, with most countries observing downward trends[2]. The potential consequences of their decline are profound and largely unpredictable, as the disappearance of honey bees could trigger cascading effects throughout entire ecosystems. This decline is driven by a multifactorial process, where a combination of factors likely acts synergistically[6]. Key contributors include habitat loss, environmental pollution, the spread of monocultures, parasites such as the unicellular microsporidian *Nosema ceranae* and the *Varroa destructor* mite, and pesticide exposure[4].

Beyond geological, political, and socio-economic factors, climate change also significantly impacts agriculture, and these challenges have driven the rapid expansion of competitive, intensive agricultural practices[7]. Such high-yield agricultural systems are increasingly rely on the extensive use of pesticides to meet the demands and expectations of the global population[8]. Neonicotinoids, in particular, target the nicotinic acetylcholine receptors in the central nervous system of insects, making them selective for certain pest species[9]. The newer generation of neonicotinoids, such as acetamiprid, has a more favorable toxicity profile compared to earlier

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versions, making it safer to use[10]. However, multiple studies have shown that, in addition to their primary effects, neonicotinoid pesticides can induce oxidative stress, depleting both enzymatic and non-enzymatic antioxidant defense systems[11].

Lipid molecules, particularly PUFAs, are highly susceptible to oxidative damage, as the free radicals generated during oxidative stress can rapidly initiate chain reactions, leading to widespread molecular damage[12]. Changes in the fatty acid profile of honey bee central nervous systems following acetamiprid exposure are of particular concern. As key components of cellular membranes, fatty acids play a critical role in maintaining cell structure and function, and alterations in their composition can affect cell survival[13].

Under normal physiological conditions, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced during processes such as mitochondrial respiration[14]. Typically, these species are neutralized by the antioxidant defense systems. However, when the balance shifts toward oxidation, due to biogenic or abiotic factors, the defense mechanisms can become overwhelmed[15]. In the case of lipid peroxidation, free radicals attack the carbon-carbon double bonds in molecules, especially in PUFAs[16]. Various ROS lead to the formation of lipid peroxidation products from fatty acids, which can be potentially dangerous and affect immune processes, antioxidant compounds and various enzymes[17]. Several studies have highlighted the significance of fatty acid profile changes in certain human psychiatric and cardiovascular diseases, which are believed to stem from oxidative stress[18]. Fatty acid metabolites may contribute to the pathogenesis of these diseases by introducing new biological functions[19]. In colony collapse disorder (CCD), one of the most significant bee health crises of recent decades, worker bees fail to return to their hives, often without exhibiting overt signs of disease[20]. This phenomenon may be linked to cognitive impairments, potentially analogous to neurodegenerative conditions observed in humans[13,21].

The relationship between changes in fatty acid profiles and pesticide exposure, particularly to neonicotinoids, in honey bees remains underexplored. While some studies have investigated these effects in other insect species or model organisms, research specifically focusing on honey bees is limited. Given the critical role of fatty acids in the central nervous system, further research in honey bees is essential. Alterations in fatty acid concentrations have been linked to oxidative stress and impairments in central nervous system function in various insect models, such as *Drosophila melanogaster (Diptera: Drosophilidae)* and other pollinators, such as bumblebees (*Bombus terrestris*, *Hymenoptera: Apidae*)[22,23]. These findings highlight the need for more detailed investigations to fully understand the complex interactions between pesticide exposure and neural health in honey bees. Our previous results, combined with research on redox homeostasis from our research team, aim to shed light on neonicotinoids' effects on bees and advance the goal of bee conservation. We hypothesize that oxidative stress induced by acetamiprid exposure disrupts the fatty acid composition in the honey bee central nervous system, potentially leading to cellular damage and metabolic disturbances, including imbalances in redox and lipid homeostasis[24]. The objective of this study is to assess fatty acid composition in the central nervous system of honey bees following acute, sublethal, field-relevant exposure to acetamiprid.

Materials and methods
Collection and acclimation of honey bees
Adult western honey bees were collected following the method described by Williams et al., 2013 and current OECD guidelines[25–27]. To ensure uniform health and origin, a single *Apis mellifera carnica* (*Hymenoptera: Apidae*) colony was included in the study. The colony had not been exposed to any treatments for three months prior to the experiment, and no signs of disease were observed during veterinary examination prior to and during the experiment. The experimental colonies were kept on freshly built combs in a predominantly wooded area with limited agricultural exposure and were regularly monitored to ensure they were free of diseases before and during the study.

Forager bees were collected from non-brood frames during the morning hours. The bees were then randomly divided into groups (three replicates/treatment group), with approximately 200 individuals/replicate. The study included four treatment groups: one control group and three groups exposed to different concentrations of acetamiprid. The size of the applied cages were 30 cm × 20 cm and were kept in a dark room at 25 ± 2 °C with 50–65% relative humidity. Bees were provided *ad libitum* access to a 50% sucrose solution and water. A short acclimation period of 36 h was included at the start of the study, during which the bees received only sucrose solution and water, without any additional treatment.

Acetamiprid treatment of honey bees
After the acclimation period, the feeding solutions were supplemented with acetamiprid (Merck KGaA, Darmstadt, Germany, product number: 33674) at the appropriate concentrations. These solutions were replaced every 8 hours to prevent degradation of the active ingredients. The treatment phase lasted for 48 hours, during which the feeding solutions contained acetamiprid at concentrations of 35 μg/mL, 17.5 μg/mL and 8.75 μg/mL, corresponding to the 'AcetHigh', 'AcetMedium', and 'AcetLow' groups, respectively.

Considering that the daily consumption of sucrose solution by bees has been reported to be approximately 40 μL/day[28], the doses used in this study are referred to oral LD50/10 (lethal dose 50) ("AcetHigh": 1.453 μg/bee/day), LD50/20 ("AcetMedium": 0.727 μg/bee/day), and LD50/40 ("AcetLow": 0.363 μg/bee/day)[29,30]. These treatments are classified as acute sub-lethal doses, additionally, the study design aligns with field-realistic doses of acetamiprid exposure[31–33]. Mortality was recorded every 12 h before and during treatment and did not exceed 2% in any cage.

Preparation of central nervous system homogenates
After the treatment phase, cages were placed on dry ice and transported to the laboratory, where the samples were stored at -80 °C until further processing. Individual samples for homogenization and measurement were

randomly selected from replicates within each treatment group ($n = 10$, for each sampling, we pooled brain samples from four individuals for analysis). After careful thawing, bee heads were removed and dissected under a stereomicroscope on ice. The dissected brain specimens included the protocerebrum, antennal lobe, optic lobe, and suboesophageal ganglion.

Fatty acids extraction

For each experimental group, 30 mg of homogenized brain tissue (comprising 10 samples /group, with each sample derived from four bees) was weighed into vials. Subsequently, 40 mL of methanol and 6 mL of 50% w/v sodium hydroxide were added to each sample. Nonadecanoic acid (C19:0) was used as an internal standard, with 1.6 mL of a 500.2 mg/100 mL solution pipetted to each sample. Thereafter, the mixture was heated at 80 °C for 60 min. Following cooling of the samples, 50 mL of distilled water and 35 mL of hydrochloric acid (4 M) were added to lower the pH of the solution. Fatty acids were extracted with 2×25 mL chloroform, followed by the addition of a 0.9% sodium chloride solution for phase separation. The lower organic phase was collected, dried with anhydrous sodium sulphate, and evaporated to dryness under nitrogen[34].

The resulting dry residue was redissolved in 6 mL of 0.5 M sodium hydroxide in methanol and heated at 80 °C for 5 min. Methyl ester derivatives were then synthesized by the addition of 6 mL of 14% w/v boron trifluoride-methanol reagent, followed by a second heating step at 80 °C for 5 min. To facilitate extraction, 3 mL of hexane and 6 mL of saturated sodium chloride solution were added, and the mixture was vortexed for 1 min. The layers were allowed to separate for 5 min, after which the upper hexane layer was carefully transferred to a gas chromatography (GC) vial. Fatty acid composition was subsequently analyzed using gas chromatography–mass spectrometry (GC-MS)[35].

GC-MS analysis

Gas chromatography (GC) analysis was performed using a Zebron BPX-70 column (30 m × 0.25 mm × 0.25 μm film thickness; Phenomenex, Torrance, CA, USA) with a GCMS-QP2010 SE system (Shimadzu Co., Kyoto, Japan). Helium was applied as the carrier gas, with a column head pressure of 60.1 kPa. The total flow rate at the split vent was 45.4 mL/min, while the flow rate through the column was maintained at 1.03 mL/min, and the septum purge flow was set at 3 mL/min.

Samples were introduced via splitless injection at an injector temperature of 220 °C, with an injection volume of 1 μL. The temperature program commenced at an initial temperature of 60 °C, followed by a linear increase to 120 °C at a rate of 13 °C/min. Subsequently, the temperature was raised to 240 °C at a rate of 2 °C/min, with a final hold at 240 °C for 8 min. The ion source temperature was maintained at 200 °C, while the interface temperature was set at 240 °C.

Statistics

Data were processed and analyzed using GraphPad Prism 9 software (GraphPad Software Inc., San Diego, CA, USA) as well as R. The homogeneity of variance and normality of distribution were assessed using the Levene test and Shapiro-Wilk test, respectively. Group differences were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test for pairwise comparisons. Differences were considered statistically significant at $P < 0.05$. Significance levels were indicated as follows: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ throughout the analyses. For all measurements in the study, the sample size was $n = 10$/group. Principal component analysis and heatmap were performed with MetaboAnalyst 6.0 (https://www.metaboanalyst.ca).

Results

Fatty acid composition

The concentrations of the 15 most abundant fatty acids have been measured and the results are summarised in Table 1. The Supplementary Table A1, A2 and the Supplementary Figure A1 of the article present the statistical parameters for both absolute and relative concentrations, along with pie chart visualizations depicting the percentage data.

A significant increase in the concentration of lauric acid (C12:0) was observed in the high-dose acetamiprid-treated group compared to the control group ($P = 0.017$; Fig. 1A). There was also a significant elevation in the concentration of myristic acid (C14:0) in the low-dose acetamiprid-treated group ($P = 0.008$; Fig. 1B). Treatment with low and high doses of acetamiprid caused a significant increase in the concentration of palmitic acid (C16:0) ($P = 0.004$ and $P < 0.001$; Fig. 1C). Examining the concentration of stearic acid (C18:0), a similar result to the previous one was observed, i.e. a significant rise in the low and high acetamiprid treated groups compared to the control ($P = 0.002$ and $P < 0.001$; Fig. 1D). The concentration of alpha-linolenic acid (C18:3, n-3) was significantly increased in the low-dose acetamiprid-treated group compared with the control group ($P < 0.001$; Fig. 1E).

Further analysis of the data also revealed a significant elevation in total fatty acid concentration of the low-dose acetamiprid-treated group ($P = 0.012$; Fig. 2A). The proportion of saturated fatty acids increased significantly in the low- and high-dose acetamiprid-treated groups compared to the control ($P = 0.001$ and $P < 0.001$; Fig. 2B). A significant elevated level in the concentration of PUFAs was detected with exposure to low-dose acetamiprid compared to the control group ($P = 0.001$; Fig. 2C). The ratio of monounsaturated fatty acids to saturated fatty acids (MUFA-SFA ratio, monounsaturated fatty acids to saturated fatty acids ratio) showed a significant decrease in the high-dose acetamiprid-treated group ($P = 0.023$; Fig. 2D). The UFA-SFA ratio (unsaturated fatty acids - saturated fatty acids ratio) also showed a significant decline in the high dose acetamiprid group compared to the control group ($P = 0.011$; Fig. 2E).

	Control mean ± SEM	AcetLow mean ± SEM	AcetMedium mean ± SEM	AcetHigh mean ± SEM
C8:0	14.42 ± 2.96	18.78 ± 2.80	19.07 ± 3.27	19.31 ± 2.67
C10:0	13.04 ± 1.40	14.38 ± 1.39	14.34 ± 0.92	17.69 ± 1.43
C12:0	26.72 ± 2.15	34.32 ± 2.57	33.19 ± 1.78	36.35*±1.99
C14:0	118.0 ± 5.05	152.0**±9.89	119.6 ± 5.95	123.6 ± 5.36
C15:0	58.14 ± 3.52	71.37 ± 6.36	56.85 ± 5.01	54.72 ± 3.21
C16:0	1215 ± 50.29	1478**±45.43	1309 ± 44.03	1522***±56.27
C16:1, n-7	104.8 ± 6.70	125.6 ± 7.20	91.57 ± 3.84	114.6 ± 5.50
C17:0	50.93 ± 1.98	57.04 ± 1.82	49.56 ± 2.96	53.97 ± 2.98
C18:0	1979 ± 61.89	2286**±48.64	2075 ± 33.05	2352***±68.47
C18:1, n-9	7731 ± 471.30	8937 ± 411.30	8596 ± 428.90	7330 ± 399.00
C18:2, n-6	511.6 ± 22.92	590.7 ± 20.67	514.2 ± 18.44	587.4 ± 21.35
C18:3, n-3	2685 ± 98.64	3224***±90.95	2644 ± 57.47	2916 ± 101.80
C20:0	87.04 ± 4.24	97.32 ± 2.72	89.90 ± 5.09	92.30 ± 3.98
C22:0	41.63 ± 1.86	44.81 ± 2.26	35.18 ± 1.57	47.48 ± 3.89
C24:0	16.68 ± 1.31	19.59 ± 1.96	14.18 ± 1.10	19.28 ± 1.53

Table 1. Fatty acid profile of honey bee brains. "Control" refers to control group with no treatment; "AcetLow", "AcetMedium" and "AcetHigh" refer to 0.363, 0.727 and 1.453 µg/bee/day acetamiprid exposures, respectively. Data is expressed as mean ± standard error of mean (SEM), as µg fatty acid/g sample. Significant differences between experimental groups (control vs. treated groups) are indicated with asterisks. *$P < 0.05$.; **$P < 0.01$.; ***$P < 0.001$.

## Principal component analysis (PCA) and heatmap

To better visualize the effects of acetamiprid treatment on the fatty acid profile of honey bee brains, a principal component analysis (PCA) was performed (Fig. 3). The PCA of the fatty acid parameters revealed that the first two principal components accounted for approximately 48% of the total variance in the experimental data (PC1: 30.3%, PC2: 20.1%). These findings indicate that no distinct treatment-associated pattern was identified in relation to the fatty acid content across the samples. Additionally, a heatmap was generated to provide a clearer visualization and overview of the fatty acid profiles of individual samples (Fig. 4).

Discussion

The effects of neonicotinoids on oxidative stress have been the focus of recent research, including studies conducted by our research group[24]. The sub-lethal effects of these compounds on non-target animal species are particularly significant, despite the fact that, if the manufacturer's instructions are followed, today's products are safer to use than their predecessors[36]. This study aimed to explore changes in the fatty acid profile of honey bees, an under-researched yet critical factor in understanding the effects of neonicotinoids on the central nervous system. Currently, very few studies have investigated changes in the central nervous system lipidome of honey bees following exposure to neonicotinoids. Moreover, most existing research tends to focus on behavioral outcomes rather than molecular-level analyses. For instance, a previous study have linked neonicotinoid exposure to a reduction in self-grooming behavior in honey bees, highlighting functional impairments, which can have detrimental effects on the viability and overall health of honey bees[37]. Highlighting the significance of this topic, previous studies on other non-target animal species have explored the sub-lethal effects of neonicotinoids on lipid metabolism and nervous system function. A study on *Drosophila melanogaster* (*Diptera: Drosophilidae*) examined the effects of low doses of imidacloprid, reporting mitochondrial dysfunction, severe damage to glial cells, and impaired vision, which collectively point to lipid dysregulation and neurodegeneration[38]. Another study on Egyptian toads (*Sclerophrys regularis*, *Anura: Bufonidae*) explored the effects of acetamiprid and observed the alteration of the plasma lipid profile and an increase in total lipid concentration[39]. This increase was associated with elevated lipid peroxidation, which can compromise cell membrane integrity. The heightened lipid levels were also linked to increased energy demands, lipid accumulation, and subsequent cell death[40]. Similarly, in an in vitro experiment using mouse Neuro-2a cells, acetamiprid was found to disrupt lipid metabolism[41].

In forager bees, researchers observed that exposure to neonicotinoids negatively impacted the proboscis extension reflex, a crucial mechanism underlying their foraging behavior[42]. In a catch-and-release experiment, it was observed that exposure to neonicotinoids significantly impaired the orientation of bees, particularly their homing memory. This disruption may play a key role in the decline of worker bee populations[43]. In a study examining the chronic effects of thiacloprid, honey bees were exposed to sub-lethal doses of this cyano-substituted compound under field conditions for several weeks. Although considered safer than its earlier counterparts, thiacloprid impaired foraging behavior, homing success, navigation performance, and social communication[44].

The aforementioned cognitive alterations have been documented in various animal species; however, the underlying mechanisms remain incompletely understood. In our previous research, we investigated the oxidative stress-inducing effects of different classes of pesticides, including acetamiprid, in honey bees. Our findings demonstrated that neonicotinoids disrupt oxidative balance, leading to an increase in reactive oxygen species
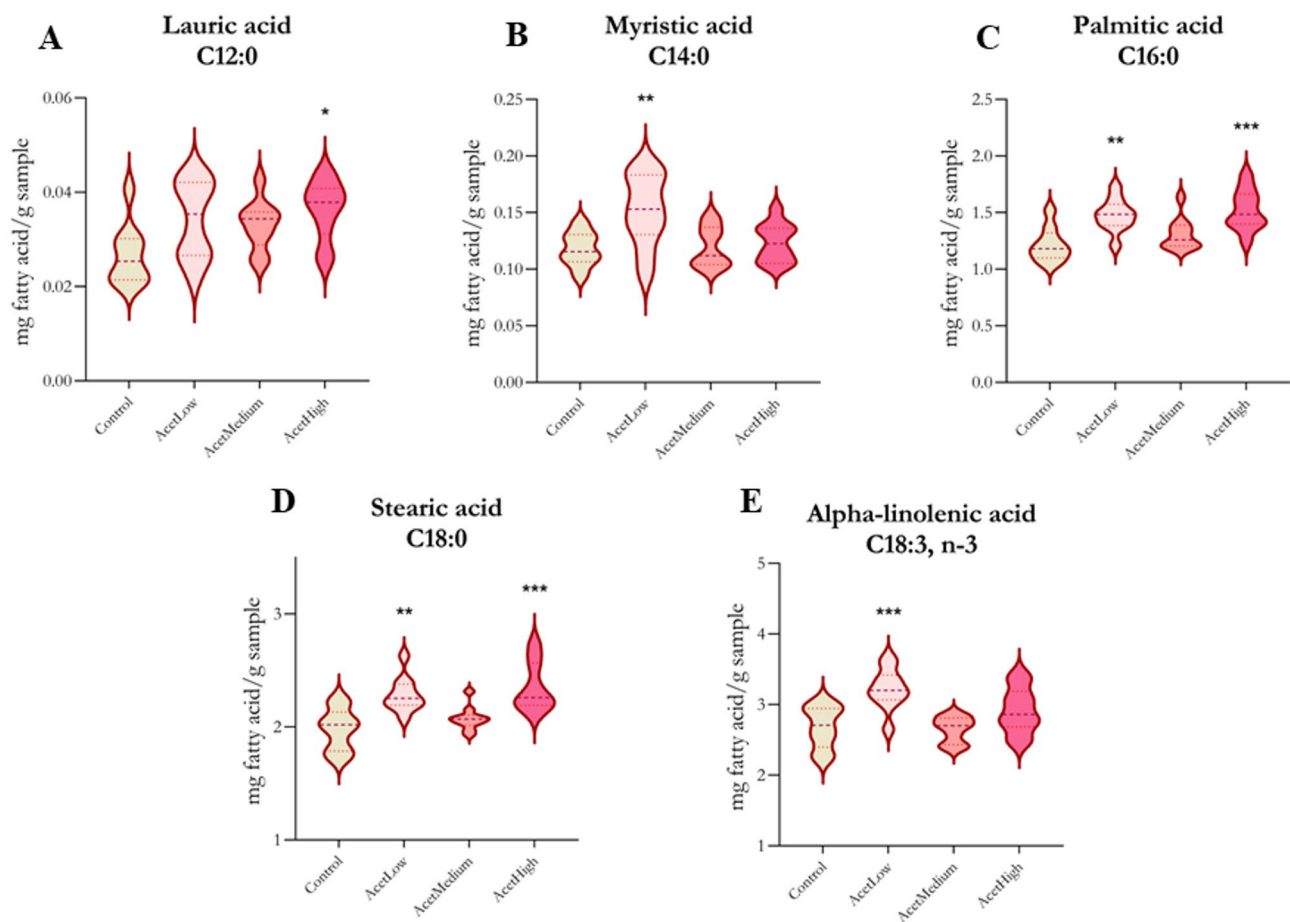
Fig. 1. Changes in the concentration of different fatty acids compared to the control group in the low-dose acetamiprid-treated (AcetLow), medium-dose acetamiprid-treated (AcetMedium) and high-dose acetamiprid-treated (AcetHigh) groups. (**A**) Lauric acid (C12:0), (**B**) Myristic acid (C14:0), (**C**) Palmitic acid (C16:0), (**D**) Stearic acid (C18:0), (**E**) Alpha-linolenic acid (C18:3, n-3). Dashed lines indicate the median and dotted lines indicated the first (Q1) and third (Q3) quartiles. Asterisks indicate significance relative to the control group. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

(ROS) levels, an elevation in malondialdehyde (MDA) concentration – a key indicator of lipid peroxidation – and an upregulation of enzymatic activity within several antioxidant defense systems[24]. Furthermore, studies conducted on other species suggest that pesticides may influence fatty acid metabolism, yet this aspect has not been explored in honey bees to date[45,46]. Based on all these considerations, our aim was to assess the changes in the fatty acid profile of the honey bee central nervous system induced by neonicotinoid exposure. These molecular alterations may be linked to various behavioral changes that could ultimately contribute to the decline in honey bee populations.

Notably, according to our results, a significant increase in lauric acid levels was observed in the group exposed to high doses of acetamiprid. Lauric acid has been recognized for its antioxidant properties[47] suggesting that it's elevated levels may reflect the activation of antioxidant defense mechanisms, potentially serving as a compensatory response to oxidative stress. However, it is crucial to consider its concentration-dependent prooxidant effects, a characteristic shared by many compounds with antioxidant activity[14]. Based on these findings, the effects of increased lauric acid concentrations cannot be categorically classified as either positive or negative. Instead, they must be assessed within the broader context of changes in the entire fatty acid profile to provide a comprehensive understanding of their implications.

Myristic acid, alongside other fatty acids, has been extensively studied in the context of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases[18,21,48–51]. We observed an increase in myristic acid concentrations in the low-dose acetamiprid-treated group, indicating its significance as a component of the fatty acid profile undergoing alteration. This indicates its potential role in the development of neurodegenerative diseases and, similar to lauric acid, its involvement in initiating compensatory mechanisms; however, no changes were observed in the groups receiving higher levels of acetamiprid treatment.

Palmitic acid concentrations showed a significant increase in both the low- and high-dose acetamiprid-treated groups in our study. Evidence from several studies suggests that palmitic acid plays a role in the pathogenesis of neurodegenerative diseases[50]. Under oxidative stress, the normal cellular redox balance is disrupted, leading to increased lipid peroxidation[17]. As an adaptive mechanism, cells often alter their lipid composition, accelerating
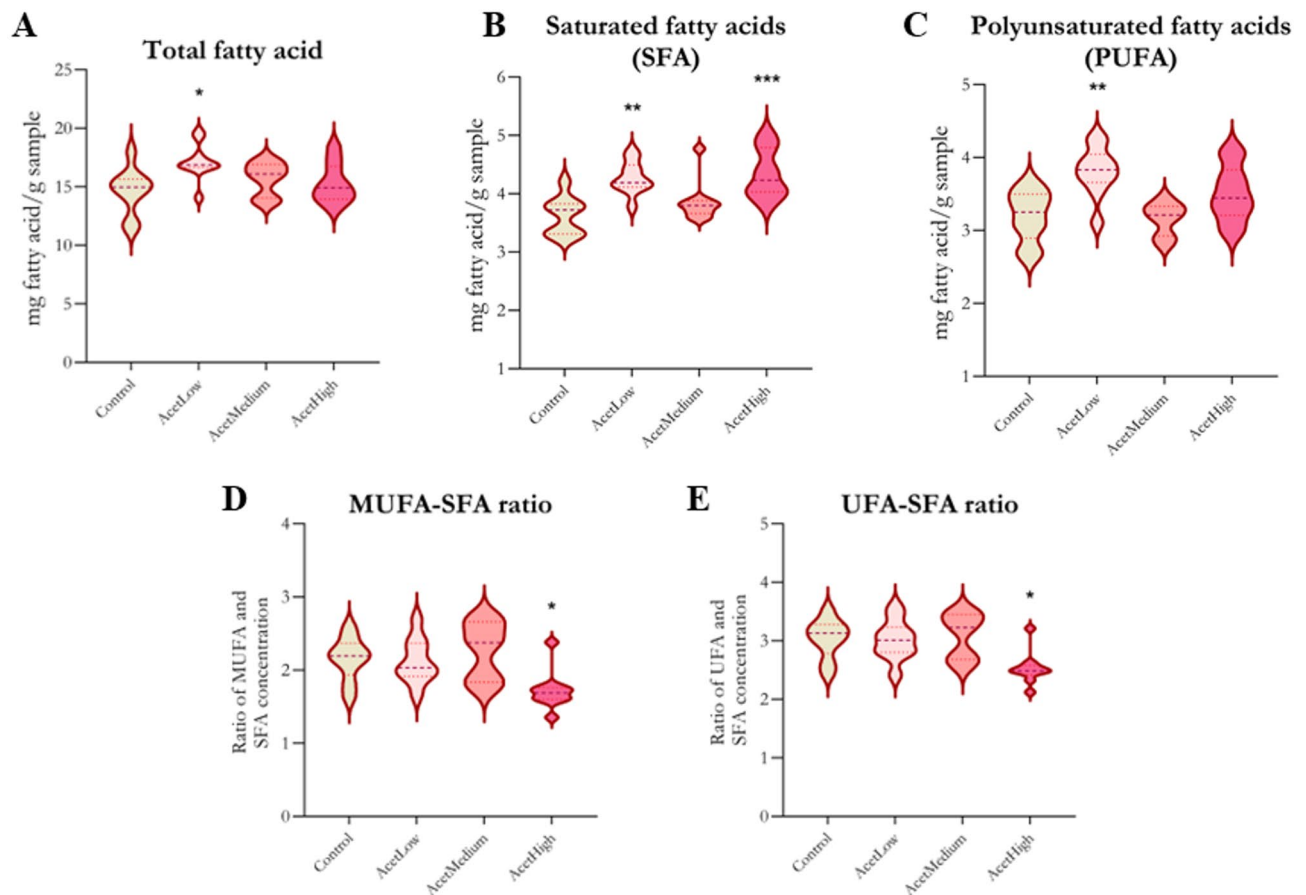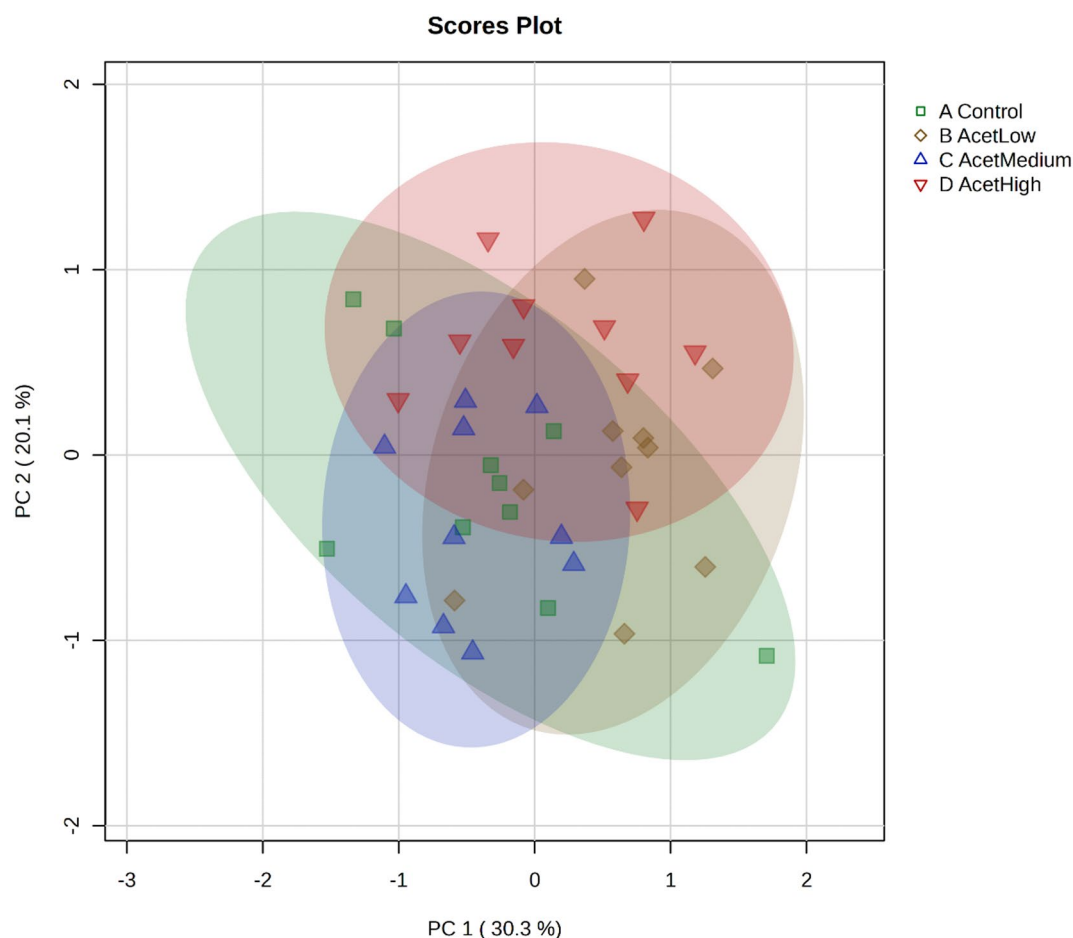
Fig. 2. Variation of fatty acid profile parameters in the low-dose acetamiprid group (AcetLow), the medium-dose acetamiprid group (AcetMedium) and the high-dose acetamiprid group (AcetHigh) compared to the control group. (**A**) Total fatty acid concentration, (**B**) Saturated fatty acid concentration (SFA), (**C**) Polyunsaturated fatty acid concentration (PUFAs), (**D**) Monounsaturated to saturated fatty acid ratio (MUFA-SFA ratio), (**E**) Unsaturated to saturated fatty acid ratio (UFA-SFA ratio). Dashed lines indicated median and dotted lines indicate first (Q1) and third (Q3) quartiles. Asterisks indicate significance relative to the control group. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

the production of SFAs like palmitic acid, because they are more stable and less susceptible to peroxidation than PUFAs, providing enhanced structural resilience[52]. Consequently, the observed increase in palmitic acid concentrations may also represent an adaptive response to oxidative stress. By increasing the production of more rigid and stable saturated fatty acids, honey bee central nervous system cells may aim to stabilize cellular membranes and mitigate damage[53]. Additionally, such increases are associated with the disruption of neural pathways, potentially leading to a range of complex consequences[54]. These findings suggest that a sustained rise in palmitic acid concentrations may contribute to central nervous system dysfunction in honey bees. However, further research is necessary to fully elucidate these effects and their broader implications for bee health and behavior.

Stearic acid, another saturated fatty acid, was identified as the most abundant saturated fatty acid in the central nervous system of honey bees. The trends in stearic acid concentrations mirrored those observed for palmitic acid, with increases detected in both the low- and high-dose acetamiprid-treated groups. Similarly to these findings, it was also previously demonstrated that stearic acid affects the cellular repair processes in a dose- and time-dependent manner, leading to lipotoxicity and elevated levels of certain pro-inflammatory cytokines. This may be the basis for the possibility, that elevated levels of stearic acid may interfere with the central nervous system of bees as described in the case of palmitic acid[55].

Alpha-linolenic acid, a polyunsaturated omega-3 fatty acid containing three C-C double bonds, is known for its neuroprotective and anti-inflammatory properties. Significant changes in the concentrations were described following low-dose acetamiprid treatment. The observed increase in its concentration is likely the part of the abovementioned adaptive response to acetamiprid-induced stress. This response may represent a cellular mechanism aimed at maintaining membrane fluidity and restoring the fatty acid composition disrupted by neonicotinoid treatment[56].

The total fatty acid concentration increased significantly in the group treated with low-dose acetamiprid. This aligns with the observation that all significant changes mentioned earlier showed upward trends, suggesting a

Scores Plot

Fig. 3. Principal component analysis (PCA) of the combined data set of fatty acid profile in honey bee brains. The 2 major components (PC 1 and PC 2) that accounted for the most variation of the metabolite abundance were used to plot. Each dot in the figure represents a single sample, and different colors indicate the different treatments. "Control" refers to control group with no treatment (red); "AcetLow" (green), "AcetMedium" (violet) and "AcetHigh" (blue) refer to 0.363, 0.727 and 1.453 μg/bee/day acetamiprid exposure, respectively.

cellular mechanism in which new fatty acids are synthesized to replace damaged ones. This process may help restore the altered fatty acid profile and enable adaptation to acetamiprid exposure[46]. Fatty acids are not only essential components of cellular membranes and signaling pathways but also play a critical role in energy metabolism, which could be especially important under acetamiprid-induced stress[37].

Several studies have shown that elevated concentrations of SFAs can lead to endoplasmic reticulum (ER) dysfunction, thereby inducing cellular stress[57]. Excess SFAs are incorporated into phospholipids, which are subsequently integrated into the ER membrane bilayer. However, this membrane typically contains phosphatidylcholine, a phospholipid derived from unsaturated fatty acids[52]. The presence of SFAs reduces membrane fluidity, which is essential for the ER to perform critical functions such as protein folding, spatial organization, and the regulation of intracellular calcium levels, among other membrane-bound processes[58]. In our experiment, SFA concentrations increased in both the low- and high-dose acetamiprid-treated groups. This rise in the quantity and proportion of SFAs may compromise these ER functions, potentially disrupting the regulation of various cellular signaling processes.

Significant changes in PUFA concentrations were observed in the group treated with low-dose acetamiprid. As discussed earlier, polyunsaturated fatty acids are more prone to oxidative stress due to their molecular structure, but they also enhance membrane fluidity, therefore, it is plausible that their increase may help compensate for the reduced fluidity caused by the higher concentration of saturated fatty acids[59]. Changes in the fatty acid profile are hypothesized to influence cellular functions in various ways, beyond merely heightening vulnerability to oxidative stress[60].

Among the parameters examining the ratios of different types of fatty acids, significant differences were observed in the MUFA-SFA and UFA-SFA ratios, both of which showed a marked decrease in the high-dose acetamiprid-treated group. As previously discussed, the increase in the proportion of SFAs is likely the part of a response mechanism aimed to maintain membrane stability[61]. At the same time, the concentrations of UFA and MUFA, such as oleic acid, did not exhibit significant changes, resulting in a shift in the ratios in favor of saturated fatty acids. While there was a notable increase in polyunsaturated fatty acids, this change may not have
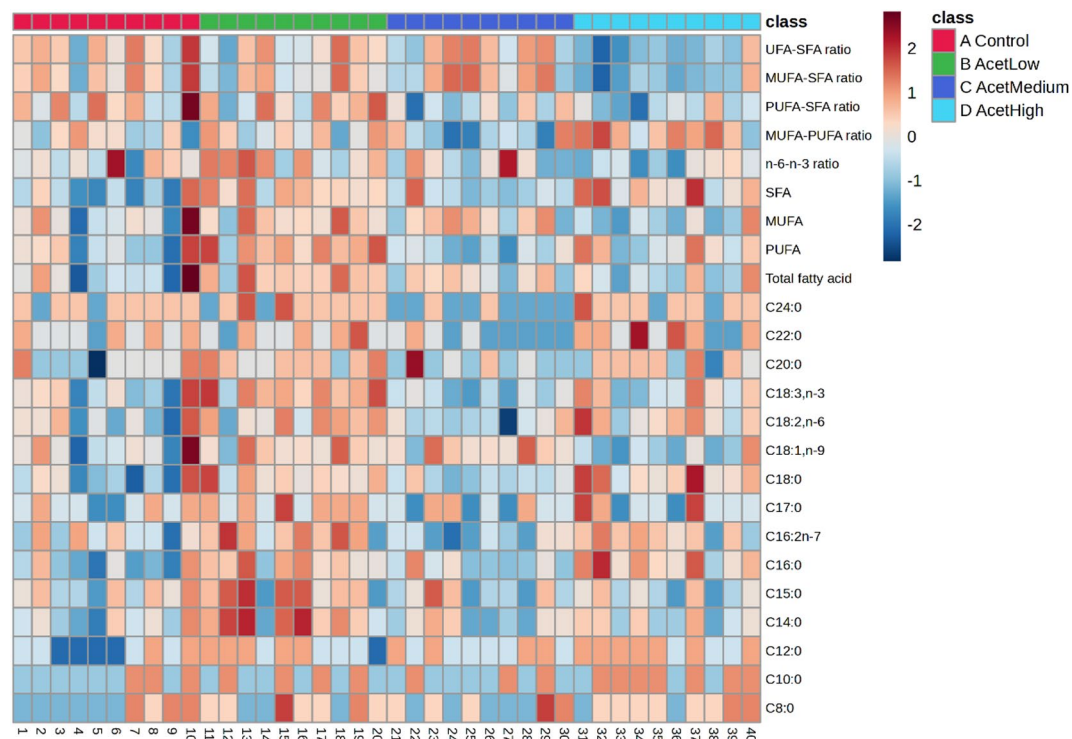
Fig. 4. Heatmap of the measured fatty acid parameters. The heatmap was generated using autoscaled data and used color coding to show relative concentration averages for treatment groups. Samples are presented individually identified by numbers and groups are color-coded according to the treatments. UFA-SFA ratio = unsaturated fatty acid – saturated fatty acid ratio, MUFA-SFA ratio = monounsaturated fatty acid – saturated fatty acid ratio, PUFA-SFA ratio = polyunsaturated fatty acid – saturated fatty acid ratio, MUFA-PUFA ratio = monounsaturated fatty acid – polyunsaturated fatty acid ratio, n-6/n-3 = n-6 polyunsaturated fatty acid – n-3 polyunsaturated fatty acid ratio, SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, Total fatty acid = Total fatty acid, C24:0 = Lignoceric acid, C22:0 = Behenic acid, C20:0 = Arachidic acid, C18:3, n-3 = Alpha-linolenic acid, C18:2, n-6 = Linoleic acid, C18:1, n-9 = Oleic acid, C18:0 = Stearic acid, C17:0 = Heptadecanoic acid, C16:1, n-7 = Palmitoleic acid, C16:0 = Palmitic acid, C15:0 = Pentadecanoic acid, C14:0 = Myristic acid, C12:0 = Lauric acid, C10:0 = Capric acid, C8:0 = Caprylic acid.

been sufficient to effectively elevate the total concentration of unsaturated fatty acids. This mechanism, driven by alterations in the fatty acid profile, likely involves various cellular processes that may be linked to changes in cognitive function as described by other research groups[59].

The evaluation of the fatty acid profile's percentage distribution revealed that, while no widespread significant differences were observed between the control and treated groups, the relative proportions of stearic acid and palmitic acid increased significantly in the high-dose acetamiprid group compared to other fatty acids. This suggests that acetamiprid exposure may selectively influence the composition of certain SFAs in the central nervous system.

While our discussion primarily centers on individual fatty acid changes, it is important to consider the broader patterns that emerged across the treatment groups. In the low-dose acetamiprid group, we detected the most notable shifts in the fatty acid profile, characterized by significant increases in both saturated fatty acids, specifically myristic acid, palmitic acid, and stearic acid, and in the polyunsaturated fatty acid alpha-linolenic acid, as well as an overall elevation in total fatty acid content. These results point to a strong cellular response, likely reflecting compensatory mechanisms aimed at counteracting oxidative stress and preserving membrane stability. Conversely, the high-dose group displayed more limited changes, with significant alterations largely confined to the saturated fatty acids lauric acid, palmitic acid, and stearic acid, while also exhibiting a marked reduction in the ratios of monounsaturated and unsaturated fatty acids to saturated fatty acids (MUFA-SFA and UFA-SFA). This difference in response suggests that higher acetamiprid concentrations may trigger a distinct or possibly suppressed physiological adaptation compared to lower doses. This suggests that acetamiprid exhibits non-monotonic dose responses, meaning its effects do not follow a simple linear relationship with concentration; instead, lower doses may induce significant biological changes that are absent or even reversed at higher doses.

In honey bees, sublethal exposure to acetamiprid has been shown to alter neurophysiological functions, including neurotransmission and lipid metabolism, in ways that are not necessarily exacerbated at higher concentrations. This phenomenon suggests that certain physiological pathways, such as oxidative stress responses and enzyme activity, may be more sensitive to low-dose exposure, leading to disruptions that are not predictable

based on traditional high-dose toxicity assessments. Notably, low-dose acetamiprid exposure has been associated with significant alterations in the fatty acid profile of the honey bee central nervous system, particularly in the levels of saturated, monounsaturated, and polyunsaturated fatty acids. These findings challenge conventional toxicological assumptions, as they imply that regulatory thresholds based solely on high-dose toxicity may underestimate the risks posed by chronic low-dose exposure to acetamiprid in pollinators.

In conclusion, our findings indicate that acetamiprid treatment significantly altered the central nervous system fatty acid profile of honey bees. We observed increased levels of several SFAs, including lauric acid, myristic acid, palmitic acid, and stearic acid, as well as the PUFA alpha-linolenic acid, total fatty acid concentrations, and both total PUFAs and SFAs parameters. Conversely, the MUFA-SFA and UFA-SFA ratios showed a marked decrease. These results suggest the activation of an adaptive mechanism in response to acetamiprid exposure, characterized by increased concentrations of both SFAs and PUFAs. Saturated fatty acids contribute to membrane stability and rigidity, enhancing resilience to various stressors, while PUFAs promote membrane fluidity, which is essential for maintaining normal cellular metabolism and signaling. Together, these changes represent a dual cellular response aimed at adapting to the stress induced by acetamiprid exposure.

Importantly, a key limitation of our experiment is that measurements were made at a single time point, making it difficult to accurately extrapolate the time course of these processes. Additionally, all experimental bees were sourced from a single colony. While this approach allowed for control over genetic and environmental variability, it may limit the generalizability of our findings, as colony-level variation is a recognized factor in honey bee research. Future studies should aim to include multiple colonies to enhance the robustness and applicability of the results. Another limitation of the present study is the use of worker bees of varying ages, collected directly from hive frames without precise age determination. While efforts were made to target foraging bees, which tend to have a narrower age distribution, some age-related variability may still be present. Addressing this limitation in future studies by controlling for worker age and including multiple colonies would further strengthen the reliability and applicability of the results.

These changes in the fatty acid profile may be intricately connected to the behavioral alterations previously described in the literature, such as impaired self-grooming and other neurological dysfunctions. Such behavioral shifts are critical because they can directly affect the overall fitness and survival of honey bees. Disruptions in lipid metabolism within the central nervous system could lead to altered neural signaling and cognitive functions, which in turn manifest as changes in behaviors essential for colony maintenance and individual health. Understanding these molecular-behavioral links is therefore vital, as they may contribute to broader ecological consequences, including the observed decline in honey bee populations worldwide.

Data availability
The data supporting the findings of this study are available from the corresponding author upon reasonable request.

References
1. Hung, K. L. J., Kingston, J. M., Albrecht, M., Holway, D. A. & Kohn, J. R. The worldwide importance of honey bees as pollinators in natural habitats. *Proc. R Soc. B.* **285**, 20172140 (2018).
2. Neov, B., Shumkova, R., Palova, N. & Hristov, P. The health crisis in managed honey bees (Apis mellifera). Which factors are involved in this phenomenon? *Biologia* **76**, 2173–2180 (2021).
3. Patel, V., Pauli, N., Biggs, E., Barbour, L. & Boruff, B. Why bees are critical for achieving sustainable development. *Ambio* **50**, 49–59 (2021).
4. Potts, S. G. et al. Safeguarding pollinators and their values to human well-being. *Nature* **540**, 220–229 (2016).
5. Murthy, J. S. V. et al. Perspective Chapter: Wild Bees – *Importance, Threats, and Conservation Challenges*IntechOpen,. (2024). https://doi.org/10.5772/intechopen.1004403
6. vanEngelsdorp, D. & Meixner, M. D. A historical review of managed honey bee populations in Europe and the united States and the factors that May affect them. *J. Invertebr. Pathol.* **103**, S80–S95 (2010).
7. Tudi, M. et al. Agriculture development, pesticide application and its impact on the environment. *IJERPH* **18**, 1112 (2021).
8. Goulson, D. R. E. V. I. E. W. An overview of the environmental risks posed by neonicotinoid insecticides. *J. Appl. Ecol.* **50**, 977–987 (2013).
9. Wang, X. et al. Mechanism of neonicotinoid toxicity: impact on oxidative stress and metabolism. *Annu. Rev. Pharmacol. Toxicol.* **58**, 471–507 (2018).
10. Fairbrother, A., Purdy, J., Anderson, T. & Fell, R. Risks of neonicotinoid insecticides to honeybees. *Enviro Toxic. Chem.* **33**, 719–731 (2014).
11. Zuščíková, L. et al. Screening of toxic effects of neonicotinoid insecticides with a focus on acetamiprid: A review. *Toxics* **11**, 598 (2023).
12. Ayala, A., Muñoz, M. F. & Argüelles, S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxidative Med. Cell. Longev.* **2014**, 1–31 (2014).
13. Tsaluchidu, S., Cocchi, M., Tonello, L. & Puri, B. K. Fatty acids and oxidative stress in psychiatric disorders. *BMC Psychiatry.* **8**, S5 (2008).
14. Pisoschi, A. M. & Pop, A. The role of antioxidants in the chemistry of oxidative stress: A review. *Eur. J. Med. Chem.* **97**, 55–74 (2015).
15. Preiser, J. Oxidative stress. *J. Parenter. Enter. Nutr.* **36**, 147–154 (2012).
16. Tsikas, D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: analytical and biological challenges. *Anal. Biochem.* **524**, 13–30 (2017).
17. Reed, T. T. Lipid peroxidation and neurodegenerative disease. *Free Radic. Biol. Med.* **51**, 1302–1319 (2011).
18. Vesga-Jiménez, D. J. et al. Fatty acids: an insight into the pathogenesis of neurodegenerative diseases and therapeutic potential. *Int. J. Mol. Sci.* **23**, 2577 (2022).
19. Assies, J. et al. Effects of oxidative stress on fatty acid- and one-carbon-metabolism in psychiatric and cardiovascular disease comorbidity. *Acta Psychiatry. Scand.* **130**, 163–180 (2014).

20. vanEngelsdorp, D. et al. Colony collapse disorder: A descriptive study. *PLoS ONE.* **4**, e6481 (2009).
21. Kastaniotis, A. J., Autio, K. J. & Nair, R. Mitochondrial fatty acids and neurodegenerative disorders. *Neuroscientist* **27**, 143–158 (2021).
22. Azizpor, P. et al. Polyunsaturated fatty acids stimulate immunity and eicosanoid production in drosophila melanogaster. *J. Lipid Res.* **65**, 100608 (2024).
23. Erban, T. et al. Chronic exposure of bumblebees to neonicotinoid Imidacloprid suppresses the entire mevalonate pathway and fatty acid synthesis. *J. Proteom.* **196**, 69–80 (2019).
24. Mackei, M. et al. Unraveling the acute sublethal effects of Acetamiprid on honey bee neurological redox equilibrium. *Sci. Rep.* **14**, 27514 (2024).
25. Williams, G. R. et al. Standard methods for maintaining adult *Apis mellifera* in cages under *in vitro* laboratory conditions. *J. Apic. Res.* **52**, 1–36 (2013).
26. Test No. 213: Honeybees, Acute oral toxicity test. *OECD* (1998). https://www.oecd.org/en/publications/test-no-213-honeybees-acute-oral-toxicity-test_9789264070165-en.html
27. Test No. 245: Honey Bee (Apis Mellifera L.), Chronic oral toxicity test (10-Day Feeding). *OECD* (2017). https://www.oecd.org/en/publications/test-no-245-honey-bee-apis-mellifera-l-chronic-oral-toxicity-test-10-day-feeding_9789264284081-en.html
28. Helmer, S. H., Kerbaol, A., Aras, P., Jumarie, C. & Boily, M. Effects of realistic doses of atrazine, metolachlor, and glyphosate on lipid peroxidation and diet-derived antioxidants in caged honey bees (Apis mellifera). *Environ. Sci. Pollut Res.* **22**, 8010–8021 (2015).
29. Gauthier, M., Aras, P., Paquin, J. & Boily, M. Chronic exposure to Imidacloprid or Thiamethoxam neonicotinoid causes oxidative damages and alters carotenoid-retinoid levels in caged honey bees (Apis mellifera). *Sci. Rep.* **8**, 16274 (2018).
30. Jumarie, C., Aras, P. & Boily, M. Mixtures of herbicides and metals affect the redox system of honey bees. *Chemosphere* **168**, 163–170 (2017).
31. Iwasa, T., Motoyama, N., Ambrose, J. T. & Roe, R. M. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, apis mellifera. *Crop Prot.* **23**, 371–378 (2004).
32. Mackei, M. et al. Detrimental consequences of Tebuconazole on redox homeostasis and fatty acid profile of honeybee brain. *Insect Biochem. Mol. Biol.* **159**, 103990 (2023).
33. Su, Y., Shi, J., Hu, Y., Liu, J. & Wu, X. Acetamiprid exposure disrupts gut microbiota in adult and larval worker honeybees (Apis mellifera L). *Insects* **15**, 927 (2024).
34. Chiu, H. H. & Kuo, C. H. Gas chromatography-mass spectrometry-based analytical strategies for fatty acid analysis in biological samples. *J. Food Drug Anal.* **28**, 60–73 (2020).
35. Sparkman, O. D., Penton, Z. & Kitson, F. G. *Gas Chromatography and Mass Spectrometry: A Practical Guide* (Academic, 2011).
36. Lu, C., Hung, Y. T. & Cheng, Q. A. Review of Sub-lethal neonicotinoid insecticides exposure and effects on pollinators. *Curr. Pollution Rep.* **6**, 137–151 (2020).
37. Morfin, N. et al. First insights into the honey bee (*Apis mellifera*) brain lipidome and its neonicotinoid-induced alterations associated with reduced self-grooming behavior. *J. Adv. Res.* **37**, 75–89 (2022).
38. Martelli, F. et al. Low doses of the neonicotinoid insecticide Imidacloprid induce ROS triggering neurological and metabolic impairments in drosophila. *Proc. Natl. Acad. Sci. U S A.* **117**, 25840–25850 (2020).
39. Saad, E. M., Elassy, N. M. & Salah-Eldein, A. M. Effect of induced sublethal intoxication with neonicotinoid insecticides on Egyptian toads (Sclerophrys regularis). *Environ. Sci. Pollut Res.* **29**, 5762–5770 (2022).
40. Dornelles, M. F. & Oliveira, G. T. Effect of atrazine, glyphosate and quinclorac on biochemical parameters, lipid peroxidation and survival in bullfrog tadpoles (Lithobates catesbeianus). *Arch. Environ. Contam. Toxicol.* **66**, 415–429 (2014).
41. Wang, X. et al. Integrated non-targeted lipidomics and metabolomics analyses for fluctuations of neonicotinoids Imidacloprid and Acetamiprid on Neuro-2a cells. *Environ. Pollut.* **284**, 117327 (2021).
42. Thany, S. H. et al. Similar comparative low and high doses of deltamethrin and Acetamiprid differently impair the retrieval of the proboscis extension reflex in the forager honey bee (Apis mellifera). *Insects* **6**, 805–814 (2015).
43. Fischer, J. et al. Neonicotinoids interfere with specific components of navigation in honeybees. *PLoS One.* **9**, e91364 (2014).
44. Tison, L. et al. Honey bees' behavior is impaired by chronic exposure to the neonicotinoid thiacloprid in the field. *Environ. Sci. Technol.* **50**, 7218–7227 (2016).
45. Sun, J. et al. Association between urinary neonicotinoid insecticide levels and dyslipidemia risk: A cross-sectional study in Chinese community-dwelling elderly. *J. Hazard. Mater.* **459**, 132159 (2023).
46. Clements, J., Olson, J. M., Sanchez-Sedillo, B., Bradford, B. & Groves, R. L. Changes in emergence phenology, fatty acid composition, and xenobiotic-metabolizing enzyme expression is associated with increased insecticide resistance in the Colorado potato beetle. *Arch. Insect Biochem. Physiol.* **103**, e21630 (2020).
47. Namachivayam, A. & Gopalakrishnan, A. V. Effect of lauric acid against ethanol-induced hepatotoxicity by modulating oxidative stress/apoptosis signalling and HNF4α in Wistar albino rats. *Heliyon* **9**, (2023).
48. Ciesielski, J., Su, T. P. & Tsai, S. Y. Myristic acid hitchhiking on sigma-1 receptor to fend off neurodegeneration. *Receptors Clin. Investig.* **3**, e1114 (2016).
49. Shah, A., Han, P., Wong, M. Y., Chang, R. C. C. & Legido-Quigley, C. Palmitate and stearate are increased in the plasma in a 6-OHDA model of parkinson's disease. *Metabolites* **9**, 31 (2019).
50. Zhou, H. & Chang, S. L. Meta-analysis of the effects of palmitic acid on microglia activation and neurodegeneration. *NeuroImmune Pharmacol. Ther.* **2**, 281–291 (2023).
51. Shamim, A., Mahmood, T., Ahsan, F., Kumar, A. & Bagga, P. Lipids: an insight into the neurodegenerative disorders. *Clin. Nutr. Experimental.* **20**, 1–19 (2018).
52. Leamy, A. K., Egnatchik, R. A. & Young, J. D. Molecular mechanisms and the role of saturated fatty acids in the progression of non-alcoholic fatty liver disease. *Prog. Lipid Res.* **52**, 165–174 (2013).
53. Maulucci, G. et al. Fatty acid-related modulations of membrane fluidity in cells: detection and implications. *Free Radical. Res.* (2016).
54. Smith, M. E. & Bazinet, R. P. Unraveling brain palmitic acid: origin, levels and metabolic fate. *Prog. Lipid Res.* **96**, 101300 (2024).
55. Spigoni, V. et al. Stearic acid at physiologic concentrations induces *in vitro* lipotoxicity in Circulating angiogenic cells. *Atherosclerosis* **265**, 162–171 (2017).
56. Pan, H. et al. Alpha-linolenic acid is a potent neuroprotective agent against soman-induced neuropathology. *NeuroToxicology* **33**, 1219–1229 (2012).
57. Mai, X., Yin, X., Chen, P., Zhang, M. & Salvianolic Acid, B. Protects against fatty Acid-Induced renal tubular injury via Inhibition of Endoplasmic reticulum stress. *Front Pharmacol* **11**, (2020).
58. Paschen, W. & Mengesdorf, T. Endoplasmic reticulum stress response and neurodegeneration. *Cell. Calcium.* **38**, 409–415 (2005).
59. Nagy, K., Tiuca, I. D., Nagy, K. & Tiuca, I. D. Importance of fatty acids in physiopathology of human body. In *Fatty Acids* IntechOpen, (2017). https://doi.org/10.5772/67407
60. Mett, J. The impact of medium chain and polyunsaturated ω-3-Fatty acids on Amyloid-β deposition, oxidative stress and metabolic dysfunction associated with alzheimer's disease. *Antioxidants* **10**, 1991 (2021).
61. Hąc-Wydro, K. & Wydro, P. The influence of fatty acids on model cholesterol/phospholipid membranes. *Chem. Phys. Lipids.* **150**, 66–81 (2007).

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Declarations

Competing interests
The authors declare no competing interests.

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