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Neuroactive metabolites modulated by the gut microbiota in honey bees

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Abstract

Honey bees have emerged as a new model to study the gut–brain axis, as they exhibit complex social behaviors and cognitive abilities, while experiments with gnotobiotic bees have revealed that their gut microbiota alters both brain and behavioral phenotypes. Furthermore, while honey bee brain functions supporting a broad range of behaviors have been intensively studied for over 50 years, the gut microbiota of bees has been experimentally characterized only recently. Here, we combined six published datasets from metabolomic analyses to provide an overview of the neuroactive metabolites whose abundance in the gut, hemolymph and brain varies in presence of the gut microbiota. Such metabolites may either be produced by gut bacteria, released from the pollen grains during their decomposition by bacteria, or produced by other organs in response to different bacterial products. We describe the current state of knowledge regarding the impact of such metabolites on brain function and behavior and provide further hypotheses to explore in this emerging field of research.

KEYWORDS
Apis, bacteria, behavior, brain, nutrition, symbiosis

1 | INTRODUCTION

Modulation of animal behavior by the gut microbiota along the so-called gut microbiota–brain axis has been increasingly studied in vertebrate systems over the past decade. Severe disruptions to the bacterial community in the gut were shown to negatively impact the host's cognitive abilities and social behavior, and were associated with several neurological disorders (Cryan & Dinan, 2012; Morais et al., 2020; Needham et al., 2020; Xia et al., 2022). Gut bacteria influence such host phenotypes by producing a plethora of metabolites that modulate brain function either by acting locally on enteric neurons or enterocytes, or by reaching the systemic circulation, that is the hemolymph, and eventually crossing the blood–brain barrier (Kuraishi et al., 2015; Morais et al., 2020). Yet, disentangling the intricate metabolic interactions within highly complex gut communities and their effects on host biology is challenging. The cumulative impact of microbe-microbe and host-microbe interactions involving hundreds to thousands of microbial species or strains makes it difficult to discriminate bacterial metabolites from host metabolites that can be produced in adjacent and remote tissues in response to gut microbes.

Insect models with relatively simple community composition and experimental ease can aid in the unveiling of such intricacies. The honey bee gut microbiota is composed of nine predominant genera comprising 15–20 species (Ellegaard & Engel, 2019) which can be grown under laboratory conditions and inoculated into

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newborn gut microbiota-free (MF) bees that emerged from late pupal stage under semi-sterile conditions to generate gnotobiotic individuals (Box 1) (Engel et al., 2016; Kwong & Moran, 2016). Most of these bacteria colonize the hindgut and include six Proteobacteria genera (*Snodgrassella*, *Gilliamella*, *Bartonella*, *Frischella*, *Bombella*, and *Commensalibacter*), two Firmicutes (*Bombilactobacillus* and *Lactobacillus*) and one Actinobacterium (*Bifidobacterium*). Recent findings advocate for the existence of a gut microbiota–brain axis in honey bees (Liberti & Engel, 2020). Several independent studies reported that MF bees exhibit altered gene expression profiles in the brain and abnormal behaviors relative to bees colonized with the native microbiota (Cabirol et al., 2023; Liberti et al., 2022; Zhang, Mu, Cao, et al., 2022; Zhang, Mu, Shi, et al., 2022). In particular, MF bees showed deficits in social behaviors and olfactory learning and memory performances. Furthermore, over the past decade, important advances in metagenomics and metabolomics led to the accumulation of data related to the metabolic pathways encoded in the genomes of different gut bacterial species and the metabolites they produce (reviewed in Bonilla-Rosso & Engel, 2018). Yet, our understanding of the role of these metabolites on bees' brain function and behavior remains limited. As in vertebrates, gut microbiota derived metabolites might act locally or systemically, but our understanding of these processes in honey bees is limited (Huang et al., 2015; Kuraishi et al., 2015). While neural innervations in the gut have been observed (Kuraishi et al., 2015) and the blood–brain barrier has been described in the fruit fly (*Drosophila*) insect model (Carlson et al., 2000; Limmer et al., 2014), these physiological parameters have not yet been characterized in the honey bee.

Here, we provide a comprehensive review of the metabolites that might be important in the gut–brain communication in this invertebrate model. First, we describe the behavioral alterations reported in MF bees and the associated neurological changes in their brain. Then, we highlight candidate metabolites for such host phenotypes based on the combination of published datasets showing their differential abundance in the gut, hemolymph or brain of MF bees compared to bees colonized with a complex microbiota (CL; gut homogenate from hive bees, a defined community of isolates, or the natural community in the hive) or with individual community members (mono-colonized, MC) (Kešnerová et al., 2017; Liberti et al., 2022; Wu et al., 2021; Zhang, Mu, Shi, et al., 2022; Zheng et al., 2017, 2019) (Table S1). Along with showing the localization of metabolic changes, we discuss the origin of neuroactive metabolites (i.e., microbe or host) and their role in generating microbiota-dependent neurological and behavioral alterations. Finally, we provide insights as to which additional bee brain or behavioral phenotypes might be affected by the gut microbiota.

BOX 1 Gnotobiotic bee experiments to measure the impact of the gut microbiota

The establishment of a gnotobiotic bee model has been essential for studying the impact of the gut microbiota on the honey bee host. Adult honey bees acquire their gut microbiota only upon eclosion from the pupal stage, via either social interactions with older nestmates or contacts with the hive environment (Martinson et al., 2012; Powell et al., 2014). Thus, adult MF bees can be readily produced by carefully removing pupae from their wax cells and letting them eclose in semi-sterile laboratory conditions. Microbiota-free bees can be subsequently colonized with the gut microbiota and kept under semi-sterile laboratory conditions for up to 3–5 weeks, in groups of 10–50 bees in different types of cages, with unlimited access to sterile sugar water and (often) bee pollen. Different methods have been established to inoculate the MF bees with the gut microbiota. For example, bacterial suspensions can be pipetted onto the sterile pollen or mixed with the sterile sugar water provided to each cage. Alternatively, hand feeding each individual bee an exact volume (e.g., 5 μL, with a pipette) of the bacterial suspension provides the advantage that each bee will be inoculated with the same amount of bacteria. The inoculum may consist of a gut homogenate of bees collected from the hive, or it may contain specific bacterial strains alone (MC bees) or in defined communities. While the use of a gut homogenate enables inoculation of bees with the complete native gut microbiota (containing all species and different strains of each species), its composition varies between individuals, which may make it difficult to reproduce experimental results. This method also precludes identification of the relative contributions of different bacterial species to the host phenotype. Furthermore, gut homogenates also contain fungi, viruses, host tissue, and fermented food which might contribute to the behavioral differences observed between MF and CL bees (Evans & Schwarz, 2011). In this sense, working with defined communities is more controlled and reproducible but, to date, they have not encompassed the genomic diversity of the complete microbiota, and hence important functions may be missing. The gnotobiotic status of MF and CL bees is usually confirmed by qPCR and amplicon sequencing methods. Such gnotobiotic bee experiments have not only demonstrated the existence of a gut–brain axis, but also revealed that the honey bee gut microbiota confers colonization resistance against pathogens and facilitates dietary breakdown and nutrient availability (Raymann & Moran, 2018).

2 | PHENOTYPIC ALTERATIONS INDUCED BY THE GUT MICROBIOTA

Gut microbiota manipulation in honey bees has been shown to modulate neurological and behavioral processes (Figure 1). While alterations in both the structure and function of the brain may explain the distinct behavioral phenotypes of MF and CL bees, few studies have established a causal link between gut microbiota-dependent brain and behavioral changes.

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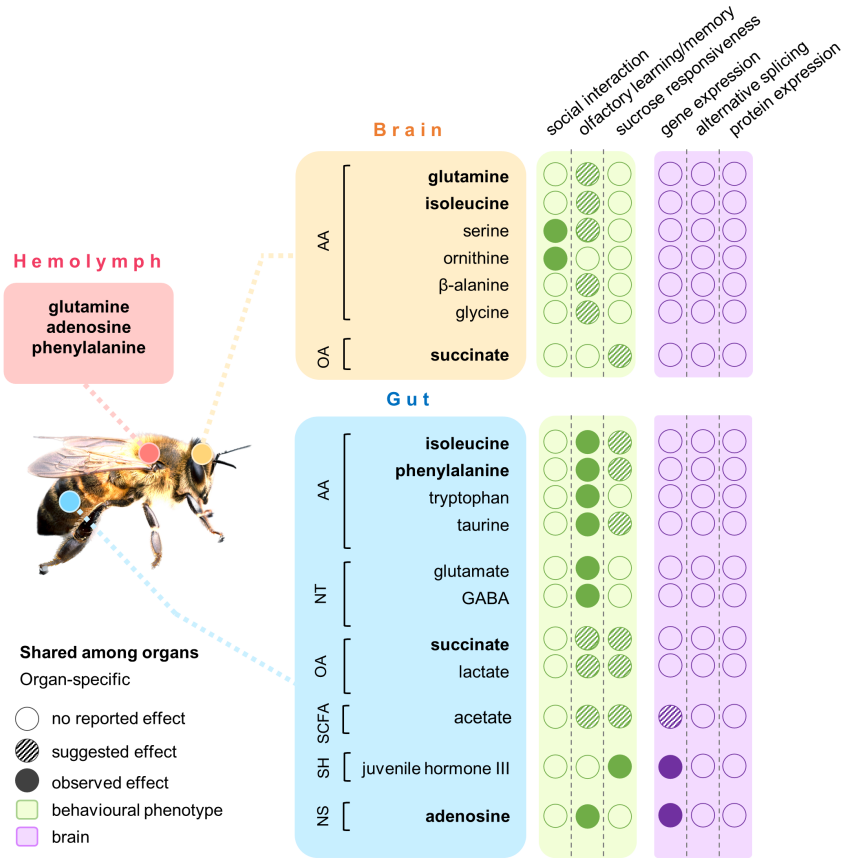

FIGURE 1 Gut microbiota-modulated neuroactive metabolites. Left panel shows the metabolites found enriched in the gut, brain and hemolymph of bees colonized with either a gut homogenate of hive bees or a defined community of gut bacteria (CL bees) compared to microbiota-free (MF) bees. Metabolites enriched in at least two locations are shown in bold. On the right panel, behavioral (green) and brain (purple) phenotypes which differ between CL and MF bees are displayed and linked to microbiota-modulated metabolites. Observed (filled circle) or suggested (stripped circle) effects are shown. AA, amino acid; NS, nucleoside; NT, neurotransmitter; OA, organic acid; SCFA, short-chained fatty acid; SH, sesquiterpenoid hormone.

2.1 | Behavioral phenotypes

Increasing evidence supports the role of the microbiota–gut–brain axis in the modulation of behaviors in honey bees (Liberti et al., 2022; Zhang, Mu, Cao, et al., 2022; Zhang, Mu, Shi, et al., 2022). Microbiota-free bees show deficits in appetitive olfactory learning and memory (Zhang, Mu, Cao, et al., 2022), sucrose responsiveness (Zhang, Mu, Shi, et al., 2022; Zheng et al., 2017) and social interactions (Liberti et al., 2022) compared to CL bees inoculated with a gut homogenate from hive bees. While the decreased sucrose responsiveness of MF bees suggests gut bacteria may modulate appetite as they do in *Drosophila melanogaster* (Kim et al., 2021), future studies in honey bees should assess the impact of gut microbiota manipulation on feeding behavior. We recently showed that a defined community of five "core" gut bacteria genera improved learning and memory function relative to MF bees (Cabirol et al., 2023). Interestingly, single members of the defined community could not recapitulate these effects suggesting that it is mediated by an emerging community property or an additive effect of the individual community member's presence. The use of such defined communities is a promising approach to identify and model metabolic interactions among community members that modulate the abundance of neuroactive metabolites reaching the host brain.

2.2 | Brain phenotypes

So far, most studies investigating differences in the brains of MF and CL bees relied on omics approaches to provide a global assessment of differences in gene expression or protein profiles, finding regulation of epigenetic processes by the gut microbiota as a possible mechanism mediating behavioral effects (Liberti et al., 2022; Wu et al., 2021; Zhang, Mu, Cao, et al., 2022; Zhang, Mu, Shi, et al., 2022). Liberti and colleagues found that differentially expressed genes between MF and CL bees were enriched for gene ontology terms related to epigenetic regulations of chromosome packaging and conformation (Liberti et al., 2022). Using proteomic analyses, Zhang and colleagues observed the upregulation of a splicing factor, U2af28, in the brain of CL bees (Zhang, Mu, Cao, et al., 2022). Alternative splicing is another regulatory process for gene expression whereby various mature mRNA isoforms are generated per gene, leading to functionally different proteins (Chen & Manley, 2009). Interestingly, changes in alternative splicing of genes in the honey bee brain were linked to gut colonization by members of the gut microbiota in a strain specific manner (Wu et al., 2021; Zhang, Mu, Shi, et al., 2022). Although experimental validation is needed, it suggests that the brain proteomic profiles, shown to differ between MF and CL bees (Zhang, Mu, Cao, et al., 2022), might be specifically shaped by the bacterial species and strains colonizing the gut.

The identity of genes and proteins whose expression or abundance in the brain differ between MF, CL and MC bees allows us to identify neural processes that might be affected by the gut microbiota. For instance, multiple neurotransmission systems seem to be modulated by gut bacteria (Wu et al., 2021; Zhang, Mu, Cao, et al., 2022; Zhang, Mu, Shi, et al., 2022). Zhang and colleagues observed that proteins found to be unique to the CL brain were related to synaptic neurotransmission and transmembrane transport

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of cations/ions (Zhang, Mu, Cao, et al., 2022). They also detected an upregulation of the muscarinic acetylcholine receptor, important for olfactory memory in honey bees (Lozano et al., 2001). Additionally, genes involved in GABAergic, dopaminergic, serotoninergic, or glutamatergic synapses showed differences in alternative splicing events in the brains of MF bees and bees mono-colonized with *Gilliamella*, *Bombilactobacillus*, *Lactobacillus* Firm-5, or different strains of *Bifidobacterium* (Wu et al., 2021; Zhang, Mu, Shi, et al., 2022). Each of these neurotransmission pathways regulate diverse animal behaviors, including the ones known to be affected by the gut microbiota in bees.

Other differentially expressed genes might support the behavioral differences observed between MF and CL bees. Two genes involved in olfactory perception were upregulated in CL bees, which may explain their reported increase in olfactory learning and memory performances (Zhang, Mu, Cao, et al., 2022). Differences in sucrose responsiveness between MF and CL bees might be supported by the increased expression of genes coding for peptides of the insulin/insulin-like signaling pathway (Zheng et al., 2017), known to promote sucrose responsiveness in honey bees (Mott & Breed, 2012). The deficits in social interactions of MF bees could be linked to a decreased amino acid metabolism in their brain (Liberti et al., 2022). Liberti and colleagues not only found that the expression of genes for amino acid metabolism was downregulated, but also that indeed fewer amino acids were detectable in the brain of MF bees compared to CL bees. Moreover, the abundances of several amino acids in the brain were positively correlated with the number of head-to-head interactions per bee, independent of the microbiota treatment, suggesting a direct link between these metabolites and social behavior (see "Amino acids" paragraph below) (Liberti et al., 2022). Alternatively, such deficits could also be linked to the presence of differentially spliced genes related to the human autism spectrum disorder in the brain of MF bees as detected by Zhang, Mu, Shi, et al. (2022). Finally, the expression levels of several genes of the major royal jelly protein family (*mrjp* genes), which play crucial roles in caste determination and reproductive maturation in honey bees (Drapeau et al., 2006), were significantly higher in the brains of CL compared to MF bees. Interestingly, these genes were differentially affected by different community members when mono-colonized (Zhang, Mu, Cao, et al., 2022; Zhang, Mu, Shi, et al., 2022): While gut colonization with *Lactobacillus* Firm-4 or Firm-5 upregulated several *mrjp* family genes, colonization with *Gilliamella* decreased their expression in the brain of MC bees compared to MF bees. At this point, we can only speculate about the consequences of these gene expression changes.

In summary, the reported omics analyses highlight candidate neurobiological pathways mediating the impact of the gut microbiota on bees' behavior. Other neurobiological processes known to support social behavior and cognitive performances in honey bees and other animals have not yet been investigated in the context of the bee microbiota–gut–brain axis (for reviews see Anton & Rossler, 2021; Caroni et al., 2014; Turrigiano & Nelson, 2000). In honey bees, such processes include, but are not restricted to, brain structural and functional maturation during early life (Cabirol et al., 2017; Grosso et al., 2018; Wang et al., 2005), plasticity of synaptic weights (Menzel, 2014; Szyszka et al., 2008) and experience-dependent structural rearrangements of the neuronal circuitry (Andrione et al., 2017; Hourcade et al., 2010). While brain metabolites involved in such a broad range of phenotypes have been partly identified, the causal relationship between gut bacteria, metabolite levels in the brain, neuronal function and behavior still needs to be uncovered.

3 | LINKING GUT MICROBIOTA-MODULATED METABOLITES TO NEUROLOGICAL AND BEHAVIORAL PHENOTYPES

Metabolites detected in the gut depend on a complex interplay of environmental, microbial and host factors, such as the dietary substrate availability, composition of bacterial species in the gut, host variability, and exposure to phytochemicals and other environmental toxins (Daisley et al., 2020). When comparing colonized bees (CL or MC) against MF bees, it is possible to determine metabolites whose abundances are modulated by the gut microbiota. Combining the differentially abundant metabolites provided in published studies on the topic (Kešnerová et al., 2017; Liberti et al., 2022; Wu et al., 2021; Zhang, Mu, Shi, et al., 2022; Zheng et al., 2017, 2019), we found 321 metabolites whose abundance in either the gut, hemolymph or brain was reported as significantly increased in MF bees compared to CL bees, and 284 metabolites whose abundance was significantly decreased in MF bees (Table S1). For simplicity, we focus below on metabolite classes that are known, or have been suspected, to modulate neural functions and behavior and discuss their possible role in mediating such effects in the honey bee model (Figure 1). The microbial or host origin of metabolites found to be differentially abundant in the brain of MF and CL bees is discussed for each metabolite class, though in many cases this is inferred from genome annotations and still requires experimental validation.

3.1 | Amino acids

In vertebrates, several amino acids were identified as neuromodulators or precursors of neurotransmitters (Needham et al., 2020). **Tryptophan**, for instance, can be converted by gut bacteria into indole derivatives, tryptamine and kynurenine, which are known neuroactive molecules. It is also a precursor for the neurotransmitter serotonin (Needham et al., 2020). Honey bee gut bacteria, especially strains of *Lactobacillus* Firm-5, convert tryptophan into indole derivatives such as kynurenic acid (Zheng et al., 2017), indole-3-acetate (Kešnerová et al., 2017; Zheng et al., 2017) and xanthurenic acid (Kešnerová et al., 2017) which all accumulate in the gut of CL bees (Table S1). *Lactobacillus apis* was shown to produce high levels

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of indole derivatives in the gut of MC bees when dietary tryptophan was administered, which was coupled with increased olfactory memory performances (Zhang, Mu, Cao, et al., 2022). Tryptophan itself was found in higher abundance in the gut of CL individuals compared to MF individuals (Figure 1). Recent studies have shown that honey bee gut bacteria could modulate tryptophan levels through the kynurenine pathway (Quinn et al., 2023; Zhang, Mu, Shi, et al., 2022). Specifically, *S. alvi* encodes a kynureninase that converts host- and/or diet-derived kynurenine into anthranilate which was found to accumulate in the gut lumen of bees mono-colonized with *S. alvi* (Kešnerová et al., 2017; Quinn et al., 2023) and in the gut of CL bees (Kešnerová et al., 2017). Anthranilate serves as a precursor for tryptophan, along with several neurotransmitters including serotonin, tryptamine, and various indole derivatives (Kaur et al., 2019).

Another interesting neuroactive amino acid upregulated in the brain of CL bees is **beta-alanine** (Liberti et al., 2022). In insects, beta-alanine was shown to modulate a wide range of neuronal and brain functions (Mustard, 2020). It acts as substrate for an enzyme inactivating biogenic amines (Richardt et al., 2003) and an agonist of $GABA_A$ receptors (Mustard, 2020). In *Drosophila*, beta-alanine was shown to play an important role in visual processing and the recycling of histamine, the visual neurotransmitter (Borycz et al., 2012; Mustard, 2020). Oral administration of beta-alanine or **taurine** decreased olfactory learning performance and increased memory retention in bees (Carlesso et al., 2021). Taurine itself was also among the metabolites found in higher abundance in the gut of CL bees compared to MF bees (Kešnerová et al., 2017; Zheng et al., 2017). Brain and behavioral effects of orally administered compounds suggest they may reach the brain and are consistent with previous studies showing that essential amino acids as well as glutamate could cross the insect blood–brain barrier (Limmer et al., 2014; Maleszka et al., 2000).

Overall, several amino acids were more abundant in the gut of CL bees compared to MF bees (Kešnerová et al., 2017; Quinn et al., 2023; Zheng et al., 2017). The core members *G. apicola*, *S. alvi*, *B. asteroides*, *Bartonella apis* and *Bombella apis* contain the pathways for synthesis of most amino acids (Bonilla-Rosso & Engel, 2018; Zheng et al., 2019). Moreover, certain strains of *Gilliamella* and *Snodgrassella* harbor urease genes that allow these bacteria to recycle nitrogen from excretory urea and transform into both essential and non-essential amino acids (Kwong et al., 2014; Kwong & Moran, 2016; Li et al., 2022; Quinn et al., 2023). While it is still unknown if all amino acids produced by the gut microbiota are absorbed by the host, the levels of many amino acids were reported to be increased in the hemolymph and brain of CL bees (Liberti et al., 2022; Zheng et al., 2017). Among them, **isoleucine** and **phenylalanine** were shown to modulate bees' feeding behavior and learning performance when supplemented in the diet (Simcock et al., 2014). This effect might be mediated by an increased expression of peptides from the nutrient sensing insulin/insulin-like pathway in the fat body as observed in bees fed or injected with a mixture of essential and non-essential amino acids (Ihle et al., 2014; Nilsen et al., 2011). Meanwhile, the abundance of **serine**, **ornithine** and **tyrosine** in the brain correlated with the number of social interactions in honey bees (Liberti et al., 2022). Serine and ornithine were also more abundant in the brains of CL bees, who exhibited increased social interactions, compared to MF bees (Figure 1). Serine plays an important role in excitatory glutamatergic neurotransmission as a co-agonist required for the binding of the excitatory neurotransmitter glutamate on its N-Methyl-D-aspartate-type (NMDA) receptors (Wolosker, 2006). Interestingly, **glycine**, which is also a co-agonist of these receptors, was upregulated in the brains of CL bees (Liberti et al., 2022). Glycine was shown to modulate circadian rhythms in *Drosophila* (Frenkel et al., 2017), but has received little attention in honey bees so far. **Glutamine**, a precursor and metabolite of glutamate (Aldana et al., 2020), was more abundant in the hemolymph and brain of CL bees while **glutamate** itself was only found more abundant in their gut (Figure 1). Altogether, the available evidence suggests that gut bacteria regulate glutamatergic neurotransmission via the glutamine-glutamate shuttle and by providing amino acid that act as co-agonists of glutamate receptors. Upregulated glutamatergic neurotransmission might support the increased olfactory learning and memory performances of CL bees (Leboulle, 2013).

3.2 | Neurotransmitters

Numerous studies have provided evidence that gut microbes can produce neurotransmitters, such as dopamine, serotonin, and γ-aminobutyric acid (**GABA**) (Otaru et al., 2021; Strandwitz et al., 2019; Valles-Colomer et al., 2019). In the human gut, GABA can be produced from glutamate by *Lactobacillus* and *Bifidobacterium* (Strandwitz et al., 2019; Yunes et al., 2016). In honey bees, contradictive results have been reported. Consistently with humans, increased levels of GABA have been observed in the gut of bees mono-colonized with *B. asteroides* W8111 (Wu et al., 2021) and *Bombilactobacillus* W8089 (Zhang, Mu, Shi, et al., 2022), as well as in the gut of CL bees relative to MF bees (Zheng et al., 2017). However, a different study did not detect any significant differences in the levels of GABA in the gut of CL bees, or bees mono-colonized with different strains of *B. asteroides* or *Bombilactobacillus* compared to MF bees (Kešnerová et al., 2017). Furthermore, in vitro assays did not show production of GABA by these bacteria (Kešnerová et al., 2017). While strain variability in the possession of the enzymatic machinery required to produce GABA, as demonstrated in *B. asteroides* (Wu et al., 2021), is a possible explanation for the contradictory results, another plausible contributor is the metabolism of GABA by other gut symbionts like *Snodgrassella* (Quinn et al., 2023).

GABA is a major inhibitory neurotransmitter in the bee brain and its receptors have been detected in various brain regions (Bicker, 1999). As such, it orchestrates a broad range of behavior including odor discrimination and memory formation in honey bees (Froese et al., 2014; Mustard, 2020; Szyszka et al., 2005). Behavioral effects of orally administered GABA suggest that bacterial GABA can act on the nervous system, either locally or via the systemic

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circulation (Carlesso et al., 2021). Addition of GABA in the diet before an appetitive olfactory conditioning significantly reduces bees' olfactory learning performance (Carlesso et al., 2021). A single study so far compared the brain metabolic profiles of MF and CL bees and did not find any significant difference in the abundance of GABA (Liberti et al., 2022). Yet, it was significantly more abundant in the brain of bees mono-colonized with *B. asteroides* W8111 (Wu et al., 2021) and *Bombilactobacillus* (Zhang, Mu, Shi, et al., 2022) suggesting either that bacterial GABA can travel to the brain or that these bacteria triggered GABA production by the host. Differences in experimental design may explain that GABA levels were not increased in the brain of bees colonized with the native gut microbiota, naturally containing *Bifidobacterium* and *Bombilactobacillus* strains (Liberti et al., 2022). The neurotransmitter GABA can also be converted into succinate (Fox & Larsen, 1972) or glutamine (Hertz, 2013) in the bee brain, which would explain the observed increased levels of both amino acids (but not GABA) in the brain of CL bees (Liberti et al., 2022).

The neurotransmitters **dopamine** and **serotonin** were found less abundant in the brains of bees mono-colonized with *Gilliamella*, *Bombilactobacillus* and *Lactobacillus* Firm-5 in the study by Zhang, Mu, Shi, et al. (2022). While their abundance in the brain of CL bees has not been assessed using such targeted metabolomics, decreased levels of these neurotransmitters would explain some of the behavioral differences observed between MF and CL bees. Indeed, brain injections of dopamine and serotonin reduce appetitive olfactory memory retention in hive bees and dopamine also reduces bees' sucrose responsiveness (Scheiner et al., 2006). Other behaviors such as defense behavior and food wanting are under the control of serotonin or dopamine but their modulation by the gut microbiota has never been demonstrated (Huang et al., 2022; Nouvian et al., 2018).

3.3 | Hormones

Juvenile hormones are an important class of sesquiterpenoids that regulate insect development and behavior, including their division of labor (Robinson, 1987). In bees, **juvenile hormone III (JHIII)** regulates the age-related transition from young nurse bees to older forager bees, with higher titers in foragers compared to nurses. Intriguingly, Kešnerová and colleagues found that CL bees have increased levels of JHIII derivatives in the gut, specifically by *B. asteroides* (Kešnerová et al., 2017). This may be an indication that the gut microbiota regulates behavioral maturation in bees. JHIII was also shown to increase sucrose responsiveness, which is consistent with both the high sucrose responsiveness of foragers compared to nurses (Pankiw & Page, 2003; Scheiner et al., 2017; Wang et al., 2012) as well as the increased sucrose responsiveness of CL bees compared to MF bees. These effects of JHIII might be mediated by changes in the expression of genes belonging to the insulin/insulin-like signaling pathway in the fat body of bees (Nilsen et al., 2011; Wang et al., 2012). This pathway is involved in the regulation of division of labor in honey bees, promoting the transition from nursing to foraging (Ament et al., 2008; Mott & Breed, 2012). Yet, the impact of the gut microbiota on the nurse to forager transition has not been investigated.

3.4 | Organic acids

In honey bees, the gut microbiota core members *Gilliamella*, *Bifidobacterium*, and *Lactobacillus* Firm-5 are particularly efficient at breaking down diet polysaccharides into smaller, more easily digestible molecules (Bonilla-Rosso & Engel, 2018; Brochet et al., 2021; Kešnerová et al., 2017; Zheng et al., 2017). By fermentation they produce organic acids such as lactate, succinate, and the short-chain fatty acid (SCFA) acetate, which accumulate in the hindgut (Figure 1).

While little is known about how SCFAs affect neuronal function in honey bees, this class of metabolites has been highlighted as a key player of the gut–brain communication in mammals (Dalile et al., 2019; Krautkramer & Fan, 2021). Butyrate, propionate, and acetate are known inhibitors of histone deacetylases (HDAC) and therefore promote histone acetylation and gene expression (Dalile et al., 2019; Krautkramer & Fan, 2021). Butyrate is commonly used to inhibit HDAC activity in rodents and was shown to promote memory retention (Dalile et al., 2019). In contrast, injections of sodium butyrate in the honey bee brain led to aversive memory impairments (Lockett et al., 2014). Moreover, SCFAs were not detected as differentially abundant in the brains of CL bees compared to MF bees, raising the question of their absorption through the blood–brain barrier in bees (Table S1). SCFAs might instead act locally on the enteric neurons or intestinal epithelial cells. A single study so far suggested that organic acids, including SCFAs, may modulate food intake via their local action on enteroendocrine cells (Ricigliano & Anderson, 2020). Feeding a mixture of organic acids (acetate, lactate, butyrate, formate, and succinate) to pollen-deprived honey bees modified the expression of genes coding for the neuropeptide-F and allatostatins, which are neuropeptides regulating food intake in bees. Allatostatins are also inhibitors of JHIII production and important mediators of stress response (Sánchez-Morales et al., 2022) and stress-induced olfactory learning deficits (Urlacher et al., 2017).

3.5 | Nucleosides

Finally, the nucleoside **adenosine**, whose abundance was higher in the gut of CL bees (Figure 1), is also an important neuromodulator found in the extracellular space in mammalian brain tissues (Dunwiddie & Masino, 2001). Adenosine and other nucleosides can be produced by gut bacteria from the breakdown of available nucleotides, in particular, members of *Bifidobacterium* and *Lactobacillus* have been described as adenosine producers (Li et al., 2023; Mager et al., 2020). Furthermore, Chen et al. (2021) found that adenosine modulates brain function in honey bees (Chen et al., 2021) and that infection with the common deformed wing virus reduced adenosine concentration in the brain, altered the expression profile of genes related to neurotransmission and impaired olfactory long-term memory (Chen et al., 2021). Oral

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administration of adenosine to infected bees restored gene expression and memory performance to the control level.

4 | CONCLUSIONS AND PERSPECTIVES

There is now evidence that the gut microbiota influences the behavior of bees and may be key for the social lifestyle of this animal, making it a highly interesting field of study. The simplicity of the microbiota's composition and its ease of manipulation offer exciting opportunities to unravel the underlying biological mechanisms that govern bees' behavior. We provided an overview of the microbial and host-derived metabolites regulated by gut bacteria that are known to affect brain functions and behavior in honey bees. The identification of differentially abundant metabolites in the gut of MC and CL bees compared to MF bees shed light on the metabolic activity of the gut microbiota. Metabolites present in the gut of bees colonized with a defined community do not seem to be the sum of those present in MC bees (Kešnerová et al., 2017). Consistent with this hypothesis, a recent study showed that the increased learning and memory performances of bees colonized by a defined and simplified community of core gut bacteria could not be recapitulated in bees mono-colonized by single members of this community (Cabirol et al., 2023). Thus, even use of simplified communities to generate gnotobiotic bees will help disentangling the metabolic interactions in the gut and their effects on the host. However, selecting representative strains of a genus to create a community for gnotobiotic bee experiments remains challenging, as closely related strains sharing high nucleotide identity may still harbor different functional capabilities (Brochet et al., 2021).

A single study has so far compared the metabolic profiles in the brain of CL and MF bees (Liberti et al., 2022). Additional data using a combination of metabolomic techniques are required to detect most metabolites whose abundances in the gut, hemolymph and brain are modified by the gut microbiota. While omics approaches are descriptive and correlative, the most definitive methods for proving the bacterial origin of metabolites differentially abundant in the brain of CL and MF bees would utilize stable isotopic tracers (i.e., ^{13}C, ^{15}N, or ^{2}H) (Neufeld et al., 2007). To establish causation between a given bacterial metabolite and behavioral or brain phenotypes, honey bees can be fed a simple sugar diet supplemented with the metabolite of interest as previously shown (Zhang, Mu, Cao, et al., 2022). Also, recent advances in genome editing of honey bees and their gut symbionts offer new approaches to identify the genetic basis of metabolic interactions influencing the gut–brain axis (Carcaud et al., 2023; Chhun et al., 2023; Lang et al., 2023; Leonard et al., 2020; Schulte et al., 2014).

Finally, diet plays an immense role in shaping the gut environment, not only because it dictates the immediate amount and quality of bacterial substrates, but also because it can modify the microbiota community composition (Krautkramer & Fan, 2021). Changes in macronutrient levels, in particular the dietary protein-to-carbohydrate ratio, influence sucrose responsiveness, feeding behavior and learning and memory in honey bees (Bouchebti et al., 2022; Ihle et al., 2014). Future research should therefore consider diet, the gut microbiota and the host gut environment as a tripartite system modulating the level of neuroactive metabolites.

AUTHOR CONTRIBUTIONS

Amélie Cabirol: Conceptualization; investigation; methodology; validation; visualization; writing – review and editing; writing – original draft. **Silvia Moriano-Gutierrez:** Investigation; writing – original draft; visualization; writing – review and editing; validation; methodology; conceptualization. **Philipp Engel:** Conceptualization; writing – review and editing; supervision; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ETHICS STATEMENT

No formal consent or approval was required for this review.

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SUPPORTING INFORMATION

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