REVIEW PAPER

Neuroactive nectar: compounds in nectar that interact with neurons

Julie A. Mustard[1] ⓘ

Abstract

As well as sugars to entice pollinators, nectar contains many other chemicals including amino acids and plant secondary compounds such as phenolics, alkaloids, and glycosides. Rather than simply the byproducts of plant metabolism or contamination by compounds meant to deter herbivory, it is clear that these chemicals may have important roles in nectar. Proposed functions of non-sugar components of nectar include pollinator nutrition, reducing nectar robbing, and defense against microbes. Additionally, some of these compounds are able to interact directly with the nervous system via binding to receptor proteins found on the surface of neurons. Thus, these neuroactive components of nectar may be able to manipulate pollinator behavior. To increase our ability to analyze the many functions of nectar, it is important to understand how specific components may interact with neurons. This review examines the neurotransmitter receptors that are targets of some of the chemicals present in nectar. Although these compounds also affect the nervous systems of vertebrates, the focus of this review is on the interactions between nectar and insect pollinators.

Keywords Neurotransmitter · Nectar · Pollinator · Caffeine · Nicotine · Bee

Introduction

Nectar is a complex mixture that may contain sugars, amino acids, and secondary compounds such as alkaloids, phenolics, and glycosides (Stevenson et al. 2017). Some of the compounds found in nectar are bitter tasting or may be toxic, effects that appear to be at odds with the function of nectar as an attractive reward for pollinators (Adler 2001). This apparent paradox has led to multiple hypotheses about the functions of different compounds. Nectar components may influence pollinator behavior, affect the microbial community both in the plant and in the insect gut, and/or impact pollinator parasites (Nepi et al. 2018; Stevenson et al. 2017). In addition, some compounds may act to deter nectar robbers, select for specific pollinators, or enhance the movement of pollen between flowers (Irwin et al. 2010; Jacobsen and Raguso 2018; Kessler and Baldwin 2007). Given that nectar components belong to many different classes of chemicals,

it is not unexpected that there would be specific functions played by distinct compounds. Several recent reviews provide excellent discussions of the variety of compounds discovered so far and some of their proposed functions in nectar (Adler 2001; Irwin et al. 2010; Jacobsen and Raguso 2018; Nepi et al. 2018; Stevenson et al. 2017).

This review focuses on compounds found in nectar that can directly interact with the nervous system and discusses the underlying mechanisms via which they may affect the behavior of insect pollinators (Table 1). Three of these compounds, gamma-aminobutyric acid (GABA), glutamate, and glycine, act as neurotransmitters in both insects and vertebrates. The other compounds discussed are not neurotransmitters themselves, but are known to interact with receptor proteins found on neurons. The receptors used for intercellular signaling in the nervous system are of two different types (Fig. 1). The ionotropic receptors are ion channels that allow the flow of ions across the membrane down the ion's electrochemical gradient. Binding of a neurotransmitter to the channel increases the probability of channel opening. Movement of Na^+ or Ca^{2+} through a channel works to depolarize the membrane potential of the neuron making it more likely to generate an action potential, thereby producing an excitatory effect, whereas the flow of K^+ or Cl^- through a channel hyperpolarizes the neuron decreasing

✉ Julie A. Mustard
  julie.mustard@utrgv.edu

[1] Department of Biology, University of Texas Rio Grande Valley, Brownsville, TX 78520, USA

Table 1 The targets and concentration of neuroactive compounds found in nectar

| Compound (Function)	Target	Concentration in nectar
GABA (Neurotransmitter)	GABA$_A$ receptor (inhibitory ion channel) GABA$_B$ receptor (GPCR)	0.57 to 750 μM
Taurine (Agonist)	GABA$_A$ receptor (inhibitory ion channel)	90 to 325 mM
β-Alanine (Agonist)	GABA$_A$ receptor (inhibitory ion channel)	up to 200 mM
Glutamate (Neurotransmitter)	mGlu receptor (GPCR) GluR receptors (excitatory ion channels) GluCl receptors (inhibitory ion channels)	up to 0.5 mM
Glycine (Neurotransmitter)	GlyR (inhibitory ion channel)	up to 1 mM
Nicotine (Agonist)	nAChR (excitatory ion channel)	1 to 20 μM
Scopolamine (Antagonist)	muscarinic acetylcholine receptor (GPCR)	9 μM to 1.3 mM
Caffeine (Multiple actions)	AdoR (adenosine receptor antagonist) RyR (ryanodine receptor—increases cytosolic Ca^{2+}) Phosphodiesterase inhibitor (increases cytosolic cAMP)	1 to 100 μM
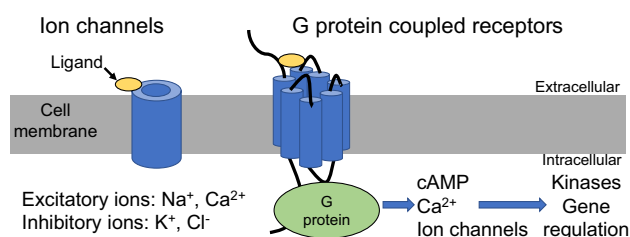
Fig. 1 The two main types of neurotransmitter receptors. A molecule (a ligand) can bind to a receptor and either activate it (an agonist) or block activation (an antagonist). Binding of an agonist to ion channels allows ions to flow across the membrane. The flow of Na$^+$ or Ca^{2+} works to depolarize the membrane, whereas passage of K$^+$ or Cl$^-$ hyperpolarizes the membrane. Ion channels only allow specific ions across. Binding of an agonist to a G protein-coupled receptor leads to the activation of the G protein which then disassociates from the receptor and goes on to regulate the activity of other proteins. There are several different kinds of G proteins, so the downstream events depend on the type of G protein that binds to the receptor

the probability of it firing, resulting in an inhibitory effect. The second main class of receptors are G protein-coupled receptors (GPCRs) also called metabotropic receptors. Binding of a ligand to the receptor leads to the activation of G protein which then disassociates from the receptor and interacts with other proteins. Activated G proteins have functions such as binding to ion channels affecting their likelihood of opening or regulating the activity of enzymes leading to changes in the levels of intracellular signaling molecules such as cAMP and Ca^{2+}. These molecules then go on to regulate the activity of other proteins, which can lead to

changes in gene expression, modify ion channel activity, and affect protein function. Thus, compounds that can activate or inhibit GPCRs can have a profound effect on pollinator behavior in both the short and the long term.

Localizing neurotransmitters to specific neurons and circuits in the nervous system can provide insight into the neurotransmitters' functions. For example, the presence of specific transmitters or receptors in the antennal lobes suggest that the transmitter plays an important role in olfaction, while those located in the optic lobes are involved in vision. Two important brain regions believed to regulate higher-order functions in insects are the mushroom bodies and the central complex. The mushroom bodies are made up of Kenyon cells which receive and integrate sensory information and are important for learning and memory (Heisenberg 1998; Menzel 2001). The central complex is involved in locomotion, place learning, and sun compass orientation (Pfeiffer and Homberg 2014; Strauss 2002). Amongst insect pollinators, the honey bee, *Apis mellifera*, and the sphinx moth, *Manduca sexta*, have the most well-characterized neuroanatomy and will mainly be used as examples.

Targets of neuroactive compounds found in nectar

Actions at gamma-aminobutyric acid (GABA) receptors

GABA is an amino acid synthesized from the amino acid glutamate, although it is not incorporated into proteins.

GABA is the most abundant inhibitory neurotransmitter in both vertebrates and invertebrates. In the brain of the honey bee and the sphinx moth, neurons containing GABA (GABAergic neurons) are found in the antennal lobes, optic lobes, mushroom bodies, central complex, and the subesophageal ganglion (Bicker 1999; Homberg et al. 1987; Schäfer and Bicker 1986). In honey bees, GABA within the antennal lobes is important for olfactory processing (Choudhary et al. 2012; Dupuis et al. 2010; Sachse and Galizia 2002), and GABA signaling in the antennal lobes and mushroom bodies is important for olfactory learning (Raccuglia and Mueller 2013). In addition, GABA is found at the insect neuromuscular junction where it acts on leg and body wall muscles (Wolf 2014) and has been shown to be involved in motor function (Mustard et al. 2020). In both vertebrates and insects, GABA acts at two different types of receptors: inhibitory ion channels, known as $GABA_A$ receptors, and $GABA_B$ type receptors, which are GPCRs. There are two or three distinct $GABA_A$ receptors, encoded by different genes, in the insects examined so far (Jones 2018). The $GABA_A$-type RDL receptor is the target of the pesticides dieldrin and fipronil, (see Ffrench-Constant et al. 2016 for a recent review). Thus, GABA-containing nectar may produce distinct effects on pollinators exposed to these pesticides. For example, analysis of the dieldrin resistant allele of the RDL receptor suggests that dieldrin and fipronil bind preferentially to the desensitized conformation of the receptor (Le Goff et al. 2005; Zhang et al. 1994). There are several different mechanisms that can lead to desensitization, but the outcome is the inactivation of a receptor even though it is bound to an agonist. Desensitization prevents the receptor from continuous signaling. Pollinators exposed to GABA or other $GABA_A$ receptor agonists in nectar may have more of their receptors in the desensitized state and thus may be more sensitive to pesticides that bind to the desensitized conformation of the receptor. The possible interaction between neuroactive components of nectar and pesticides should be considered when evaluating the impact of pesticides on pollinators.

GABA appears to be a fairly common component of nectar and has been quantified in a number of plant species including *Nicotiana* and more than 60% of plant species tested in a Mediterranean scrubland plant community (Petanidou et al. 2006; Kaczorowski et al. 2005; Nepi 2014). When present, GABA nectar concentrations ranged from 0.57 to 750 µM (Kaczorowski et al. 2005; Nepi 2014; Petanidou et al. 2006). In a preference test, freely foraging honey bees avoided consuming GABA, although this was at concentrations (22 mM or greater) higher than those found in nectar (Inouye and Waller 1984). Chronic consumption of GABA by caged bees did not affect the survival of honey bees, but increased the survival of the bumble bee *Bombus terrestris,* although the effect was not significant

at concentrations in the natural range (Bogo et al. 2019). Furthermore, the presence of GABA did not affect the amount of food consumed by either honey bees or bumble bees (Bogo et al. 2019). In behavioral assays examining time spent walking, feeding, flying, or still, GABA in its natural range reduced flying in bumble bees and time spent feeding in honey bees (Bogo et al. 2019). Given its prevalence in nectar and the potential for GABA to influence pollinator behavior, very little has been done to characterize its effects.

In addition to GABA itself, two other non-protein amino acids found in nectar, taurine and beta-alanine, activate $GABA_A$ receptors. Taurine is relatively abundant in insect tissues, and is found in the mushroom bodies, and in regions associated with sensory processing, such as photoreceptors and other neurons in the visual system, the antennal lobes, and neurons in feeding structures (Schäfer et al. 1988; Bicker 1991). Taurine acts as an agonist at $GABA_A$ receptors (Palmer and Harvey 2014; Whitton et al. 1994; Wafford and Sattelle 1986) and would be expected to increase inhibition in a similar way to GABA. Indeed, in *Drosophila*, taurine reduces locomotion and increases the amount of time the flies sleep (Lin et al. 2010); however, relatively little is known about its functions in insects. Taurine has been found in the nectar of a wide variety of plants including multiple members of the genus *Fritillaria* which contained concentrations up to 106 mM (Roguz et al. 2019). The Mediterranean herb *Cerinthe major* had nectar concentrations of 325 mM (Nocentini et al. 2012), and approximately 50% of a sample of 30 different species growing wild in the United Kingdom contained up to 90 mM taurine in their nectar (Gardener and Gillman 2001). Addition of taurine in a range of 14 to 257 mM to sucrose solutions did not produce either preference or deter feeding of *Apis mellifera* (Inouye and Waller 1984).

Beta-Alanine is found at relatively high concentrations in the insect visual system where it may play an important role in the recycling of histamine, the neurotransmitter of the insect visual system (Borycz et al. 2012). More generally, the enzyme encoded by the *ebony* gene uses beta-alanine as a substrate for the inactivation of biogenic amine neurotransmitters including dopamine, tyramine, octopamine, serotonin, and histamine (Richardt et al. 2003). The biogenic amines are involved in many behaviors in the honey bee that may impact foraging such as learning and memory (Hammer and Menzel 1998), foraging behavior (Schulz and Robinson, 2001), sucrose responsiveness (Scheiner et al. 2002), olfactory processing (Rein et al. 2013), and conditioned taste aversion (Wright et al. 2010); therefore, any compound that affects their levels may have a significant impact on behavior. Additionally, beta-alanine activates $GABA_A$ receptors (Wafford and Sattelle 1986; Lees et al. 2014) and would be expected to increase inhibition similarly to GABA and taurine. Beta-Alanine of up to 200 mM is found in *Fritillaria* nectar (Roguz et al. 2019) and occurred in 4 of 73 species in

a Mediterranean plant community (Petanidou et al. 2006). Bumble bees (*Bombus terrestris)* consumed more of solutions that contained 2.4 mM beta-alanine, even though it also significantly increased mortality, while honey bees increase consumption of solutions at a higher concentration, 46 mM (Bogo et al. 2019). In contrast to its expected role as a GABA$_A$ receptor agonist, feeding on beta-alanine increased the amount of time the bumble bees spent walking (Bogo et al. 2019). Given the potential targets of beta-alanine, study of the impacts of its consumption warrant further investigation.

Signaling at glutamate receptors

Glutamate (glutamic acid) is an amino acid that also acts as a neurotransmitter. In insects, glutamate is the major excitatory neurotransmitter at the neuromuscular junction (Jan and Jan 1976), a function carried out by acetylcholine in vertebrates. Glutamate-releasing neurons are also found in the central nervous system of the honey bee, including the optic lobes, central complex, and the subesophageal ganglion (Bicker et al. 1988; see Leboulle 2013 for a comprehensive review on glutamate in honey bees). There are three distinct types of glutamate receptors: excitatory channels, inhibitory channels, and GPCRs. The excitatory channels fall into two main classes that have significant functional differences: the NMDA- and AMPA-type receptors. These two types of receptors are essential for the neural plasticity that underlies the formation of memories in mammals, and there is also substantial evidence that these receptors play important roles in learning and memory in insects (Mussig et al. 2010; Si et al. 2004). Inhibitory glutamate-gated channels, referred to as GluCl receptors because they are selectively permeable to chloride, are found in multiple insect species (Barbara et al. 2005; El Hassani et al. 2012; Jones 2018), although they do not appear to be present in mammals. This allows glutamate to either excite or inhibit neurons, depending on which receptor type is expressed. As with vertebrates, glutamate also activates GPCRs in insects. Experiments examining the release of glutamate and the activity of different glutamate receptors have shown important roles for glutamate signaling in learning and memory in the honey bee (El Hassani et al. 2012; Kucharski et al. 2007; Locatelli et al. 2005; Mussig et al. 2010).

A survey of numerous plant species shows that the concentration of amino acids varies widely amongst nectar samples, but glutamate values as high as 0.5 mM were observed (Gardener and Gillman 2001; Gottsberger et al. 1984; Nepi et al. 2012). In an assay where free-flying bees could choose to forage on solutions containing sucrose or solutions of sucrose plus an amino acid, glutamate at 7.3 mM acted as a deterrent (Hendriksma et al. 2014). Glutamate reduced foraging by the tropical stingless bee *Melipona fuliginosa*,

although it did not affect other Meliponini species (Roubik et al. 1995). Bumble bees (*Bombus terrestris*) appear to be able to perceive amino acids, including glutamate, in water, although a high concentration outside of the naturally occurring range (68 mM) was used (Ruedenauer et al. 2019). Interestingly, it is unclear if the bumble bees can distinguish between distinct amino acids or if they were detecting the presence of amino acids in general.

Impacts on glycine receptors

Glycine is another amino acid that also acts as a neurotransmitter. In both insects and mammals, glycine activates ionotropic chloride channels resulting in the inhibition of neuronal activity (Jones 2018; Lynch 2004). However, little is known about the possible functions of these receptors in insects. A recent study in *Drosophila* has revealed an important role for glycine signaling in circadian rhythms (Frenkel et al. 2017), which would be expected to impact foraging behavior (Bloch 2010).

Glycine levels of up to 1 mM in nectar have been reported (Gardener and Gillman 2001; Gottsberger et al. 1984; Nepi et al. 2012). As with glutamate, glycine reduced foraging by *Melipona fuliginosa* (Roubik et al. 1995). Experiments examining the effects of glycine on honey bees have yielded variable results. In one study, glycine appeared to act as a deterrent as free-flying honey bees preferred sucrose alone to sucrose containing 14 mM glycine (Hendriksma et al. 2014). However, in a study examining the effects of glycine over a wide range of concentrations, from 27 mM to 27 nM, a choice assay with confined bees did not show reduced feeding on glycine. Furthermore, the addition of glycine to the reward during olfactory conditioning increased learning in restrained honey bees (Kim and Smith 2000). Given the prevalence of glycine in nectar, more work should explore its possible functions influencing pollinator behavior.

Compounds that act on acetylcholine receptors

Acetylcholine (ACh) is present throughout the honey bee and sphinx moth brain (Homberg et al. 1995; Kreissl and Bicker 1989) and is most likely the neurotransmitter used in the mushroom body Kenyon cells (Barnstedt et al. 2016). In both mammals and insects, ACh acts at both excitatory ion channels, the nicotinic receptors (nAChR; Jones 2018), and GPCRs, the muscarinic receptors (mAChR; Collin et al. 2013; Xia et al. 2016). In honey bees, the nAChR is expressed in the antennal lobes and mushroom bodies (Barbara et al. 2008; Goldberg et al. 1999; Kreissl and Bicker 1989; Wüstenberg and Grünewald 2004). Honey bee nAChR receptors are involved in sensitivity to sucrose, learning, and memory (Dacher et al. 2005; Gauthier et al. 2006; Thany et al.

2005), while the mAChR receptors play an important role in memory (Lozano et al. 2001). Furthermore, signaling through mAChR receptors is important for the changes in mushroom body neurons associated with the transition from nursing to foraging behavior in honey bees (Dobrin et al. 2011; Ismail et al. 2006).

Nicotine acts as an agonist at the nAChR; indeed, the receptor is named after the fact that it is nicotine's target. The natural range of nicotine in *Nicotiana* nectar ranges from 1 to 20 μM (Detzel and Wink 1993; Haverkamp et al. 2018; Tadmor-Melamed et al. 2004). Both honey bees and bumble bees show a preference for consuming nicotine-containing solutions over sucrose alone within the naturally occurring range (Baracchi et al. 2017; Singaravelan et al. 2005). However, bees find the taste aversive at higher concentrations (Baracchi et al. 2017; Kohler et al. 2012; Singaravelan et al. 2005; Tiedeken et al. 2014). In a visual learning task, free-flying bumble bees learned to visit the artificial flowers of the color containing a reward more quickly when the reward contained nicotine plus sucrose than sucrose alone, suggesting that nicotine may enhance learning. Furthermore, the bumble bees persisted in choosing the flower color associated with the nicotine reward, even when that flower no longer contained any food, much longer than bees trained on flowers with sucrose alone (Baracchi et al. 2017). Persistent foraging on unrewarding flowers suggests that nicotine in nectar may have negative impacts on pollinators. The nAChR is also the target of the neonicotinoid pesticides which mimic nicotine in activating the receptor. Although the presence of nicotine in nectar is relatively restricted, pollinators foraging on nicotine-containing nectar may be differentially impacted by exposure to neonicotinoids. As with the GABA$_A$ receptor, the nAChR undergoes desensitization (Palmer et al. 2013) and some neonicotinoids may bind preferentially to the desensitized receptor (Nauen et al. 2003), so the combination of nicotine and neonicotinoids may have synergistic effects.

Nectar components may also interact with the muscarinic acetylcholine receptor. The alkaloid scopolamine acts an antagonist at insect mAChR receptors (Collin et al. 2013; Terazima and Yoshino 2010; Xia et al. 2016). Scopolamine has been reported in nectar from several solanaceous species in the range of 9 μM to 0.5 mM (Kerchner et al. 2015) and in *Datura* at somewhat higher concentrations of 0.5 to 1.3 mM (Boros et al. 2010). Concentrations of 1 mM were shown to deter feeding by honey bees (Detzel and Wink 1993). Significantly, feeding of 1 mM scopolamine was shown to increase aggressive behavior of honey bees towards both nestmates and non-nestmates (Ismail et al. 2008). Given that feeding of scopolamine within the naturally occurring range also affected the growth of mushroom body neurons in the honey bee brain (Dobrin et al. 2011), the impact of scopolamine on pollinator behavior is worthy of investigation.

Possible actions of caffeine

Caffeine has multiple cellular targets which would be expected to affect the nervous system (Mustard 2014). In mammals, the main target is currently believed to be adenosine receptors which are GPCRs present on some neurons. Intercellular levels of adenosine are usually high enough to cause constitutive activation of these receptors. Caffeine acts as an antagonist, blocking signaling though these receptors. Electrophysiological experiments examining cells in the mushroom bodies of the honey bee brain showed that caffeine and another adenosine receptor antagonist had similar effects, suggesting caffeine also works through the adenosine receptors in insects (Wright et al. 2013). However, caffeine has been shown to affect sleep in the fruit fly, *Drosophila melanogaster*, and when the adenosine receptor was genetically knocked out, caffeine maintained its effects on sleep (Wu et al. 2009). Thus, whether or not the adenosine receptor underlies the actions of caffeine in insects remains to be addressed. Two other targets have been shown to be affected by caffeine in insects. Caffeine binds to ryanodine receptors leading to increases in intracellular Ca^{2+} levels, and it also acts as a phosphodiesterase inhibitor resulting in increases in cAMP (Mustard 2014). Both Ca^{2+} and cAMP act as second messengers in the intracellular signaling pathways activated by neurotransmitter G protein-coupled receptors and thus effecting their levels would be expected to impact behavior.

Caffeine has been found in the nectar of several plant species including coffee, citrus, and linden with a concentration range of 1 to 100 μM (Kretschmar and Baumann 1999; Naef et al. 2004; Wright et al. 2013). In free-flying honey bees, the addition of ecologically relevant concentrations of caffeine to a feeder increases the number of visits compared to sucrose alone (Couvillon et al. 2015; Singaravelan et al. 2005). However, both honey bees and bumble bees find higher concentrations aversive (Mustard et al. 2012; Singaravelan et al. 2005; Tiedeken et al. 2014; Wright et al. 2013). Using olfactory associative conditioning with restrained honey bees, caffeine in the nectar-relevant range increased the rate of learning and memory retention (Wright et al. 2013). The presence of caffeine in a feeder increased waggle dancing in honey bees, suggesting that the bees found caffeine to be more rewarding than sucrose alone. Additionally, the bees continue to visit the feeder even when the food is removed for much longer and even when another food source is available (Couvillon et al. 2015). In an assay using artificial flowers, bumble bees (*B. impatiens*) increased pollination at flowers with sucrose plus caffeine versus those without caffeine (Thomson et al. 2015). Taken together, these results suggest that caffeine in nectar may increase foraging by pollinators. In contrast, the stingless bee *Plebeia droryana* did not show a preference for feeding on caffeinated sucrose over sucrose alone, highlighting the

importance of examining the effects of neuroactive compounds on different species (Peng et al. 2019).

Conclusions

Understanding the roles of non-sugar components of nectar is a complex question, in part, because compounds may affect pollinators in multiple ways. For example, the amino acids glutamate and glycine are used in the construction of proteins, and glutamate is also a key intermediate in the synthesis of other amino acids. Therefore, glycine and glutamate might be important sources of nutrition for pollinators, as well as being neurotransmitters that can directly influence insect nervous systems (Paoli et al. 2014; Stabler et al. 2015). Nectar compounds may be detoxified via several routes, significantly reducing the concentration found in the hemolymph (Berenbaum and Johnson 2015). However, relatively little work has been done to investigate these mechanisms in pollinators compared to herbivores (Irwin et al. 2014). Reductionist studies in which particular compounds are studied in isolation, rather than in the mixture of chemicals present in nectar, will help to disentangle these different roles. Additionally, it is essential that nectar-relevant concentrations of the compound are examined. However, fully understanding the impacts of different components of nectar requires studying them together as well, as different compounds may interact and also influence the perception of nectar.

Honey bees and bumble bees are excellent model systems to study these questions as there are well-characterized assays for their behavior in both the controlled laboratory environment and in the field. However, it is clear that not all pollinators will be affected in the same way. For example, differences in the ability of different species to taste specific compounds would affect their willingness to consume nectar. Furthermore, some species may have evolved mechanisms to deal with particular plant compounds. Improvements in instrumentation and techniques have increased the ability to detect and identify compounds present in nectar. As the nectar of more plant species is analyzed, the number and diversity of compounds discovered will undoubtedly increase. Recognizing that some nectar components may bind to neurotransmitter receptors and thereby directly influence neural activity may suggest mechanisms through which nectar can influence pollinator behavior. Pharmacological and molecular tools can then be used to test the effects of nectar components on the proposed pathways, providing a better understanding of the relationship between plant and pollinator.

References

Adler LS (2001) The ecological significance of toxic nectar. Oikos 91:409–420

Baracchi D, Marples A, Jenkins AJ, Leitch AR, Chittka L (2017) Nicotine in floral nectar pharmacologically influences bumblebee learning of floral features. Sci Rep 7:1951. https://doi.org/10.1038/s41598-017-01980-1

Barbara GS, Zube C, Rybak J, Gauthier M, Grunewald B (2005) Acetylcholine, GABA and glutamate induce ionic currents in cultured antennal lobe neurons of the honeybee, *Apis mellifera*. J Comp Physiol A 191:823–836. https://doi.org/10.1007/s00359-005-0007-3

Barbara GS, Grunewald B, Paute S, Gauthier M, Raymond-Delpech V (2008) Study of nicotinic acetylcholine receptors on cultured antennal lobe neurones from adult honeybee brains. Invert Neurosci 8:19–29. https://doi.org/10.1007/s10158-007-0062-2

Barnstedt O et al (2016) Memory-relevant mushroom body output synapses are cholinergic. Neuron 89:1237–1247. https://doi.org/10.1016/j.neuron.2016.02.015

Berenbaum MR, Johnson RM (2015) Xenobiotic detoxification pathways in honey bees. Curr Opin Insect Sci 10:51–58

Bicker G (1991) Taurine-like immunoreactivity in photoreceptor cells and mushroom bodies: a comparison of the chemical architecture of insect nervous systems. Brain Res 560:201–206

Bicker G (1999) Histochemistry of classical neurotransmitters in antennal lobes and mushroom bodies of the honeybee. Microsc Res Tech 45:174–183

Bicker G, Schafer S, Ottersen OP, Storm-Mathisen J (1988) Glutamate-like immunoreactivity in identified neuronal populations of insect nervous systems. J Neurosci 8:2108–2122

Bloch G (2010) The social clock of the honeybee. J Biol Rhythms 25:307–317. https://doi.org/10.1177/0748730410380149

Bogo G, Bortolotti L, Sagona S, Felicioli A, Galloni M, Barberis M, Nepi M (2019) Effects of non-protein amino acids in nectar on bee survival and behavior. J Chem Ecol 45:278–285. https://doi.org/10.1007/s10886-018-01044-2

Boros B, Farkas A, Jakabova S, Bacskay I, Kilar F, Felinger A (2010) LC-MS Quantitative determination of atropine and scopolamine in the Floral Nectar of *Datura* Species. Chromatographia 71:S43–S49. https://doi.org/10.1365/s10337-010-1524-y

Borycz J, Borycz JA, Edwards TN, Boulianne GL, Meinertzhagen IA (2012) The metabolism of histamine in the *Drosophila* optic lobe involves an ommatidial pathway: β-alanine recycles through the retina. J Exp Biol 215:1399–1411. https://doi.org/10.1242/jeb.060699

Choudhary AF, Laycock I, Wright GA (2012) gamma-Aminobutyric acid receptor A-mediated inhibition in the honeybee's antennal lobe is necessary for the formation of configural olfactory percepts. Eur J Neurosci 35:1718–1724. https://doi.org/10.1111/j.1460-9568.2012.08090.x

Collin C, Hauser F, Gonzalez de Valdivia E, Li S, Reisenberger J, Carlsen EM, Khan Z, Hansen NO, Puhm F, Søndergaard L, Niemiec J, Heninger M, Ren GR, Grimmelikhuijzen CJ (2013) Two types of muscarinic acetylcholine receptors in *Drosophila* and other arthropods. Cell Mol Life Sci 70:3231–3242. https://doi.org/10.1007/s00018-013-1334-0

Couvillon MJ, Al Toufailia H, Butterfield TM, Schrell F, Ratnieks FLW, Schurch R (2015) Caffeinated forage tricks honeybees into increasing foraging and recruitment behaviors. Curr Biol 25:2815–2818. https://doi.org/10.1016/j.cub.2015.08.052

Dacher M, Lagarrigue A, Gauthier M (2005) Antennal tactile learning in the honeybee: effect of nicotinic antagonists on memory dynamics. Neuroscience 130:37–50. https://doi.org/10.1016/j.neuroscience.2004.09.006

Detzel A, Wink M (1993) Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. Chemoecology 4:8–18

Dobrin SE, Herlihy JD, Robinson GE, Fahrbach SE (2011) Muscarinic regulation of Kenyon cell dendritic arborizations in adult worker honey bees. Arthropod Struct Develop 40:409–419

Dupuis JP, Bazelot M, Barbara GS, Paute S, Gauthier M, Raymond-Delpech V (2010) Homomeric RDL and heteromeric RDL/LCCH3 GABA receptors in the honeybee antennal lobes: two candidates for inhibitory transmission in olfactory processing. J Neurophysiol 103:458–468. https://doi.org/10.1152/jn.00798.2009

El Hassani AK, Schuster S, Dyck Y, Demares F, Leboulle G, Armengaud C (2012) Identification, localization and function of glutamate-gated chloride channel receptors in the honeybee brain. Eur J Neurosci 36:2409–2420. https://doi.org/10.1111/j.1460-9568.2012.08144.x

Ffrench-Constant RH, Williamson MS, Davies TG, Bass C (2016) Ion channels as insecticide targets. J Neurogenet 30:163–177. https://doi.org/10.1080/01677063.2016.1229781

Frenkel L et al (2017) Organization of circadian behavior relies on glycinergic transmission. Cell Rep 19:72–85. https://doi.org/10.1016/j.celrep.2017.03.034

Gardener MC, Gillman MP (2001) Analyzing variability in nectar amino acids: composition is less variable than concentration. J Chem Ecol 27:2545–2558

Gauthier M et al (2006) Involvement of alpha-bungarotoxin-sensitive nicotinic receptors in long-term memory formation in the honeybee (*Apis mellifera*). Neurobiol Learn Mem 86:164–174. https://doi.org/10.1016/j.nlm.2006.02.003

Goldberg F, Grunewald B, Rosenboom H, Menzel R (1999) Nicotinic acetylcholine currents of cultured Kkenyon cells from the mushroom bodies of the honey bee *Apis mellifera*. J Physiol 514(Pt 3):759–768. https://doi.org/10.1111/j.1469-7793.1999.759ad.x

Gottsberger G, Schrauwen J, Linskens HF (1984) Amino acids and sugars in nectar, and their putative evolutionary significance. Plant Syst Evol 145:55–77

Hammer M, Menzel R (1998) Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. Learn Mem 5:146–156

Haverkamp A, Hansson BS, Baldwin IT, Knaden M, Yon F (2018) Floral trait variations among wild tobacco populations influence the foraging behavior of hawkmoth pollinators. Front Ecol Evol 6:1–10. https://doi.org/10.3389/fevo.2018.00019

Heisenberg M (1998) What do the mushroom bodies do for the insect brain? An introduction. Learn Mem 5:1–10

Hendriksma HP, Oxman KL, Shafir S (2014) Amino acid and carbohydrate tradeoffs by honey bee nectar foragers and their implications for plant-pollinator interactions. J Insect Physiol 69:56–64. https://doi.org/10.1016/j.jinsphys.2014.05.025

Homberg U, Kingan TG, Hildebrand JG (1987) Immunocytochemistry of GABA in the brain and suboesophageal ganglion of *Manduca sexta*. Cell Tissue Res 248:1–24. https://doi.org/10.1007/bf01239957

Homberg U, Hoskins SG, Hildebrand JG (1995) Distribution of acetylcholinesterase activity in the deutocerebrum of the sphinx moth *Manduca sexta*. Cell Tissue Res 279:249–259. https://doi.org/10.1007/bf00318481

Inouye DW, Waller GD (1984) Responses of honey bees (*Apis mellifera*) to amino acid solutions mimicking floral nectars. Ecology 65:618–625

Irwin RE, Bronstein JL, Manson JS, Richardson L (2010) Nectar robbing: ecological and evolutionary perspectives. Annu Rev Ecol Evol Syst 41:271–292

Irwin RE, Cook D, Richardson LL, Gardner DL (2014) Secondary compounds in floral rewards of toxic rangeland plants: impacts on pollinators. J Agric Food Chem 62:7335–7344

Ismail N, Robinson GE, Fahrbach SE (2006) Stimulation of muscarinic receptors mimics experience-dependent plasticity in the honey bee brain. Proc Natl Acad USA 103:207–211

Ismail N, Christine S, Robinson GE, Fahrbach SE (2008) Pilocarpine improves recognition of nestmates in young honey bees. Neurosci Lett 439:178–181

Jacobsen DJ, Raguso RA (2018) Lingering effects of herbivory and plant defenses on pollinators. Curr Biol 28:R1164–R1169. https://doi.org/10.1016/j.cub.2018.08.010

Jan LY, Jan YN (1976) L-glutamate as an excitatory transmitter at the *Drosophila* larval neuromuscular junction. J Physiol 262:215–236

Jones AK (2018) Genomics, cys-loop ligand-gated ion channels and new targets for the control of insect pests and vectors. Curr Opin Insect Sci 30:1–7. https://doi.org/10.1016/j.cois.2018.07.016

Kaczorowski RL, Gardener MC, Holtsford TP (2005) Nectar traits in nicotiana section Alatae (Solanaceae) in relation to floral traits, pollinators, and mating system. Am J Bot 92:1270–1283. https://doi.org/10.3732/ajb.92.8.1270

Kerchner A, Darók J, Bacskay I, Felinger A, Jakab G, Farkas A (2015) Protein and alkaloid patterns of the floral nectar in some solanaceous species. Acta Biol Hung 66:304–315. https://doi.org/10.1556/018.66.2015.3.6

Kessler D, Baldwin IT (2007) Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of *Nicotiana attenuata*. Plant J 49:840–854. https://doi.org/10.1111/j.1365-313X.2006.02995.x

Kim YS, Smith BH (2000) Effect of an amino acid on feeding preferences and learning behavior in the honey bee, *Apis mellifera*. J Insect Physiol 46:793–801

Kohler A, Pirk CW, Nicolson SW (2012) Honeybees and nectar nicotine: deterrence and reduced survival versus potential health benefits. J Insect Physiol 58:286–292. https://doi.org/10.1016/j.jinsphys.2011.12.002

Kreissl S, Bicker G (1989) Histochemistry of acetylcholinesterase and immunocytochemistry of an acetylcholine receptor-like antigen in the brain of the honeybee. J Comp Neurol 286:71–84. https://doi.org/10.1002/cne.902860105

Kretschmar J, Baumann T (1999) Caffeine in Citrus flowers. Phytochemistry 52:19–23

Kucharski R, Mitri C, Grau Y, Maleszka R (2007) Characterization of a metabotropic glutamate receptor in the honeybee (*Apis mellifera*): implications for memory formation. Invert Neurosci 7:99–108. https://doi.org/10.1007/s10158-007-0045-3

Le Goff G, Hamon A, Bergé JB, Amichot M (2005) Resistance to fipronil in *Drosophila simulans*: influence of two point mutations in the RDL GABA receptor subunit. J Neurochem 92:1295–1305

Leboulle G (2013) Glutamate neurotransmission and appetitive olfactory conditioning in the honeybee. In: Menzel R, Benjamin PR (eds) Invertebrate learning and memory. Handbook of behavioral neuroscience, vol 22. Elsevier, London. https://doi.org/10.1016/B978-0-12-415823-8.00034-4

Lees K, Musgaard M, Suwanmanee S, Buckingham SD, Biggin P et al (2014) Actions of Agonists, fipronil and ivermectin on the predominant in vivo splice and edit variant (RDL$_{bd}$, I/V) of the *Drosophila* GABA receptor expressed in *Xenopus laevis* oocytes. PLoS ONE 9(5):e97468. https://doi.org/10.1371/journal.pone.0097468

Lin FJ, Pierce MM, Sehgal A, Wu T, Skipper DC, Chabba R (2010) Effect of taurine and caffeine on sleep-wake activity in *Drosophila melanogaster*. Nat Sci Sleep. 2:221–231. https://doi.org/10.2147/NSS.S13034

Locatelli F, Bundrock G, Muller U (2005) Focal and temporal release of glutamate in the mushroom bodies improves olfactory memory in *Apis mellifera*. J Neurosci 25:11614–11618. https://doi.org/10.1523/JNEUROSCI.3180-05.2005

Lozano VC, Armengaud C, Gauthier M (2001) Memory impairment induced by cholinergic antagonists injected into the mushroom bodies of the honeybee. J Comp Physiol A 187:249–254

Lynch JW (2004) Molecular structure and function of the glycine receptor chloride channel. Physiol Rev 84:1051–1095. https://doi.org/10.1152/physrev.00042.2003

Menzel R (2001) Searching for the memory trace in a mini-brain, the honeybee. Learn Mem 8:53–62. https://doi.org/10.1101/lm.38801

Mussig L, Richlitzki A, Rossler R, Eisenhardt D, Menzel R, Leboulle G (2010) Acute disruption of the NMDA receptor subunit NR1 in the honeybee brain selectively impairs memory formation. J Neurosci 30:7817–7825. https://doi.org/10.1523/JNEUROSCI.5543-09.2010

Mustard JA (2014) The buzz on caffeine in invertebrates: effects on behavior and molecular mechanisms. Cell Mol Life Sci 71:1375–1382. https://doi.org/10.1007/s00018-013-1497-8

Mustard JA, Dews L, Brugato A, Dey K, Wright GA (2012) Consumption of an acute dose of caffeine reduces acquisition but not memory in the honey bee. Behav Brain Res 232:217–224. https://doi.org/10.1016/j.bbr.2012.04.014

Mustard JA, Jones L, Wright GA (2020) GABA signaling affects motor function in the honey bee. J Insect Physiol 120:103989. https://doi.org/10.1016/j.jinsphys.2019.103989

Naef R, Jaquier A, Velluz A, Bachofen B (2004) From the linden flower to linden honey–volatile constituents of linden nectar, the extract of bee-stomach and ripe honey. Chem Biodivers 1:1870–1879. https://doi.org/10.1002/cbdv.200490143

Nauen R, Ebbinghaus-Kintscher U, Salgado VL, Kaussmann M (2003) Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. Pestic Biochem Physiol 76:55–69

Nepi M (2014) Beyond nectar sweetness: the hidden ecological role of non-protein amino acids in nectar. J Ecol 102:108–115

Nepi M et al (2012) Amino acids and protein profile in floral nectar: much more than a simple reward. Flora 207:475–481

Nepi M, Grasso DA, Mancuso S (2018) Nectar in plant-insect mutualistic relationships: from food reward to partner manipulation. Front Plant Sci 9:1063. https://doi.org/10.3389/fpls.2018.01063

Nocentini D, Pacini E, Guarnieri M, Nepi M (2012) Flower morphology, nectar traits and pollinators of Cerinthe major (Boraginaceae-Lithospermeae). Flora 207:186–196. https://doi.org/10.1016/j.flora.2012.01.004

Palmer MJ, Harvey J (2014) Honeybee Kenyon cells are regulated by a tonic GABA receptor conductance. J Neurophysiol 112:2026–2035

Palmer MJ, Moffat C, Saranzewa N, Harvey J, Wright GA, Connolly CN (2013) Cholinergic pesticides cause mushroom body neuronal inactivation in honeybees. Nat Commun 4:1634. https://doi.org/10.1038/ncomms2648

Paoli PP, Donley D, Stabler D, Saseendranath A, Nicolson SW, Simpson SJ, Wright GA (2014) Nutritional balance of essential amino acids and carbohydrates of the adult worker honeybee depends on age. Amino Acids 46:1449–1458. https://doi.org/10.1007/s00726-014-1706-2

Peng T, Segers F, Nascimento F, Gruter C (2019) Resource profitability, but not caffeine, affects individual and collective foraging in the stingless bee Plebeia droryana. J Exp Biol. https://doi.org/10.1242/jeb.195503

Petanidou T, Van Laere A, Ellis WN, Smets E (2006) What shapes amino acid and sugar composition in Mediterranean floral nectars? Oikos 115:155–169

Pfeiffer K, Homberg U (2014) Organization and functional roles of the central complex in the insect brain. Annu Rev Entomol 59:165–184. https://doi.org/10.1146/annurev-ento-011613-162031

Raccuglia D, Mueller U (2013) Focal uncaging of GABA reveals a temporally defined role for GABAergic inhibition during appetitive associative olfactory conditioning in honeybees. Learn Mem 20:410–416. https://doi.org/10.1101/lm.030205.112

Rein J, Mustard JA, Strauch M, Smith BH, Galizia CG (2013) Octopamine modulates activity of neural networks in the honey bee antennal lobe. J Comp Physiol A 199:947–962. https://doi.org/10.1007/s00359-013-0805-y

Richardt A, Kemme T, Wagner S, Schwarzer D, Marahiel MA, Hovemann BT (2003) Ebony, a novel nonribosomal peptide synthetase for beta-alanine conjugation with biogenic amines in Drosophila. J Biol Chem. 278(42):41160–41166. https://doi.org/10.1074/jbc.M304303200

Roguz K, Bajguz A, Chmur M, Gołębiewska A, Roguz A, Zych M (2019) Diversity of nectar amino acids in the Fritillaria (Liliaceae) genus: ecological and evolutionary implications. Sci Rep 9:15209. https://doi.org/10.1038/s41598-019-51170-4

Roubik D, Yanega D, Aluja M, Buchmann S, Inouye DW (1995) On optimal nectar foraging by some tropical bees (Hymenoptera: Apidae). Apidologie 26:197–211

Ruedenauer FA, Leonhardt SD, Lunau K, Spaethe J (2019) Bumblebees are able to perceive amino acids via chemotactile antennal stimulation. J Comp Physiol A 205:321–331. https://doi.org/10.1007/s00359-019-01321-9

Sachse S, Galizia CG (2002) Role of inhibition for temporal and spatial odor representation in olfactory output neurons: a calcium imaging study. J Neurophysiol 87:1106–1117. https://doi.org/10.1152/jn.00325.2001

Schäfer S, Bicker G (1986) Distribution of GABA-like immunoreactivity in the brain of the honeybee. J Comp Neurol 246:287–300. https://doi.org/10.1002/cne.902460302

Schäfer S, Bicker G, Ottersen OP, Stormmathisen J (1988) Taurine-like immunoreactivity in the brain of the honeybee. J Comp Neurol 268:60–70

Scheiner R, Pluckhahn S, Oney B, Blenau W, Erber J (2002) Behavioural pharmacology of octopamine, tyramine and dopamine in honey bees. Behav Brain Res 136:545–553

Schulz DJ, Robinson GE (2001) Octopamine influences division of labor in honey bee colonies. J Comp Physiol A 187:53–61

Si A, Helliwell P, Maleszka R (2004) Effects of NMDA receptor antagonists on olfactory learning and memory in the honeybee (Apis mellifera). Pharmacol Biochem Behav 77:191–197. https://doi.org/10.1016/j.pbb.2003.09.023

Singaravelan N, Nee'man G, Inbar M, Izhaki I (2005) Feeding responses of free-flying honeybees to secondary compounds mimicking floral nectars. J Chem Ecol 31:2791–2804. https://doi.org/10.1007/s10886-005-8394-z

Stabler D, Paoli PP, Nicolson SW, Wright GA (2015) Nutrient balancing of the adult worker bumblebee (Bombus terrestris) depends on the dietary source of essential amino acids. J Exp Biol 218:793–802. https://doi.org/10.1242/jeb.114249

Stevenson PC, Nicolson SW, Wright GA (2017) Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. Funct Ecol 31:65–75

Strauss R (2002) The central complex and the genetic dissection of locomotor behaviour. Curr Opin Neurobiol 12:633–638

Tadmor-Melamed H, Markman S, Arieli A, Distl M, Wink M, Izhaki I (2004) Limited ability of Palestine Sunbirds Nectarinia osea to cope with pyridine alkaloids in nectar of Tree Tobacco Nicotiana glauca. Funct Ecol 18:844–850

Terazima E, Yoshino M (2010) Modulatory action of acetylcholine on the Na^+-dependent action potentials in Kenyon cells isolated from the mushroom body of the cricket brain. J Insect Physiol 56:1746–1754. https://doi.org/10.1016/j.jinsphys.2010.07.001

Thany SH, Crozatier M, Raymond-Delpech V, Gauthier M, Lenaers G (2005) Apisalpha2, Apisalpha7-1 and Apisalpha7-2: three new neuronal nicotinic acetylcholine receptor alpha-subunits in the

honeybee brain. Gene 344:125–132. https://doi.org/10.1016/j.gene.2004.09.010

Thomson JD, Draguleasa MA, Tan MG (2015) Flowers with caffeinated nectar receive more pollination. Arthropod-Plant Interactions 9:1–7

Tiedeken EJ, Stout JC, Stevenson PC, Wright GA (2014) Bumblebees are not deterred by ecologically relevant concentrations of nectar toxins. J Exp Biol 217:1620–1625. https://doi.org/10.1242/jeb.097543

Wafford KA, Sattelle DB (1986) Effects of amino acid neurotransmitter candidates on an identified insect motoneurone. Neurosci Lett 63:135–140

Whitton PS, Nicholson RA, Strang RHC (1994) GABA and taurine: electrophysiological responses of isolated locust (*Schistocerca gregaria*) somata to taurine and GABA application. J Insect Physiol 40:195–199. https://doi.org/10.1016/0022-1910(94)90042-6

Wolf H (2014) Inhibitory motoneurons in arthropod motor control: organisation, function, evolution. J Comp Physiol A 200:693–710. https://doi.org/10.1007/s00359-014-0922-2

Wright GA, Mustard JA, Simcock NK, Ross-Taylor AA, McNicholas LD, Popescu A, Marion-Poll F (2010) Parallel reinforcement pathways for conditioned food aversions in the honeybee. Curr Biol 20:2234–2240. https://doi.org/10.1016/j.cub.2010.11.040

Wright GA et al (2013) Caffeine in floral nectar enhances a pollinator's memory of reward. Science 339:1202–1204. https://doi.org/10.1126/science.1228806

Wu MN, Ho K, Crocker A, Yue Z, Koh K, Sehgal A (2009) The effects of caffeine on sleep in *Drosophila* require PKA activity, but not the adenosine receptor. J Neurosci 29:11029–11037. https://doi.org/10.1523/JNEUROSCI.1653-09.2009

Wüstenberg DG, Grünewald B (2004) Pharmacology of the neuronal nicotinic acetylcholine receptor of cultured Kenyon cells of the honeybee, *Apis mellifera*. J Comp Physiol A 190:807–821. https://doi.org/10.1007/s00359-004-0530-7

Xia RY, Li MQ, Wu YS, Qi YX, Ye GY, Huang J (2016) A new family of insect muscarinic acetylcholine receptors. Insect Mol Biol 25(4):362–369. https://doi.org/10.1111/imb.12229

Zhang HG, ffrench-Constant RH, Jackson MB (1994) A unique amino acid of the *Drosophila* GABA receptor with influence on drug sensitivity by two mechanisms. J Physiol 479:65–75