International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 17-09-2024, Accepted: 18-10-2024, Published: 02-11-2024
Volume 9, Issue 11, 2024, Page No. 13-19

Modulation of bee behavior and neural activity by nicotine and microbial infection: Investigating the relation between acetylcholine receptors and Nosema-induced stress

Sung Joo Lee
Department of Environment, Asia Pacific International School, Nowon-gu, Seoul, Repiblic of Korea

Abstract
This study investigated the effects of microorganisms on bee behavior, with a particular focus on how neurotransmitters, specifically acetylcholine, can regulate the bee nervous system. Considering the vital ecological role of bees, the objective was to analyze the potential impacts that microbial-induced behavioral changes in bees could have on the broader ecosystem. Drawing on various examples from nature where microorganisms manipulate the behavior of their hosts, this research aimed to determine whether bee behavior could similarly be influenced by microbes, providing essential foundational data to address ecological issues such as declines in bee populations. The study sought to analyze the molecular and physiological mechanisms by which microorganisms alter bee behavior, with a specific emphasis on the effects of acetylcholine and nicotine on the bee nervous system. To achieve this, the research systematically examined the behavioral changes in bees infected with microorganisms that prompted a preference for malodorous water, as well as the effects of nicotine on neural transmission. The results indicated that bees infected by the microorganisms exhibited a greater preference for malodorous water, suggesting that the infection altered their behavioral patterns. Additionally, while nicotine temporarily increased the bees' activity levels, it was found to potentially have adverse effects on their nervous system over the long term. These findings offer critical insights into how microorganisms and nicotine influence bee behavior and neural function, contributing to strategies for protecting bees within agricultural ecosystems. The study suggests that this data could serve as a basis for further research into the long-term effects of nicotine-based insecticides on bees, helping to develop strategies for safeguarding bee populations. By understanding the mechanisms behind behavioral changes in bees, this research also provides key information for assessing the broader implications for ecosystems and agriculture, ultimately guiding efforts to conserve bee populations.

Keywords: Entomology, Nicotine, bee behavior, microbial infection, Acetylcholine receptors

Introduction

In natural ecosystems, microorganisms can manipulate the behavior of their host organisms in various ways. This phenomenon is well-documented across a range of microbes, including parasites, viruses, and bacteria, which alter host behavior to enhance their own survival, reproduction, or transmission. For example, some parasites modify their hosts' behavior to make them more susceptible to predation, thereby facilitating the transfer of the parasite to its final host. Others induce specific behaviors in their hosts that promote their own reproduction. Understanding how microorganisms can regulate the behavior of ecologically important species, such as bees, provides critical insights into the complex ecological interactions that occur in nature.

Bees play a crucial role as primary pollinators, making them vital to the health of ecosystems. If microbial infections can influence bee behavior, the effects could cascade throughout the ecosystem. Therefore, understanding the mechanisms underlying behavioral changes in bees and their broader ecological impacts is a key area of ecological research. This study aims to investigate how bee behavior may be influenced by microorganisms, potentially offering new insights into maintaining ecosystem health.

If certain microorganisms are found to alter bee behavior, it is possible that these microbes act as pathogens, negatively impacting the health of bee populations. By studying the effects of microorganisms on the health and survival of bees, we can assess the risks posed by diseases and parasites that threaten bees. Given the global decline in bee populations, this line of research could provide essential foundational knowledge for developing conservation strategies aimed at protecting and preserving bees.

This study aims to analyze how microorganisms regulate the behavior of their hosts, with a specific focus on bees. Building on previous research that has demonstrated how microorganisms can alter host behavior to enhance their own survival, reproduction, or transmission, this investigation seeks to understand the mechanisms through which microbial infections affect bee behavior at molecular and physiological levels.

Given the crucial ecological role of bees as pollinators, changes in their behavior could have significant consequences for entire ecosystems. Therefore, this study evaluates how these behavioral changes might impact ecosystem health, aiming to reveal specific mechanisms by which microorganisms alter bee behavior, which could ultimately contribute to the development of ecosystem restoration and conservation strategies.

Additionally, this research investigates the effects of acetylcholine and nicotine on the bee nervous system, seeking to determine how these neurotransmitters and compounds modulate the physiological and behavioral changes in bees induced by microbial infections. Specifically, the study measures how nicotine influences the secretion of neurotransmitters and the activation of receptors in bees. Through these analyses, the research seeks to identify both the positive and negative impacts of nicotine on bee survival, behavior, and immune responses.

Ultimately, this investigation not only enhances the understanding of the neural and behavioral dynamics of bees but also contributes to evaluating the long-term effects of nicotine-based insecticides. The findings are expected to serve as essential foundational knowledge for protecting and managing bee populations within agricultural ecosystems.

Material and methods

Parasites and microorganisms have developed various mechanisms to manipulate the behavior of their hosts. These methods include direct neural manipulation, hormonal changes, and alterations in immune responses. For instance, the Gordian worm infects insects and drives them to water, where it can reproduce. This manipulation is achieved by disrupting the host's neural pathways, causing abnormal behaviors. Similarly, Toxoplasma gondii alters the hormonal signals in rodents, reducing their fear of cats, its final host, to ensure transmission.

Specific examples highlight the diversity of these behaviors. The Gordian worm manipulates grasshoppers and crickets to move toward water, while Toxoplasma gondii changes neurotransmitter levels in rodents to make them more susceptible to predation by cats. Plasmodium, the malaria parasite, affects mosquitoes, increasing their feeding frequency and enhancing its transmission to humans.

The scientific principles behind these manipulations include changes in neurotransmitter levels, gene expression, and immune signaling. For instance, parasites can alter dopamine or serotonin levels in the host's brain, directly influencing mood and behavior. Others can modify gene expression to affect hormonal systems, while some manipulate immune responses to alter neural activity, leading to behavioral changes.

Acetylcholine plays a critical role in insect neural communication. It helps coordinate movement and sensory perception, and its receptors, known as nAChRs, are vital for these functions. Nicotine, structurally similar to acetylcholine, can bind to these receptors, leading to excessive activation. While this makes nicotine an effective insecticide, prolonged exposure can disrupt the nervous systems of non-target insects, such as bees, potentially causing harmful effects.

Experiment 1

This experiment sought to explore the potential of microorganisms to modify and manipulate the natural behavior of bees. The focus was on assessing whether an infection by a specific microorganism could cause behavioral shifts, particularly by inducing a preference for malodorous water. Normally, bees avoid foul-smelling substances, as they are more inclined to seek out cleaner, sweeter sources of food and water. However, the introduction of the microorganism aimed to determine if it could override this natural aversion, leading the bees to exhibit an unusual attraction to unpleasant odors. The underlying hypothesis was that the microorganism would alter the bees' sensory perception or decision-making processes, thereby influencing their behavior.

By infecting the bees with the microorganism, the study sought to observe and document any changes in their typical foraging and drinking patterns. If the microorganism could successfully shift the bees' preference toward malodorous water, it would suggest that microbial infections have the capacity to disrupt natural behaviors and potentially lead to adverse effects on the health and functioning of the bee colony. Understanding these interactions is critical, as it can reveal broader implications for how pathogens might alter pollinator behavior, which in turn could impact pollination patterns and ecosystem stability.

The experiment focused on the Western honeybee (Apis mellifera), a species known for its essential role in pollination and ecosystem health. To investigate the effects of microbial infection on bee behavior, two distinct groups were established. The first was an experimental group, consisting of bees deliberately infected with a specific microorganism. This microorganism was known to induce changes in host behavior, including an increased attraction to malodorous water. The second group served as the control, comprising uninfected bees that had not been exposed to the microorganism, thereby maintaining their natural behavioral patterns.

Both groups were placed in an environment where they were given two choices: malodorous water, which contained unpleasant compounds typically avoided by healthy bees, and sugar water, a preferred substance that bees are naturally inclined to seek out due to its sweetness. By providing these two contrasting options, the experiment aimed to observe and compare the behavioral responses of the two groups. The goal was to determine whether the infected bees showed a significant deviation from their natural preference, indicating that the microorganism was capable of altering their typical foraging and drinking behavior.

Behavioral observations were conducted under controlled conditions, ensuring that external factors did not influence the bees' choices. This allowed for a direct assessment of the impact of the microorganism on the bees' decision-making processes. By comparing the selections made by the experimental and control groups, the study aimed to identify any shifts in behavior that could be attributed to the infection, providing insights into how pathogens might manipulate host behavior to their advantage.

Fig 1: Behavior experiment

The bees were deliberately infected with the microorganism over a 24-hour period, during which they were kept under controlled conditions to ensure consistent exposure. Following the infection phase, the bees were introduced to a test environment designed to evaluate their behavior. In this setting, two distinct water sources were provided: one containing malodorous water, which emitted unpleasant odors typically avoided by bees, and the other containing sugar water, a sweet and naturally appealing substance that bees are known to prefer.

To avoid any positional bias that might influence the bees' choices, the locations of the malodorous and sugar water were randomly assigned within the environment. This random placement ensured that the bees' behavior was not guided by any spatial preference, allowing researchers to focus solely on their reaction to the contents of each water source.

Throughout the experiment, researchers closely monitored the interactions between the bees and the two types of water. Detailed observations were made to track how many bees were drawn to each source, as well as the duration of time they spent at each. By recording both the frequency and length of visits, the study aimed to gather comprehensive data on the bees' preferences. This approach enabled a thorough analysis of any behavioral shifts, providing insights into whether the infection altered the bees' natural attraction, potentially increasing their interest in the malodorous water due to the effects of the microorganism.

Experiment 2

The experiment was designed to explore how Nosema infection could influence and potentially regulate bee behavior, with a particular focus on understanding the role of acetylcholine, a key neurotransmitter in the nervous system. Nosema is a parasitic microorganism that infects the digestive tracts of bees, often leading to noticeable changes in their activity levels, foraging patterns, and overall health. By infecting the bees with Nosema, the study aimed to induce behavioral changes that could be carefully monitored and analyzed to determine how this microorganism alters neural function.

A central aspect of the experiment was to investigate whether the administration of nicotine, via exposure to tobacco leaves, would further affect the behavior of bees infected with Nosema. Nicotine is known to interact with nicotinic acetylcholine receptors (nAChRs), which play a crucial role in neural signaling by binding to acetylcholine. Given nicotine's ability to modulate neural activity by mimicking acetylcholine, the researchers hypothesized that it could either enhance or mitigate the behavioral effects induced by Nosema infection. This interaction provided a unique opportunity to explore how external compounds, like nicotine, might influence neurotransmission in bees, especially under the stress of microbial infection.

To thoroughly assess these effects, the study compared the behaviors of two main groups of bees: those that were exposed to nicotine and those that were not. By observing changes in activity, foraging behavior, and overall responsiveness, the researchers aimed to determine if nicotine could alter the neural and behavioral impact of Nosema. This comparative approach allowed for a deeper understanding of the interaction between a microbial infection and external chemical agents, shedding light on the complex dynamics of neural regulation in bees.

Nicotine, a naturally occurring compound found in tobacco leaves, has a well-documented ability to interact with nicotinic acetylcholine receptors (nAChRs) in the nervous system of bees. These receptors are crucial for transmitting neural signals, as they bind to acetylcholine, a neurotransmitter responsible for regulating various physiological and behavioral processes. When nicotine binds to these receptors, it can mimic the action of acetylcholine, leading to changes in neural activity.

The experiment was based on the hypothesis that exposure to nicotine through tobacco leaves would modify acetylcholine neurotransmission in bees. This alteration in neural signaling was expected to have significant effects on the behavior of bees already infected with Nosema. Specifically, the study aimed to determine whether nicotine could mitigate the adverse behavioral changes associated with Nosema infection, such as reduced activity and impaired foraging, or if it would instead amplify these effects. The outcome would depend on how nicotine influenced the bees' neural pathways, either by enhancing acetylcholine-like activity or disrupting normal neurotransmission, thereby offering insights into the complex interplay between microbial infections and external chemical agents in shaping bee behavior.

The experiment was designed with three distinct groups to allow for a comprehensive analysis of how Nosema infection and nicotine exposure might interact to influence bee behavior. The first group, the control group, comprised bees that were infected with Nosema but were not exposed to any tobacco leaves. This setup allowed the researchers to observe the typical behavioral changes associated with Nosema infection without the interference of nicotine, providing a baseline for comparison.

Experimental Group 1 consisted of Nosema-infected bees that were also given a small amount of tobacco leaves. The purpose of this group was to determine whether nicotine, introduced via the tobacco leaves, would alter the behavioral effects typically seen in Nosema-infected bees. By observing this group, researchers could assess whether nicotine would mitigate the infection's negative impact on activity and foraging, or potentially exacerbate these effects.

Experimental Group 2, on the other hand, included bees that were not infected with Nosema but were provided with tobacco leaves. This group served as a control to understand the effects of nicotine alone, without the complicating factor of a microbial infection. By comparing the behavior of this group to both the control group and Experimental Group 1, the researchers could gain insights into the independent and combined impacts of nicotine and Nosema on the bees' neural function and behavior.

The experiment began by infecting the bees with Nosema, a microorganism that colonizes and reproduces within the digestive tract of bees. Nosema is known to have detrimental effects on the bees' overall health, primarily manifesting as reduced activity levels and increased fatigue. These changes in behavior are a direct result of the stress and energy drain caused by the infection, which impairs the bees' ability to forage and function effectively within their colony.

Once the bees were infected, a subset of these Nosema-infected bees, designated as Experimental Group 1, was exposed to a small quantity of tobacco leaves. This exposure was intended to introduce nicotine into their system, allowing researchers to observe how nicotine might interact with the bees' nervous system, specifically its potential to influence neurotransmission via acetylcholine. Nicotine is known to bind to nicotinic acetylcholine receptors (nAChRs), and the study aimed to see whether this interaction would alter the typical behavioral changes induced by Nosema.

The behavior of all groups, including the control group (infected bees without nicotine exposure) and Experimental Group 2 (uninfected bees exposed to nicotine), was closely monitored over a set period. Researchers focused on several key indicators: activity levels, foraging behavior, and flight capability. By comparing these behavioral metrics across the different groups, the study aimed to determine whether nicotine could counteract or enhance the effects of Nosema infection, offering new insights into how external chemical agents and microbial infections jointly influence bee behavior.

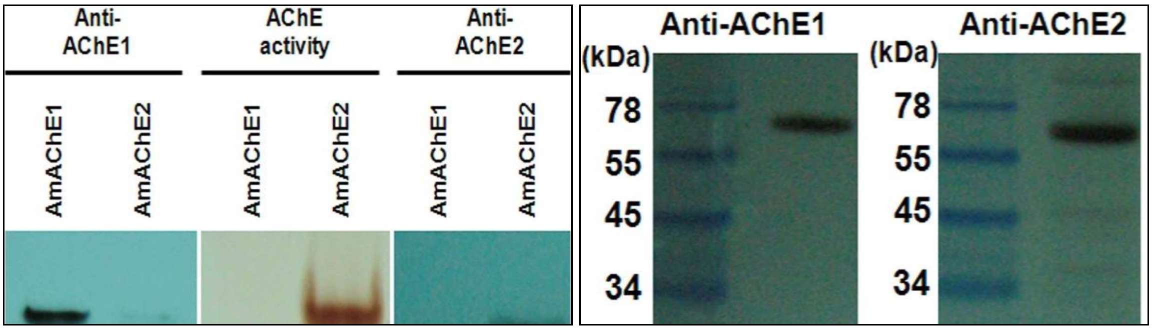


Fig 2: Measurement of Acetylcholinesterase Activity

To assess the expression of acetylcholine receptors (AChRs) in the bees' nervous tissues, the experiment utilized immunohistochemistry (IHC), a technique that allows for the precise visualization of specific proteins within tissue samples. Immunohistochemistry involves the use of antibodies that specifically bind to AChRs, enabling researchers to detect and analyze the presence and distribution of these receptors at a cellular level.

In this experiment, tissue samples from the bees' nervous systems were prepared and treated with antibodies that target acetylcholine receptors, particularly nicotinic acetylcholine receptors (nAChRs). When these antibodies bind to the receptors, they can be visualized using a marker, such as a fluorescent dye or an enzyme that produces a color reaction. This process allowed researchers to observe and measure the levels of AChR expression in the neurons.

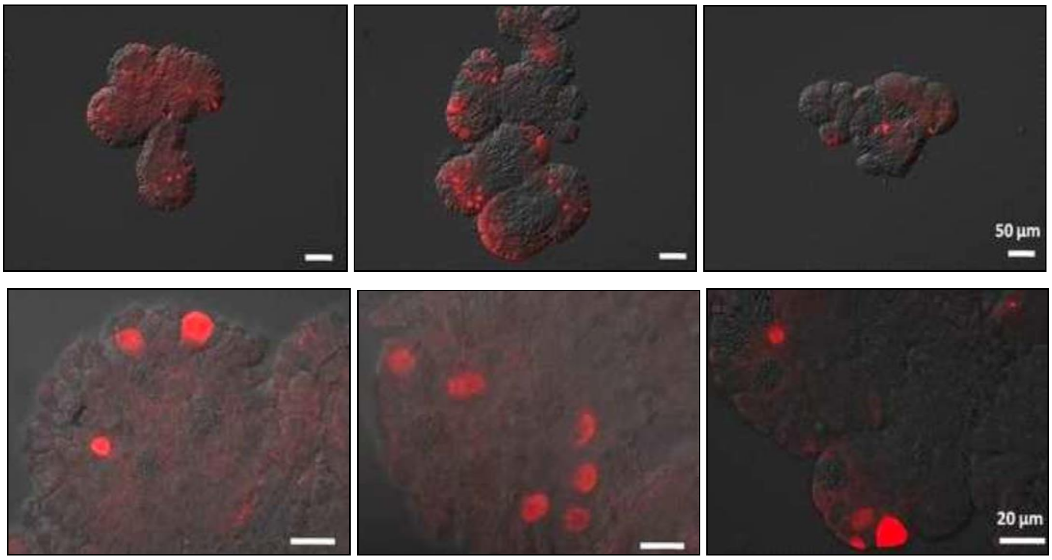
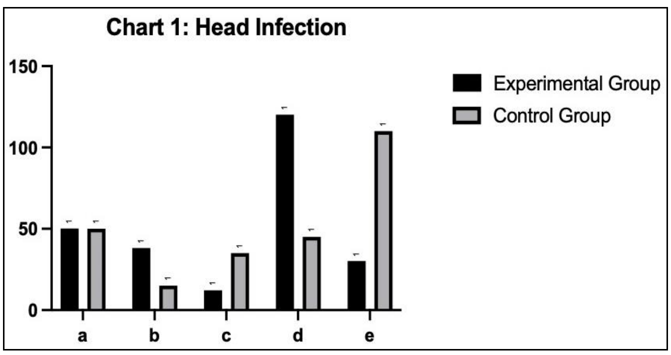
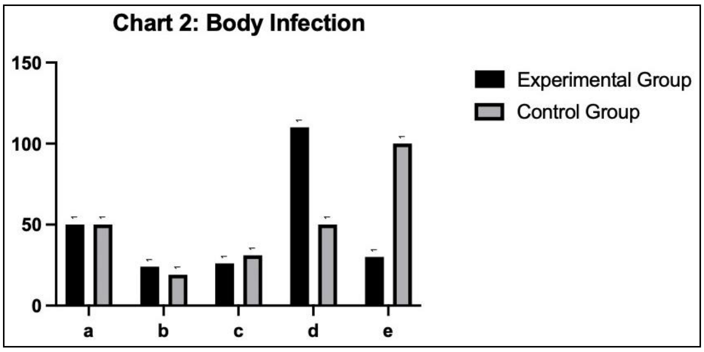


Fig 3: Expression of Acetylcholine Receptors

Results



	Total Number of Bees	Bees Near Malodorous Water	Bees Near Sugar Water	Average Time Spent Near Malodorous Water (seconds)	Average Time Spent Near Sugar Water (seconds)
Experimental Group	50	38/50	12/50	120±15 (sec)	30±10 (sec)
Control Group	50	15/50	35/50	45±10 (sec)	110±20 (sec)

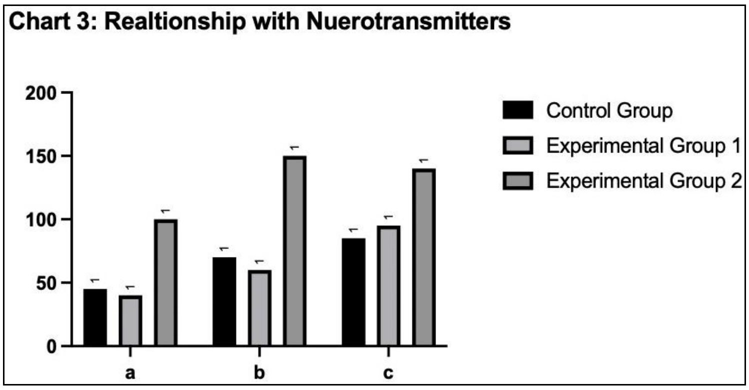


	Total Number of Bees	Bees Near Malodorous Water	Bees Near Sugar Water	Average Time Spent Near Malodorous Water (seconds)	Average Time Spent Near Sugar Water (seconds)
Experimental Group	50	24/50	26/50	110±15	30±10
Control Group	50	19/50	31/50	50±10	100±20

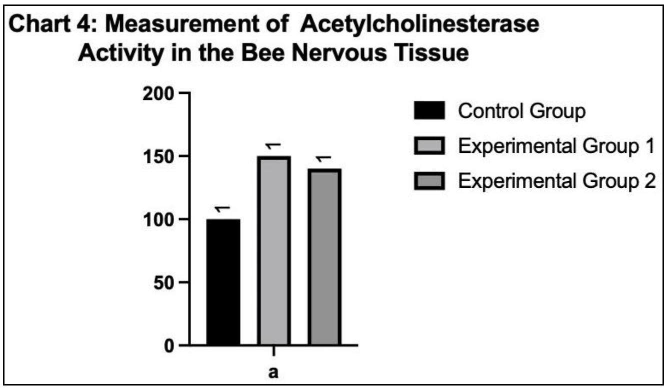
A detailed statistical analysis was conducted to determine whether bees infected with the microorganism exhibited a higher propensity to select malodorous water or spent more time near it compared to uninfected bees in the control group. The analysis aimed to uncover patterns in behavior that could suggest how the infection influenced the bees' choices. The results were clear: the experimental group, consisting of bees infected with the microorganism, displayed a significantly greater preference for malodorous water. Specifically, 76% of the infected bees chose the malodorous water, in contrast to just 30% of the bees in the control group. This substantial difference points to a direct impact of the microorganism on the bees' behavior, prompting a shift in their natural preferences and causing them to be more drawn to water sources they would typically avoid. This behavioral change provides evidence that the infection led to a modification in sensory or neural processing, causing infected bees to favor malodorous water over the more naturally attractive sugar water.

The study also analyzed how long the bees spent near each type of water, offering further insights into the effects of the microorganism. Bees in the experimental group tended to linger near the malodorous water for much longer periods, with an average duration of 120 seconds. In comparison, the control group bees, which were uninfected, spent only about 45 seconds near the same water source. This stark contrast in time spent suggests that the infection may have altered the bees' perception or behavior, making them more tolerant of or even attracted to the malodorous scent. Conversely, the infected bees spent significantly less time near the sugar water, averaging just 30 seconds, compared to their uninfected counterparts. This shift in behavior further underscores the influence of the microorganism, which appears to modify the bees' usual attraction to sweet, sugary substances, redirecting their interest toward more unpleasant scents.

Additionally, the researchers observed notable variations in behavior based on the specific areas of the body affected by the infection. Bees that had infections localized in the head region exhibited more pronounced abnormal behavior compared to those with infections in the thorax. This observation led to the hypothesis that the bacteria may have directly influenced neural functions, potentially by affecting the secretion and regulation of neurotransmitters. The presence of the microorganism in the head could have a direct effect on the bees' nervous system, altering the way signals are processed and leading to significant changes in behavior. This supports the idea that the bacteria were not merely affecting physical health but were actively manipulating neural pathways, potentially to enhance the microorganism's survival and transmission.



	Activity Level (Flight Distance, Mobility)	Survival Rate	Acetylcholine Receptor Expression
Control Group (Infected with Nosema, No Tobacco Leaves)	45%	40%	100%
Experimental Group 1 (Infected with Nosema, Given Tobacco Leaves)	70%	60%	150%
Experimental Group 2 (Not Infected with Nosema, Given Tobacco Leaves)	85%	95%	140%



	Acetylcholinesterase Activity (%)
Control Group (Infected with Nosema, No Tobacco Leaves)	100
Experimental Group 1 (Infected with Nosema, Given Tobacco Leaves)	150
Experimental Group 2 (Not Infected with Nosema, Given Tobacco Leaves)	140

Discussion & conclusion

Nicotine, a naturally occurring alkaloid found in tobacco plants, has the capacity to stimulate the nervous system of bees by binding to and activating nicotinic acetylcholine receptors (nAChRs). These receptors play a crucial role in transmitting neural signals, and their activation can lead to a temporary increase in activity and heightened survival rates in bees. When nicotine binds to these receptors, it mimics the action of the neurotransmitter acetylcholine, triggering a cascade of neural responses that enhance the bees' alertness, mobility, and overall responsiveness. This stimulatory effect can make bees more active and improve their short-term behavior, which is particularly evident in behaviors such as foraging and flight.

However, the benefits of nicotine are not without potential drawbacks. While short-term exposure may enhance the bees' behavior, prolonged or excessive activation of acetylcholine receptors can lead to neural overstimulation, commonly referred to as neural overload. This overstimulation can disrupt normal neural communication, potentially leading to adverse effects on the bees' nervous system, including reduced neural efficiency, desensitization of receptors, and even neural fatigue. The results from the experiment confirmed that exposure to nicotine through tobacco leaves had a positive, short-term impact on the bees' neural activity, stimulating their behavior and boosting their immediate responsiveness. However, these short-term gains must be weighed against potential long-term risks, and further research is essential to fully understand how extended nicotine exposure might affect the overall health, behavior, and neural stability of bees.

The experiment also investigated the effects of nicotine on bees infected with Nosema, a parasitic microorganism that impairs the bees' immune function and activity. When nicotine was administered to these infected bees, it temporarily stimulated their nervous system, leading to a noticeable improvement in their immune response and a partial recovery in their activity levels. This suggested that nicotine could potentially counteract some of the negative effects of Nosema infection by boosting the bees' neural and immune functions, at least in the short term. However, this beneficial effect appeared to be temporary, and there are concerns that prolonged exposure to nicotine might lead to adverse outcomes, such as neural fatigue and further weakening of the immune system. If nicotine causes the bees' neural pathways to remain overstimulated for extended periods, it could exhaust their nervous system, reduce their ability to recover, and ultimately worsen the effects of the Nosema infection. These findings underscore the need for caution when considering nicotine as a treatment option for infected bees, as the long-term risks may outweigh the immediate benefits.

Central to understanding the effects of nicotine on bees is the role of acetylcholine, a key neurotransmitter responsible for regulating a range of neural functions, including movement, sensory perception, and behavior. Acetylcholine plays a critical role in maintaining normal neural function in bees, and its proper regulation is essential for their survival and effective behavior. The experiment demonstrated that when nicotine activates acetylcholine receptors, there can be significant changes in the bees' neural responses, leading to altered behaviors. This indicates that acetylcholine is a vital regulator within the bee nervous system, and any disruption to its normal function—whether through overstimulation by nicotine or other factors—can have profound effects on the bees' behavior and health. The findings highlight the importance of understanding how external substances like nicotine interact with neurotransmitter systems, as this interaction can significantly alter neural communication and behavioral outcomes in bees. Further studies could help clarify these effects and inform approaches to managing the health and behavior of bee populations.

This study provides valuable data for understanding how neurotransmitters are involved in regulating the behavior and immune responses of bees. The findings highlight the critical role of acetylcholine receptors in controlling bee behavior, offering key insights that could be used for better management and protection of bee populations.

The study is also relevant to research on insecticides, as nicotinic acetylcholine receptors (nAChRs) are important targets in the development of insecticides. The results contribute to the analysis of how nicotinebased insecticides impact the nervous system of bees, providing useful data to assess the potential longterm risks of neural damage caused by these substances. This research could help in evaluating the safety of insecticides and their long-term effects on bee health.

Additionally, the study has implications for agricultural and ecosystem management. Bees play a vital role in agricultural ecosystems, and their health directly affects food production. The findings offer insights into ways to improve the health and behavior of bees, particularly by suggesting new strategies to mitigate the impact of infectious diseases on bee colonies. This could lead to the development of practices that help maintain robust bee populations, which are essential for sustaining crop pollination and agricultural productivity.

Future research can build on this study to further explore the effects of nicotine on the nervous and immune systems of bees. The data collected here serve as a foundation for examining potential long-term neural damage and immune responses and could be expanded through follow-up studies. In particular, more detailed analyses of behavioral patterns and survival rates could lead to practical strategies for ecosystem protection. By gaining a deeper understanding of how nicotine affects the neural functions and behaviors of bees, this research provides a basis for developing more effective methods for bee management and conservation.

References

1. Veits M, *et al*. Flowers respond to pollinator sound within minutes by increasing nectar sugar concentration. Ecol Lett,2019:22(9):1483–1492. doi: 10.1111/ele.13331.
2. Dodd ME, Silvertown J, Chase MW. Phylogenetic Analysis of Trait Evolution and Species Diversity Variation among Angiosperm Families. Evolution,1999:53(3):732. doi: 10.2307/2640713.
3. Kearns CA, Inouye DW, Waser NM. ENDANGERED MUTUALISMS: The Conservation of Plant-Pollinator Interactions. Annu Rev Ecol Syst,1998:29(1):83–112. doi: 10.1146/annurev.ecolsys.29.1.83.
4. Westerkamp C, Gottsberger G. Diversity Pays in Crop Pollination. Crop Sci. 2000;40(5):1209–22. doi: 10.2135/cropsci2000.4051209x.
5. Klein AM, *et al*. Importance of pollinators in changing landscapes for world crops. Proc R Soc B Biol Sci,2007:274(1608):303–313. doi: 10.1098/rspb.2006.3721.
6. The Potential Consequences of Pollinator Declines on the Conservation of Biodiversity and Stability of Food Crop Yields. Conserv Biol,2008:12(1):8–17. doi: 10.1111/j.1523-1739.1998.97154.x.
7. Vanengelsdorp D, *et al*. Colony Collapse Disorder: A Descriptive Study. PLoS ONE, 2009, 4(8). doi: 10.1371/journal.pone.0006481.
8. Steinhauer NA, *et al*. A national survey of managed honey bee 2012–2013 annual colony losses in the USA: results from the Bee Informed Partnership. J Apic Res,2014:53(1):1–18. doi: 10.3896/IBRA.1.53.1.01.
9. Hamblin AL, Youngsteadt E, Frank SD. Wild bee abundance declines with urban warming, regardless of floral density. Urban Ecosyst,2018:21(3):419–428. doi: 10.1007/s11252-018-0731-4.
10. Stout JC, Morales CL. Ecological impacts of invasive alien species on bees. Apidologie,2009:40(3):388–409. doi: 10.1051/apido/2009023.
11. Abay Z, Bezabeh A, Gela A, Tassew A. Evaluating the Impact of Commonly Used Pesticides on Honeybees (*Apis mellifera*) in North Gonder of Amhara Region, Ethiopia. J Toxicol,2023:2023:1–13. doi: 10.1155/2023/2634158.
12. Honey Bee Nutrition, 2010, August. Available from: http://nashbee.org/wp-content/uploads/Honey-Bee-Nutrition-by-Zachary-Huang.pdf.
13. Southwick EE, Loper GM, Sadwick SE. Nectar production, composition, energetics and pollinator attractiveness in spring flowers of western New York. Am J Bot. 1981;68(7):994–1002. doi: 10.1002/j.1537-2197.1981.tb07816.x.
14. Appel HM, Cocroft RB. Plants respond to leaf vibrations caused by insect herbivore chewing. Oecologia,2014:175(4):1257–1266. doi: 10.1007/s00442-014-2995-6.
15. Hamant O, Haswell ES. Life behind the wall: sensing mechanical cues in plants. BMC Biol,2017:15(1):59. doi: 10.1186/s12915-017-0403-5.
16. De Luca PA, Vallejo-Marín M. What's the 'buzz' about? The ecology and evolutionary significance of buzz-pollination. Curr Opin Plant Biol,2013:16(4):429–35. doi: 10.1016/j.pbi.2013.05.002.
17. Esch H, Wilson D. The sounds produced by flies and bees. Z für Vgl Physiol,1967:54(2):256–267. doi: 10.1007/BF00298031.