When the society dictates food search – Neural signalling underlying appetitive motivation in honey bees

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In honey bees, appetitive motivation is primarily driven by the needs of the colony rather than individual needs. The regulation of appetitive behavior is achieved through the coordinated action of neuropeptides, hormones and biogenic amines, which integrate multiple signals to ensure appropriate appetitive responses. Dopamine signalling underpins a food-related wanting system that is sensitive to aversive experiences. The short neuropeptide F (sNPF) enhances appetitive responsiveness, food intake and behavioral and neural responsiveness to food-related odorants. Additionally, it facilitates appetitive learning and memory. On the contrary, tachykinin-related peptides (TRPs) inhibit appetitive responses. Physiological changes during the transition to the foraging state lead to distinct patterns of insulin and adipokinetic hormone (AKH) signaling, different from those seen in solitary insects, indicating that social life had significant consequences on the systems controlling appetitive motivation. Overall, studying the neural bases of appetitive behavior in bees reveals unique aspects that arise from their social lifestyle.

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Animal survival depends on meeting basic needs such as food, water, reproduction, and sleep, among others. Pursuing these objectives relies on motivational drives that energize behaviors leading to desired goals, which are inherently rewarding [1]. Appetitive motivation, which drives food-seeking behavior, integrates food-related goals and excitatory drives that guide foraging [2]. The regulation of appetitive behavior is orchestrated by the coordinated action of molecules (peptides, hormones, neurotransmitters, etc.), acting within specific neural circuits that integrate multiple signals to ensure coordination between the external and internal environments [3].

In insects, extensive research on the neural bases of appetitive behavior has been conducted in the fruit fly *Drosophila melanogaster* [4–6]. Yet, relying exclusively on fruit flies may overlook fundamental evolutionary differences between them and other insects, which vary in key aspects of appetitive motivation. For example, social insects display a critical distinction from solitary insects like fruit flies: their appetitive food search is driven primarily by the needs of the colony rather than individual hunger [7–9]. While food provisioning is a key aspect of parental care found in many species beyond social insects, a crucial difference in social insects like honey bees is that the foragers are not the parents. This difference highlights a fundamental contrast with other species and presents a fascinating research area that combines sociality and individual motivation — one that cannot be fully explored through *Drosophila* research alone.

Here we focus on honey bees, which have pioneered studies on the neurobiological bases of insect behavior [10–15], and we review recent findings related to the neural regulation of their appetitive motivation. We focus on signalling pathways involving biogenic amines and neuropeptides, which are modulated according to the phases of the foraging cycle [16] and play a crucial role in the appetitive behavior of bees.

A new perspective for dopamine in appetitive motivation
Previous studies indicated that the biogenic amine octopamine (OA) mediates the reinforcing properties of

sucrose solution in appetitive olfactory learning. Activation of the sucrose-responding octopaminergic neuron VUMmx1 *via* intracellular current injection following odorant presentation resulted in olfactory learning even if no sucrose was ever delivered [17]. This result showed that OA substitutes for sucrose reward during olfactory conditioning. Accordingly, brain injections of OA following odorant presentation also supported olfactory learning [18]. OA levels also increase in foragers [19] and OA modulates reward representation during waggle dances [20], which is consistent with a role in appetitive reinforcer representation.

Dopamine (DA), on the contrary, had been shown to mediate the reinforcing properties of aversive reinforcers such as electric shock in honey bees [12,21]. In aversive olfactory conditioning, blockade of DA receptors impairs aversive learning [21]. Yet recent, studies have changed this perception by including a motivational perspective absent in previous analyses on the role of DA. This perspective separated the processes of *wanting*, *liking* and *learning-related reward* [22]. While most of the above-mentioned studies relate to the process of reward in *learning*, processes such as *liking*, which refers to the hedonic impact of a pleasurable reward, and *wanting*, which refers to the motivation to seek the reward itself, had been rarely studied separately. In mammals, wanting and liking are supported by different brain systems and neuromodulatory circuits [22]. In honey bees, two recent studies revealed that DA is a key neurotransmitter for appetitive wanting [23,24]. Huang et al. [23] showed that distinct food-related motivational and communication states determine different levels of DA in the brain of foragers, consistently with the existence of a DA-based wanting system activated by both colony and individual needs. Foragers motivated to collect food exhibit higher levels of DA in the brain and pharmacological blockade of dopaminergic signalling decreases foraging, consistently with the inhibition of a DA-based wanting system [22,25,26]. DA brain levels also increase when foragers report distance and direction of a profitable food source *via* the waggle dance, which suggests that during dances, bees reactivate motivation for the appetitive properties of the food source (Figure 1). Individual starvation also increases DA brain levels, thus showing that besides a colony-driven DA wanting, an individually hunger-driven DA wanting also exists [23]. Consistently, artificial DA enhancement rescues appetitive responsiveness in partially fed bees and improves appetitive learning and memory [23]. A technical discussion of these findings is available in Refs. [27,28].

Negative experiences in a foraging context also affect significantly appetitive dopaminergic wanting [24]. Honey bees exposed at a feeder to predatory hornets increase alarm and escape responses and decrease significantly foraging activities, staying longer in the

Figure 1
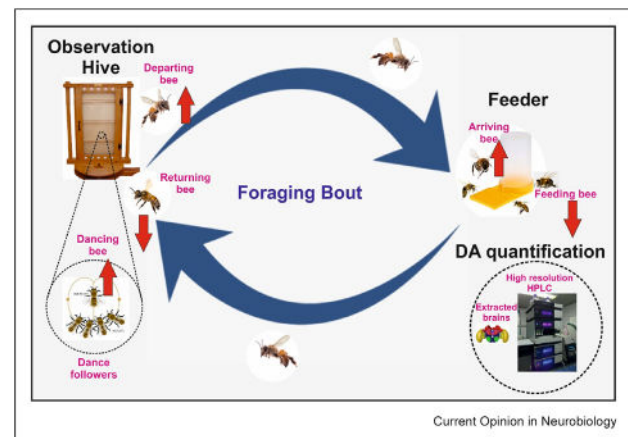
A foraging bout showing dopamine (DA) fluctuation (red arrows) quantified in individual bee brains using high resolution HPLC. Foragers leave the hive with elevated DA levels, which are present upon arrival at a known profitable food source, consistently with an appetitive DA-driven wanting system. Feeding consummates the appetitive goal and decreases DA levels so that returning bees have also lower DA levels. During dances reporting an exploited, profitable food source, dancers exhibit enhanced DA levels, which suggests that dancers recollect transiently the appetitive properties of the food source, elevating thereby DA levels in their brains. Adapted from Ref. [23].

hive. Waggle dances for that feeder are also reduced *via* stop signals used to cease dancing and recruitment to a dangerous place. As expected, DA levels in the brain of motivated dancers are higher than those of control bees. Yet, stop signallers, which experienced the presence of hornets, exhibit decreased DA levels. Besides, receivers, which did not experience hornet attacks themselves but got stop signals, also present reduced DA levels [24]. Overall, these findings show that aversive experiences decrease appetitive motivation and DA in the bee brain and that communicating about negative experiences induces the same effect.

The existence of a dopaminergic wanting system is consistent with reports showing that levels of DA reach a peak at the foraging stage [29] and that honey bee dancers have higher levels of DA [30], a fact that was originally related to the regulation of sensory information and processing of locomotory information [30] rather than to motivational levels. Different populations of dopaminergic neurons [31] may mediate appetitive wanting and aversive reinforcement signalling, reconciling the two different functions reported for DA.

As mentioned above, besides DA, OA has also been shown to increase in foragers [19] and to mediate waggle dances [20] despite being present in lower quantities compared to DA [30]. This raises the question of the specific contribution of DA and OA to appetitive motivation. A possible answer is provided by a recent work in which bees had to decide whether to rely on their own

experience of a rewarded feeder or on dances reporting an alternative, unknown feeder [32]. When bees were fed with sucrose solution containing OA, they paid less attention to the social information provided by dances indicating the unknown feeder and reactivated the visits to their known feeder; on the contrary, if they were fed with DA, they attended more the dances for the unknown source. This suggests that OA may reactivate learning-dependent circuits, which rely on OA to signal reward [17] (i.e. feeder memories), thus leading to prioritization of individual experience while DA, reflecting appetitive motivation, would be evoked by feeder reminiscences and by social information. Both processes may underlie the foraging and communication of motivated bees.

sNPF, a neuropeptide driving appetitive responses

Neuropeptides range from a few to around 100 amino acids, and are secreted by a large variety of neurons of the central nervous system. They act as neuro-modulators [33] and regulate multiple behaviors [34,35]. Among these molecules, the neuropeptide F (NPF) [36−38] and the short neuropeptide F (sNPF) [39−41] may either promote or inhibit feeding and/or food search depending on the insect species considered.

In honey bees, two peptides NPF and sNPF, have been identified. However, only a receptor gene for sNPF (*snpfR*) was found [42,43]. Accordingly, recent studies [44,45] focused on the role of sNPF for appetitive decision-making and learning, as well as for aversive responsiveness. Foragers differing in feeding status (starved *vs.* partially fed) and in sNPF levels, which were varied by subjecting partially fed bees to topical applications of sNPF on their thorax [46] were used (Figure 2a). They were subjected to tests quantifying appetitive responding either *via* individual consumption of sucrose solution or *via* proboscis extension response (PER) upon antennal stimulation with sucrose solutions of different concentrations [44]. As expected, starved bees were more responsive to sucrose and consumed more sucrose solution than partially fed bees. Yet, increasing internal levels of sNPF in partially fed bees increased their responsiveness and food consumption to the levels of their starved counterparts [44] despite the presence of food in their crops (Figure 2b,c). Appetitive responding (PER) to odorants with intrinsic appetitive value [47] yielded a similar picture: starved animals were more responsive than partially fed ones but increasing sNPF levels rescued in part responding in the latter (Figure 2d). To search for a neural correlate of this differential odor responding, the activity of the olfactory circuits in the bee brain was recorded using calcium imaging [44]. Projection neurons conveying olfactory information from the first olfactory neuropil, the

Figure 2
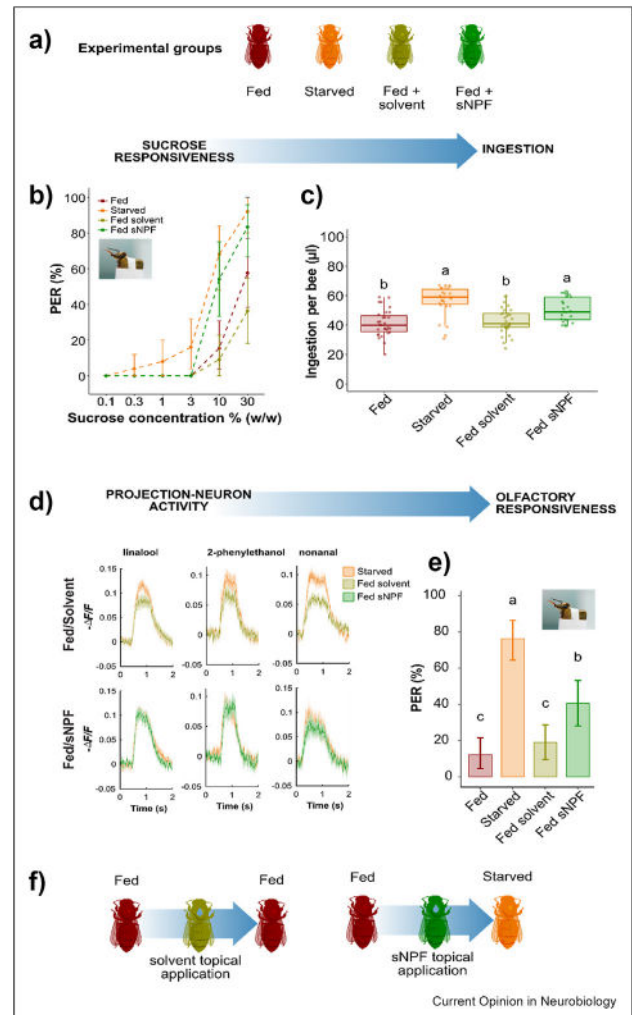
The effect of sNPF on appetitive responses of honey bee foragers. **a) Experimental groups of foragers.** Foragers were either starved or partially fed. The latter received a topical application of a solvent or of sNPF. **b) Sucrose responsiveness.** Harnessed bees were tested on a series of increasing sucrose concentrations. The proportion of proboscis extension response (PER) was higher in starved and partially fed foragers treated with sNPF than in partially fed bees, untreated or treated with the solvent. **c) Ingestion.** Starved and partially fed foragers treated with sNPF ingested more sucrose solution than partially fed bees, untreated or treated with the solvent. **d) Activity of projection neurons (PNs) upon odor stimulations**. Using *in vivo* calcium imaging, the glomerular responses to linalool, 2-phenylethanol and nonanal were visualized before and after feeding + topical application of solvent or sNPF. Feeding decreases the neural activity of olfactory PNs in the bee brain while sNPF rescues neural responses of partially fed bees to the level exhibited by starved bees. **e) Olfactory responsiveness.** Harnessed bees were stimulated with appetitive odours (linalool and 2-phenylethanol) that naturally trigger PER. The proportion of PER was higher in starved and in partially fed bees treated with sNPF. **f) General conclusions.** Topical application of solvent does not change appetitive behaviour of partially fed bees as these bees behaved like their untreated partially-fed counterparts. However, a topical application of sNPF on partially fed bees makes them behave like starved foragers. Adapted from Ref. [44].

antennal lobe, to higher-order olfactory centers, such as the mushroom bodies and the lateral horn, responded differently to odorants according to the animal's feeding state. Responses were lower in partially fed animals than in starved animals; yet, sNPF treatment rescued neural responsiveness in partially fed bees to the level of starved ones (Figure 2c), thus showing that sNPF modulates, not only feeding-related responses, but also sensory responsiveness. The role of sNPF in aversive responding was studied by recording the sting extension response (SER) exhibited by honey bees in response to nociceptive stimuli such as electric and thermal shocks. Aversive responsiveness to both kind of stimuli was unaffected by sNPF, thus showing that contrary to fruit flies, where NPF promotes resilience to stress and aversive stimuli [48], sNPF does not control aversive responding in bees [49,50].

Finally, the impact of sNPF on visual learning was studied in flying bees differing in feeding state (starved vs. partially fed) and in sNPF levels [45]. Bees were conditioned to discriminate a yellow from a blue target in a miniature maze. Each trained bee remained in the maze for the entire training as returning to the hive and unloading the food gathered would change the crop contents, the motivational state and possibly sNPF levels. After completing the training, bees were subjected to a memory test. Artificially increasing sNPF levels in partially-fed foragers with a reduced motivation to learn colors resulted in significant color learning and memory above the levels exhibited by starved foragers [45]. These results thus identify sNPF as a critical component of motivational processes involved in foraging and in the cognitive processes associated to this activity.

Tachykinin, an inhibitor of appetitive responses

Tachykinin is a member of the structurally related RF-amide peptide family referred to as tachykinin-related peptides (TRPs) [51]. In honey bees, TRPs are predominantly expressed in the mushroom bodies and in some neurons of the antennal and optic lobes [52]; some expression has also been found in the antennae [53]. Tachykinin has been recently studied in two honey bee species, *Apis mellifera* and *Apis cerana*, in which appetitive responses (PER) of nurses, pollen foragers and nectar foragers to brood, pollen and sucrose contacting their antennae were quantified upon TRP injection and RNAi-mediated knockdown of the TRP receptor (TRPR) [54] (Figure 3). TRP signalling regulated responsiveness to these three types of stimuli in a task-specific manner: for instance, injection of the tachykinin related peptide TRP2 decreased sucrose responsiveness in nectar and pollen foragers but not in nurse bees. On the contrary, the same injection decreased PER to antennal larval stimulation in nurses but neither in pollen nor in nectar foragers. RNAi-mediated

knockdown of TRPR had the opposite effect: it increased sucrose responsiveness in nectar and pollen foragers but not in nurse bees, and increased responsiveness to larval stimulation in nurse bees but neither in pollen nor in nectar foragers [54]. Thus, compared to sNPF, TRP signalling seems to act in an opposite way, inhibiting rather than promoting appetitive responses.

Leucokinin, a role to be clarified

In *D melanogaster*, leucokinin modulates taste detection [55] and signals that the crop is full, prompting the cessation of meal ingestion [56]. Its signalling occurs *via* insulin like peptides [57,58] and *via* serotonin, which diminishes the activity of leucokinin neurons [59]. In the Asian honey bee *A. cerana*, the relative expression of the leucokinin receptor gene (*Lkr*) was higher both in the antennae and brain of foragers compared to those of nurse bees, thus suggesting a role in foraging activity [60]. RNAi knockdown of *Lkr* induced a significant increase in the sucrose response threshold when sucrose solution stimulated the bees' antennae, thus suggesting that signalling through the leucokinin receptor confers high sucrose sensitivity while blockade of this signalling reduces it [60]. Intriguingly, the same results were obtained when sucrose solutions were brought close to the antennae without physical contact [60]. This result can be due to a change in sensitivity of antennal hygro-receptors, thus asking for more experiments to disentangle this effect from that reported on sucrose responsiveness.

Insulin plays a different role in honey bees than in solitary insects

The insulin/insulin-like growth factor signalling (IIS) is activated by the interaction between insulin-like peptides (ILPs) and their respective insulin receptors (InRs) [61]. Invertebrate ILPs have functions homologous to those of the insulin and insulin-like growth factor 1 ligands found in mammals, i.e. they advertise a satiated individual that it does not need food by relaying information about glycogen levels to the brain [61]. In insects, ILPs are produced in the brain and peripheral tissues such as the fat body, and circulate either as hormones or neuromodulators [61], impacting nutrient-related and nutrient-unrelated behaviors [62]. In *D. melanogaster*, elevated circulating levels of ILPs following food consumption inhibit the action of sNPF expression in the olfactory sensory neurons, resulting in less sensitivity to food associated odors and suppression of food searching behavior [63].

This scheme is different from that of honey bees, which experience a series of physiological changes before transitioning to foraging activities, which include a massive reduction of fat bodies and their associated lipid stores [64,65]. The reduction of the fat body is accompanied by an increase in juvenile hormone (JH) [66] and

Figure 3
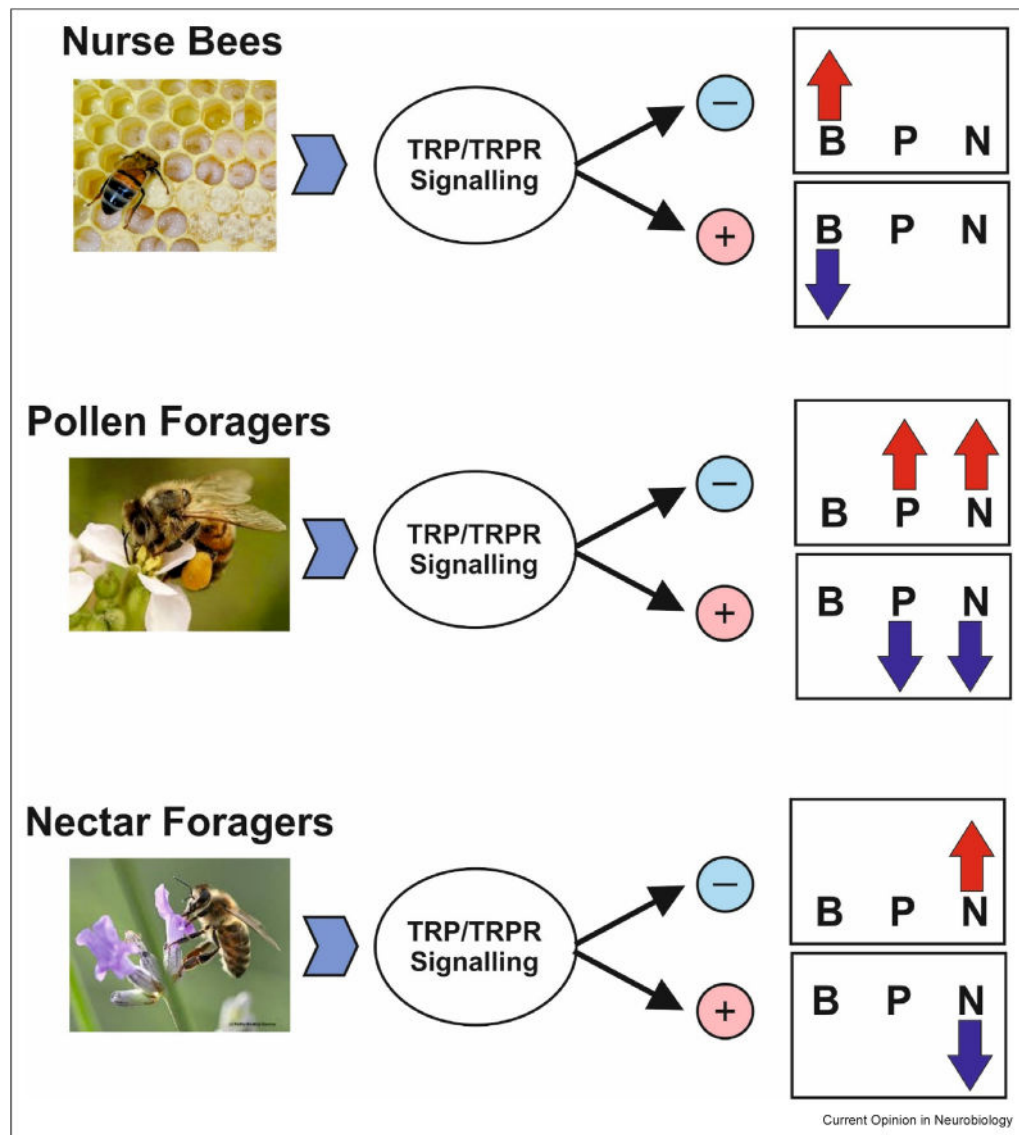
The effect of the tachykinin pathway (signalling *via* the tachykinin-related peptide [TRP] and its receptor [TRPR]) on responsiveness of nurses, pollen foragers and nectar foragers to brood (B), pollen (P) and nectar (N). The tachykinin pathway affects responsiveness in a caste and task-specific manner. Pathway activation decreases brood responsiveness in nurses but has no effect on their sucrose and pollen responsiveness; in pollen foragers, pathway activation decreases pollen and nectar responsiveness but has no effect on brood responsiveness; in nectar foragers, pathway activation decreases nectar responsiveness but has no effect on brood nor on pollen responsiveness. '-' indicates signalling inactive (light-blue circle); '+' indicates signalling active (pink circle). Vertical red arrows pointing upwards indicate increase in responsiveness; vertical blue arrows pointing downwards indicate decrease in responsiveness. Adapted from Ref. [54].

ILP levels [67,68]. Foragers have higher whole-body IIS activity compared to younger workers, which relates to their higher brain IIS [69] but lower fat body IIS [70].

Two genes encoding ILPs (*ilp1* and *ilp2*) have been found in bees, which are expressed in the brain and the fat body [67,69]. The corresponding insulin peptides ILP1 and ILP2 affect differentially female caste development, with ILP2 affecting ovary and body-mass development and ILP1 regulating JH production [71]. In bees, the role of insulin in appetitive decisions is less clear and varies with age. Insulin-related signalling seems to control food choice as knock-down of the insulin receptor substrate (*irs*) gene leads to a preference for lipid and protein-rich pollen over nectar [72]. Injection of insulin in young bees determines an increase in responsiveness to odorants and to sucrose solution but a decrease in learning abilities. In older bees, the effects of insulin injection are the opposite [73].

Adipokinetic hormone (AKH), a limited action as a consequence of the social-life style

The adipokinetic hormone (AKH) of insects is considered as an equivalent of the mammalian hormone glucagon as it induces fast mobilization of carbohydrates and lipids from the fat body upon starvation [74−76]. Yet, contrary to solitary insect species, which rely on these processes for sustaining their activities, bees do not collect food for individual consumption but bring it back to the hive, where it is processed, stored and distributed. Moreover, foragers have a reduced fat body [77] with low glycogen reserves [78] and with a significant reduction in abdominal lipids preceding the onset of foraging [64,79]. To sustain foraging flights, bees load minute amounts of honey in the crop before departing from the hive [78,80]. These features indicate that the social life of bees has important consequences for the regulation of their metabolic pathways. Accordingly, no physiological response to AKH injection was detected in active foragers [81] so that it was suggested that AKH might have lost its original function in social bees [82].

To test this hypothesis, bees differing in their energy budget (starved or partially fed) were topically exposed with different doses of AKH to determine if this hormone modified food ingestion and sucrose responsiveness [83]. As expected, starved bees were more prone to ingest and to respond to sucrose solution. Yet, no effect of AKH could be detected [83]. These results are consistent with a loss of function of AKH in honey bee foragers, in accordance with a social life that implies storing energy resources in the hive, in amounts that exceed individual needs. More experiments are needed to elucidate if AKH plays a specific role in nonforaging bees.

Open questions and outlook

Neuropeptides and neurohormones are key regulators of appetitive behavior in honey bees, influencing the motivation, reward processing, and learning associated with foraging activities (Figure 4). In this scenario, distinguishing between wanting, liking and learning-related reward processing is important to disentangle the different contributions of these different forms of signalling to appetitive decision making. Further neuropeptides such as inotocin, which modulates foraging in ants in the presence of larvae or pupae [84], await investigation in bees. Two essential questions remain to be elucidated: 1) which specific circuits in the bee nervous system provide these different forms of signalling and if and how they interact, and 2) given that appetitive motivation responds largely to colony needs, which are the social cues modulating these different forms of signalling.

The identity of dopaminergic neurons underlying motivational wanting remains to be identified among the numerous clusters of dopaminergic neurons existing

Figure 4
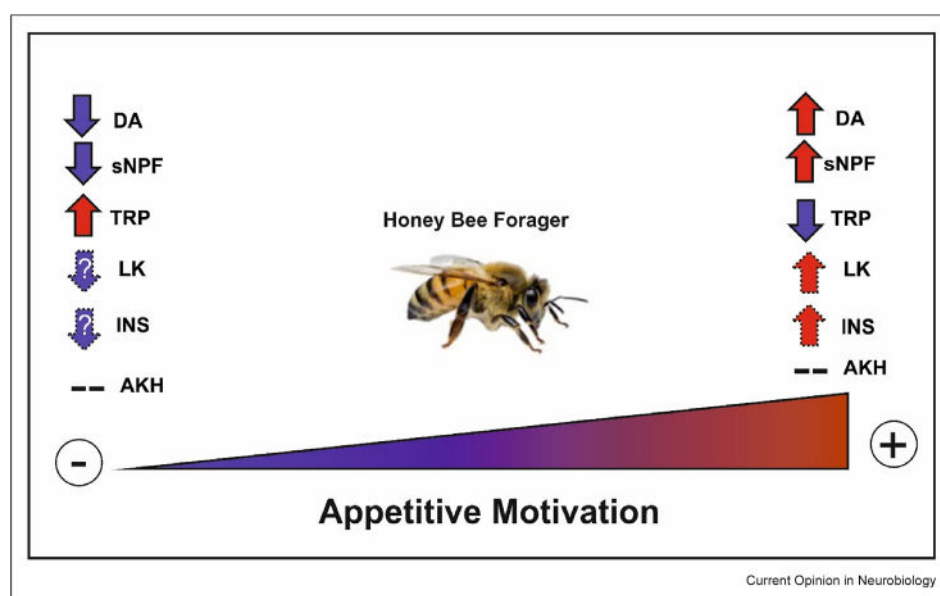
A summary of the different forms of neural signalling discussed in this work and their effect on appetitive foraging motivation. A gradient of appetitive motivation (from low, blue, to high, red) is shown at the bottom. DA: Dopaminergic signalling; sNPF: sNPF signalling; TRP: tachykinin related peptide signalling; LK: leucokinin signalling; INS: insulin-like peptide signalling; AKH: adipokinetic hormone signalling. Upward red arrows indicate increases; downward blue arrows indicate decreases; dashed arrows (upward or downward) indicate that the supposed increases or decreases remain to be verified. The question sign ('?') within an arrow indicates that the supposed effect remains to be verified. The sign '−' (AKH) indicates lack of effect in a foraging context.

in the bee brain [31]. In the same way, if and how these neurons interact with sNPF signalling awaits clarification. In fruit flies, the relationship between sNPF (or NPF) signalling and dopaminergic neurons in appetitive responding and learning has been shown both in the larva and in adult flies [85–87]; a similar knowledge is still missing in the bee. While an experimentally supported model for the relationship between midgut-derived NPF and AKH and insulin-like peptides (ILPs) exists in *Drosophila* [88], a similar model is not available for bees, given the difficulty of integrating the different function of ILPs and the loss of function of AKH.

The appealing unanswered question is if and how social cues within the hive modulate the levels of these multiple signalling channels to control appetitive motivation and foraging activities. Appetitive pheromones, i.e. pheromones promoting foraging activity, such as brood pheromones, may be responsible for modulating some of the pathways discussed in this review [89]. Pheromones affect appetitive responsiveness [90] and appetitive learning and memory [91], thus being candidates for modulating motivational pathways. If this were the case, it would constitute a fascinating example of social control of neural motivational pathways, and a cornerstone of sociality. Further research in this field, combining pheromone exposure, control of nutritional states, and molecular analyses such as spatial transcriptomics in key brain regions, promises to deepen our understanding of insect behavior and may lead to the development of strategies to enhance pollination and agriculture.

Glossary

Three main components in the processing of appetitive rewards are acknowledged [22]:

- '**Liking**': refers to the actual pleasurable impact of reward consumption.
- '**Wanting**': refers to the motivation to reach the reward
- '**Learning**': includes the information about reward acquired through individual experience

Inclusion and diversity

The authors support inclusive, diverse, and equitable conduct of research.

Author contribution

Martin Giurfa and Gabriela de Brito Sanchez: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing.

Rafael Carvalho da Silva & Louise Bestea: Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing.

Declaration of competing interest

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Data availability

No data was used for the research described in the article.

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- • of special interest
- •• of outstanding interest

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