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Review

A potential link among biogenic amines-based pesticides, learning and memory, and colony collapse disorder: A unique hypothesis

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ABSTRACT

Pesticides are substances that have been widely used throughout the world to kill, repel, or control organisms such as certain forms of plants or animals considered as pests. Depending on their type, dose, and persistence in the environment, they can have impact even on non-target species such as beneficial insects (honeybees) in different ways, including reduction in their survival rate and interference with their reproduction process. Honeybee *Apis mellifera* is a major pollinator and has substantial economical and ecological values. Colony collapse disorder (CCD) is a mysterious phenomenon in which adult honeybee workers suddenly abandon from their hives, leaving behind food, brood, and queen. It is lately drawing a lot of attention due to pollination crisis as well as global agriculture and medical demands. If the problem of CCD is not resolved soon enough, this could have a major impact on food industry affecting world's economy a big time. Causes of CCD are not known. In this overview, I discuss CCD, biogenic amines-based-pesticides (neonicotinoids and formamidines), and their disruptive effects on biogenic amine signaling causing olfactory dysfunction in honeybees. According to my hypothesis, chronic exposure of biogenic amines-based-pesticides to honeybee foragers in hives and agricultural fields can disrupt neural cholinergic and octopaminergic signaling. Abnormality in biogenic amines-mediated neuronal signaling impairs their olfactory learning and memory, therefore foragers do not return to their hive – a possible cause of CCD. This overview is an attempt to discuss a hypothetical link among biogenic amines-based pesticides, olfactory learning and memory, and CCD.

1. Introduction

Honeybees belong to the insect order Hymenoptera. The Western (European) honeybee *Apis mellifera* (genus *Apis* and specie *mellifera*) is the most commonly adaptable species and best known among all insects. Worker honeybees need to forage for a wide diversity of pollen and nectar to raise a healthy brood in the hive and maintain strong immune systems. The clustering behavior and ability to regulate the temperature within the hive irrespective of the external temperature enable honeybees to colonize through a wide variety of environments. Not all insect-dependent pollination is provided by honeybees *A. mellifera*, but the ability to easily move and manage makes them the most economically valuable pollinator of agricultural crops worldwide (vanEngelsdorp et al., 2008; Gallai et al., 2009; vanEngelsdorp and Meixner, 2010). Moreover, honeybee products such as honey, propolis, royal jelly, bee wax, and bee venom offer potential therapeutic importance (Cooper, 2007; Farooqui and Farooqui, 2010, 2011, 2012).

Colony collapse disorder (CCD) is characterized by the rapid decline of the adult bee population, leaving the brood and the queen poorly or completely unattended in the hive. Without the adult bees, colonies eventually collapse because there is no one to provide food and maintain the hive. Several reports have been published on CCD (Cox-Foster et al., 2007; vanEngelsdorp et al., 2007, 2008, 2009; vanEngelsdorp and Meixner, 2010; Bromenshenk, 2010; Bromenshenk et al., 2010; Biesmeijer et al., 2006; Oldroyd, 2007; Palacios et al., 2008; Blanchard et al., 2008; Johnson et al., 2009a; Highfield et al., 2009; VanEngelsdorp et al., 2010; Mullin et al., 2010; Williams et al., 2010; Ratnieks and Carreck, 2010; Wu et al., 2011; Core et al., 2012; Henry et al., 2012; Belzunces et al., 2012; Pareja et al., 2011; Di Prisco et al., 2011; Nazzi et al., 2012). Researchers have been struggling for years to explain CCD, but it remains unknown whether CCD is governed by one specific factor or due to the synergistic action of several factors. If the cause of CCD is not resolved soon enough then this could have a major impact on the world's economy.

My hypothesis is that the chronic exposure (low-dose, overtime) of biogenic amines-based-pesticides (neonicotinoids and formamidines) to honeybees disrupts neuronal cholinergic and octopaminergic signaling and produces excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS) that react with macromolecules and interfere with mitochondrial respiratory chain and mitochondrial Ca^{2+} metabolism, contributing to a deficiency of

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neuronal energy. Oxidative stress impairs cognitive behavior, including olfactory learning and memory, affecting orientation and navigation abilities, therefore, honeybees fail to return to their hives. In this review, I discuss CCD, biogenic amines, biogenic amines-based pesticides and signaling, and a hypothetical interrelated link among pesticides, olfactory learning and memory and CCD.

2. CCD

The term CCD was first introduced to the occurrence of severe abrupt losses in European (Western) worker honeybee *A. mellifera* colonies in North America in late 2006. Since then several substantial drops in managed population of Western honeybee colonies have been noticed throughout North America, in several European countries, portions of Canada, Middle East and Japan. In CCD the remaining honeybee population in hive consists of a queen, sudden inexplicable reduction in workers, and the presence of capped brood with plenty of food stores (honey and pollen) left in colonies. This suggests that major loss of adult workers from hive is not due to the lack of food. One of the oddest of symptoms of CCD is the lack of dead bees of workers being found in or around the hive, speculating that death takes place in the field. Large-scale losses of honeybees are not unusual. In past (1960–1970s), honeybee population has been wiped out due to extreme weather, pesticide exposure, and pest infestation (Oertel, 1965; Foote, 1966; Williams and Kauffeld, 1974; Kauffeld et al., 1976; Olley, 1976; Roberge, 1978). However, CCD differs from past colony losses because it is rapid, more severe, and results in complete loss of foragers with few or no dead bees in or around the hive (Table 1).

2.1. Theories of CCD

Several theories have been suggested for CCD. These theories include infection caused by a microsporidium parasite *Nosema ceranae* or *Nosema apis* (Cox-Foster et al., 2007; Higes et al., 2007, 2008a,b, 2009); varroa mites-*Varroa destructor* injuring both adult honeybees and brood by mite–virus association synergistically reducing host immunity and inducing virus replication (Boecking and Genersch, 2008; Bacandritsos et al., 2010; de Miranda et al., 2010; Di Prisco et al., 2011); Israeli acute paralysis virus (IAPV) and Deformed wing virus (DWV), most often transmitted by the ectoparasitic *V. destructor* (Cox-Foster et al., 2007; Yue et al., 2007; Blanchard et al., 2008); infectious disease of honeybee larvae 'chalkbrood disease' caused by the fungus *Ascosphaera apis*, affecting the overall health of bee larvae by rapidly decreasing the rate of larval feeding and substantially decreasing the expression of storage proteins, as well as affecting the differential expression of genes associated with stress-related cellular processes and immune responses, leading to gradual deterioration of the colony (Aronstein et al., 2010); honeybees parasitized by the phorid fly *Apocephalus borealis* show symptoms, such as disorientation and loss of equilibrium, could be a new threat to honeybees (Core et al., 2012); the widespread development of genetically modified (GM) crops in which herbicide resistance, pesticide resistance, and insect-killing genes are expressed, which may produce sublethal effects on honeybees (Huang et al., 2004; O'Callaghan et al., 2005; Duan et al., 2008; Eischen and Graham; 2008); electromagnetic radiation from mobile phones may be responsible for affecting the foraging behavior of honeybees (Hsu et al., 2007); nutritional stress produced on honeybees during migratory commercial beekeeping due to their use in the pollination on monocrops therefore they lack a natural defense system (Sharpe and Heyden, 2009; Spivak et al., 2011; Alaux et al., 2010b); transportation stress caused by low temperature brood rearing (Medrzycki et al., 2010); lack of genetic diversity due to mating with a single male degrades the quality of queen bee by producing offsprings of low genetic quality and such colonies provide no colonization resistance to pathogens (Mattila et al., 2012); sensitivity to numerous pesticides, disrupting several honeybee behaviors such as feeding, learning performance, orientation, and navigation (Thompson, 2003; Decourtye et al., 2003; Rortais et al., 2005; Desneux et al., 2007; Girolami et al., 2009; Maini et al., 2010; Henry et al., 2012; Belzunces et al., 2012); and combinational theory in which synergistic interactions among multiple biological, environmental, and chemical factors could derive CCD (Johnson et al., 2009a,b; Ratnieks and Carreck, 2010; Bromenshenk et al., 2010; Mullin et al., 2010; Alaux et al., 2010a; Wu et al., 2011; Spivak et al., 2011; Pettis et al., 2012; Nazzi et al., 2012; Lu et al., 2012).

The existence of high frequency of IAPV in asymptomatic hives in samples from Argentine provinces suggests that IAPV is not a good marker for CCD (Reynaldi et al., 2011). Co-infection with two pathogens (invertebrate iridovirus iridescent virus, IIV; and *N. ceranae*) was more lethal to honeybees than either pathogen alone, suggesting that such association could be critical to CCD (Bromenshenk et al., 2010). However, the validity of this relationship still remains obscure due to lack of evidence for the presence of an Iridovirus in healthy or CCD colonies in the USA and Israel (Tokarz et al., 2011; Foster, 2012). Also all parasites and pathogens could contribute to colony decline, but none seems to be the sole cause of CCD. Mixed fungal infections in honeybee brood, such as chalkbrood caused by the fungus *A. apis* and a pollen fungus *A. atra*, could enhance honeybee mortality compared to single infection (Vojvodic et al., 2012). The theory of short telomeres and premature aging, based on exhausted telomere reserves causing immune suppression leading to the death of foragers, needs further investigation (Stindl and Stindl, 2010). No conclusive data are available on other theories (GM crops, queen bee quality, electromagnetic radiation, and nutritional stress. A variety of unusual ribosomal RNA fragments abundantly found in guts of CCD bees could be linked with picorna-like viral infection, leading to arrested translation (Johnson et al., 2009a). Sublethal doses of miticides (coumaphos,

Table 1
Comparison of symptoms in large-scale colony losses between past and present.

Past Colony Losses	CCD
-Loss of 10–30% colonies	-Loss of 50 to 90%
-Dead bees in or around the hive from bee diseases	-Very few dead bees in or around the hive
	-Sudden disappearance of worker honeybees
	-Queen is healthy and still laying eggs
	-Only a few young bees survive
	-Presence of adequate food and capped brood in colonies
-Infection can be controlled with a specific antibiotic, anti-fungus and other chemicals	-No outward signs of infection, disease, pests, or parasites exist
-Multifactorial syndrome	-Multifactorial syndrome
-Inflammation may be a potential molecular mechanism involved	-ROS-induced oxidative stress may be a potential molecular mechanism involved