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Review

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Short neuropeptide F in integrated insect physiology

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Abstract: The short neuropeptide F (sNPF) family of peptides is a multifunctional group of neurohormones involved in the regulation of various physiological processes in insects. They have been found in a broad spectrum of species, but the number of isoforms in the precursor molecule varies from one to four. The receptor for sNPF (sNPFR), which belongs to the G protein-coupled receptor family, has been characterized in various insect orders and was shown to be an ortholog of the mammalian prolactin-releasing peptide receptor (PrPR). The sNPF signaling pathway interacts with other neurohormones such as insulin-like peptides, SIFamide, and pigment-dispersing factors (PDFs) to regulate various processes. The main physiological function of sNPF seems to be involved in the regulation of feeding, but the observed effects are species-specific. sNPF is also connected with the regulation of foraging behavior and the olfactory system. The influence of sNPF on feeding and thus energy metabolism may also indirectly affect other vital processes, such as reproduction and development. In addition, these neurohormones are involved in the regulation of locomotor activity and circadian rhythm in insects. This review summarizes the current state of knowledge about the sNPF system in insects.

Key words: Insect neuropeptide; Invertebrate neurobiology; Short neuropeptide F (sNPF); Feeding; Neurohormone

1 Introduction

Neuropeptides are multifunctional, relatively short, and the most diverse neurohormones, and function as signaling molecules in all animals (Nässel and Zandawala, 2019). In insects, they influence almost all physiological processes (Nässel and Zandawala, 2019). Short neuropeptides F (sNPFs) is a group of insect neurohormones excluded from the neuropeptide F (NPF) family based on their structural and physiological characteristics (Fadda et al., 2019). Both families belong to the RF family of peptides (Elphick and Mirabeau, 2014). Currently, it is known that sNPFs are involved in the regulation of biological processes such as feeding, molting, courtship, social interaction, learning, circadian clock, reproduction, and development (Fadda et al., 2019).

The structural similarities of sNPF and NPF yielded many confusing datasets and conclusions, especially shortly after their discovery, before insect genomic and transcriptomic data became available. The initial discovery of sNPF (initially designated also as the head peptide) was made in the midgut of the cockroach *Periplaneta americana* (Veenstra and Lambrou, 1995). Soon after, it was discovered in the brain of the beetle *Leptinotarsa decemlineata* (Spittaels et al., 1996) and the grasshopper *Schistocerca gregaria* (Schoofs et al., 2001). This was based on antisera raised against NPF of the tapeworm *Monieza expansa*, in which the first NPF was discovered using mammalian neuropeptide Y (NPY) antisera (Spittaels et al., 1996; Schoofs et al., 2001). As the discovered peptides share the C-terminal motif (-RLRFa) with NPF (RPRFa) but consist of only 8−10 amino acids, they were named “short” NPF and included in the same family. However, after identification of the NPF precursor in the model species *Drosophila melanogaster* and sequencing of its genome, it has been clarified

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that sNPF and NPF are separate families of neuropeptides encoded by separate genes, even if they share physiological functions—both are involved in the regulation of feeding. This was also supported by detailed analysis of whole precursor sequences (Vanden Broeck, 2001).

The involvement of sNPF and NPF in the regulation of feeding and digestion suggests that they are somehow evolutionarily related. The analysis of sNPF receptor (sNPFR) sequences and their alignment with NPF receptors and mammalian NPY receptors (NPYRs) showed that they cluster with mammalian prolactin-releasing peptide receptors (PrPRs) (Mirabeau and Joly, 2013) and are probably characteristic of protostomes (Fadda et al., 2019).

The major physiological role of sNPFs seems to be the regulation of feeding. This has been proven in various insect orders. However, the observed effects depend on the tested insect species and are contradictory, showing sNPF as a stimulator or an inhibitor of food consumption and foraging behavior. Furthermore, sNPF seems to be involved in other biological processes, such as energy homeostasis, which is directly related to feeding but also (probably indirectly) affects other physiological processes, such as reproduction and development. Other research has shown the involvement of sNPF in the regulation of locomotory activity and the circadian clock, as well as learning. This is probably connected with the interplay between sNPF and several other neurohormones, among which insulin-like peptides (ILPs) and pigment-dispersing factor (PDF) seem to be the most important. In this review, we summarize the current state of knowledge about sNPF precursor identification in various insects and what is already known about their signaling. Special emphasis has been placed on the involvement of these peptides in the regulation of various physiological processes in insects.

2 sNPF precursor structures in insects

Neuropeptides that have been identified in invertebrates are commonly encoded on precursor genes that are orthologs of genes identified in vertebrates (Hewes and Taghert, 2001; Vanden Broeck, 2001). In arthropods, a large number of peptides can be classified into the sNPF family. This is particularly true for insects and crustaceans (Walker et al., 2009). This is due to a rather short peptide sequence, yet information on the prepropeptides is scarce for arthropods other than insects. While *NPF* genes are conserved across bilaterians, the genes encoding sNPF have been characterized only in arthropods, despite the identification of an sNPF-related receptor in mollusks (Bigot et al., 2014).

All insects studied to date have peptides classified as sNPF. However, some species have a few isoforms derived from one precursor. For example, the *D. melanogaster* precursor gene encodes four sNPF isoforms (Broeck, 2001), and that of *L. decemlineata*, two isoforms (Spittaels et al., 1996). In other species, the gene encodes only one mature peptide on the prepropeptide, as in the coleopterans *Tenebrio molitor* and *Zophobas atratus* (Marciniak et al., 2022) (Fig. 1). The only exception is the mosquito *Aedes aegypti*, which has two genes encoding precursors for sNPF (Predel et al., 2010). In this species, one precursor encodes so-called head peptides, and the second precursor encodes true sNPF (Predel et al., 2010) (Fig. 1). This shows that the gene structure encoding insect sNPF precursors is highly variable, depending on the species. The number of introns presented in the gene sequence ranges from two to four, and the number of exons is also variable, ranging from three to five, depending on the tested species (Amir et al., 2022). Despite such diversity, the genes encoding sNPF have some similarities, as the first intron is always located after the signal peptide (Amir et al., 2022).

The peptides belonging to the sNPF family are highly conserved and have a characteristic xPxLRLR-Famide sequence at their C-terminus. However, a modified C-terminal RWamide sequence is present in some insects, such as flies and mosquitos (Nässel and Wegener, 2011) (Fig. 2). Studies in larvae and adults of *D. melanogaster* have demonstrated that a conserved C-terminal sequence is necessary for receptor activation (Mertens et al., 2002) and that peptides with the RFamide sequence have higher affinity for the receptor than those with the RWamide sequence (Feng et al., 2003; Reale et al., 2004). As shown in myocardial studies, the presence of proline is necessary for maintaining the bioactivity of sNPF. This amino acid most likely induces a bend in the peptide structure, which affects binding to the receptor in tissues of two tenebrionid beetles (Marciniak et al., 2008). Until recently,

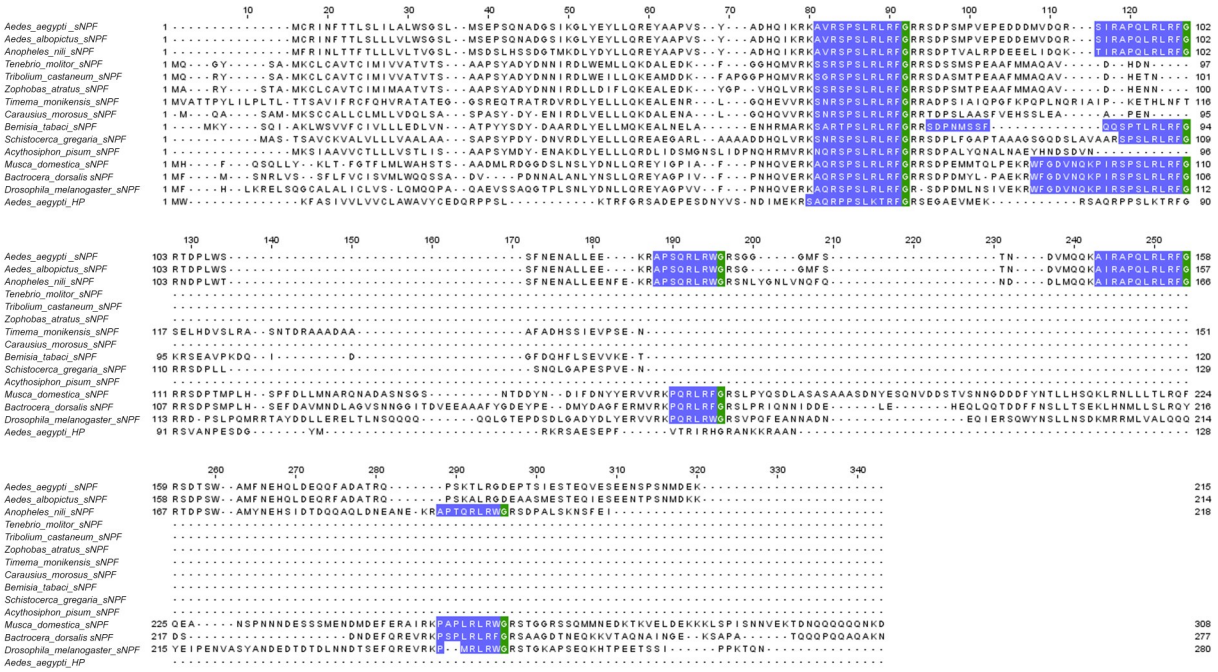

Fig. 1 Alignment of short neuropeptide F (sNPF) precursor sequences from different insect species, including *Aedes aegypti* (ABE72968.1 for sNPF precursor and AAG43377.1 for head peptide precursor (HP)), *Aedes albopictus* (XP_029728321.1), *Anopheles nili* (XP_053673911.1), *Tenebrio molitor* (ON125379), *Tribolium castaneum* (XP_008198705.1), *Zophobas atratus* (ON155957), *Timema monikensis* (CAD7431192.1), *Carausius morosus* (UES72893.1), *Bemisia tabaci* (XP_018908145.1), *Schistocerca gregaria* (AHH85823.1), *Acyrthosiphon pisum* (XP_003247250.1), *Musca domestica* (XP_011292676.1), *Bactrocera dorsalis* (XP_049302626.1), and *Drosophila melanogaster* (NP_724239.1). Note that some precursors encode one peptide while others encode several. Violet indicates mature peptides and green indicates amidation position.

it was thought that holometabolic insects such as coleopterans, dipterans, and lepidopterans have longer prepropeptides that contain multiple sNPFs, while those in hemimetabolic insects are shorter, encoding only one mature peptide (Nässel and Wegener, 2011). However, recent studies have shown that this may not be true for all holometabolic insects, such as the honeybee *Apis mellifera* and tenebrionid beetles: *Tribolium castaneum*, *T. molitor*, and *Z. atratus*, which have only one sNPF isoform (Hummon et al., 2006; Amir et al., 2022; Marciniak et al., 2022). However, one should not rely only on genomic data, and the identification of peptides using, for example, mass spectrometry is also extremely important. The precursor in *T. castaneum* encodes only one isoform, but as a result of posttranslational modifications, two active isoforms are formed: full sNPF (Trica-sNPF: SGRSPSLRLRFamide) and its truncated version (Trica-sNPF$_{4–11}$: SPSLRLRFamide) (Hauser et al., 2008). A similar pattern also occurs in all stages of *D. melanogaster*, where only Drome-sNPF1 occurs as predicted by genomic data (Nässel and Wegener, 2011).

The sNPFs are expressed in a number of tissues, with the central nervous system (CNS) having the highest and most abundant expression of this neuropeptide. In addition to the CNS, sNPFs are also expressed in other tissues, such as the gut (especially the midgut) (Liu et al., 2021) and accessory glands (Nässel and Zandawala, 2019). In most cases, neuropeptides in the insect brain are expressed in a few to several dozen neurons. However, sNPF together with proctolin is an exception, being widely distributed in the insect brain (Nässel and Zandawala, 2019). In the CNS, sNPF is expressed in 400 to a few thousand neurons, depending on the species. For example, a large portion of Kenyon cells in mushroom bodies (MBs) at all stages of *D. melanogaster* express this neuropeptide (Johard et al., 2008; Nässel et al., 2008). Except for MBs, sNPFs are localized in most parts of the insect brain: antennal lobes, optic lobes, neurons connecting the lobes, central complex, and median and lateral neurosecretory cells (MNCs and LNCs, respectively) (Nässel and Zandawala, 2019). sNPFs have also been shown to colocalize with other neuropeptides. The expression of

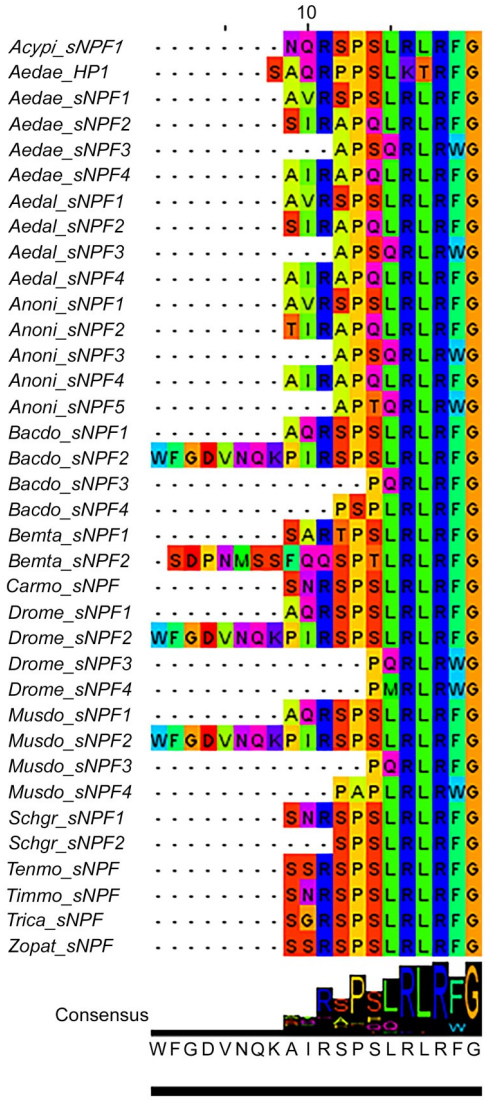

Fig. 2 Alignment of mature short neuropeptide F (sNPF) peptides from different insect species, including *Acyrthosiphon pisum* (*Acypi*), *Aedes aegypti* (*Aedae*), *Aedes albopictus* (*Aedal*), *Anopheles nili* (*Anoni*), *Bactrocera dorsalis* (*Bacdo*), *Bemisia tabaci* (*Bemta*), *Carausius morosus* (*Carmo*), *Drosophila melanogaster* (*Drome*), *Musca domestica* (*Musdo*), *Schistocerca gregaria* (*Schgr*), *Tenebrio molitor* (*Tenmo*), *Timema monikensis* (*Timmo*), *Tribolium castaneum* (*Trica*), and *Zophobas atratus* (*Zopat*). The IDs of amino acid sequences were the same as listed in Fig. 1. HP: head peptide precursor.

more than one type of neuropeptide by peptidergic neurons/neuroendocrine cells is a widespread phenomenon across different animal phyla (Nässel, 2018). sNPFs have been found to be coexpressed with corazonin (CRZ) and proctolin in MNCs (Isaac et al., 2004; Kapan et al., 2012), with tachykinin-related peptides (TRPs) and ion transport peptides (ITPs) in the pars lateralis (Kahsai et al., 2010), with PDF in clock neurons (Johard et al., 2009), and with SIFamides (SIFa) in the pars intercerebralis (Martelli et al., 2017). The coexpression/localization of these peptides suggests that they act as cotransmitters that modulate the response at the synapse (Nusbaum et al., 2017).

3 sNPFRs in insects

Due to their structure, sNPFRs belong to the rhodopsin-like superfamily of G-protein-coupled receptors (GPCRs) and show several features characteristic of this group, such as the presence of seven transmembrane regions/domains (TMs), a highly conserved DRY (aspartic acid, arginine, and tgrosine) motif in the second intracellular loop, an N-terminal extracellular segment, and a intracellular C-terminal tail responsible for interactions with G proteins (Chen and Pietrantonio, 2006; Hauser et al., 2008; Marciniak et al., 2020).

Comprehensive structural analysis of RFamide receptors, including sNPFR, in *D. melanogaster* conducted by Bass et al. (2014) predicted conserved residues that are crucial for ligand binding to the receptor. Three motifs were found in extracellular loops (ECLs). Additionally, four residues were identified in TMs, oriented to face into a putative ligand-binding pocket (Bass et al., 2014). Moreover, the predicted posttranslational modifications of the receptor protein, including the typical glycosylation of the N-terminal region and ECLs, phosphorylation by protein kinase C, cyclic adenosine monophosphate (cAMP)- and cyclic guanosine monophosphate (cGMP)-dependent kinases, and palmitoylation at the C-terminal tail, could be significant for receptor desensitization (Gainetdinov et al., 2004; Chen and Pietrantonio, 2006). Structure/activity studies concerning the activation of sNPFR showed that depending on the ligand structure, the half maximal effective concentration (EC_{50}) value can vary from the picomolar range to concentrations above 100 nmol/L (Mertens et al., 2002; Garczynski et al., 2006, 2007; Dillen et al., 2013; Christ et al., 2018). A ligand-binding assay conducted with cells from adult *D. melanogaster* and mosquito *Anopheles gambiae* showed that sNPFs consisting of nine or more amino acids are more potent than those with fewer amino acids (Garczynski et al., 2006, 2007). Moreover, research by Feng et al. (2003) and Reale et al. (2004) showed that sNPFs with

a C-terminal motif of RLRFa have a higher affinity than sNPFs with the RLRWa motif.

Compared to the sNPF precursor, sNPFR is highly conserved among insects (Fig. 2), which was proven by numerous published datasets (Christ et al., 2018; Liu et al., 2021). First, sNPFR was identified and characterized in larvae and adults of *D. melanogaster*. In subsequent research, sNPFR was found inter alia in imagoes of the fire ant *Solenopsis invicta*, the mosquito *A. gambiae*, and other insects (Nässel and Wegener, 2011). Recently, in silico analysis confirmed the presence of sNPFR in most insect species. The phylogenetic similarities between insect sNPFRs are summarized in Fig. 3. In insect species classified in different orders, the structural similarity between receptors is usually higher than 40% (BLASTp, data available in the National Center for Biotechnology Information (NCBI) database). However, across different insect families, the level of similarity can be much higher. For example, in Tenebrionidae the structural similarity of sNPFR is about 90% (Marciniak et al., 2020). Most differences in the structure of sNPFRs are observed in the N-terminal and C-terminal regions. The highest similarity is observed in sequences of transmembrane helixes (Marciniak et al., 2020).

sNPFR is widely distributed in insect tissues. The localization of sNPFR reflects the physiological actions (Nässel and Wegener, 2011), which are described in detail below. Similar to other neuropeptides, the highest abundance of sNPFR was identified in the CNS. A comprehensive analysis by Carlsson et al. (2013) of the sNPFR distribution in nervous tissues of *D. melanogaster* larvae revealed the presence of numerous neurons in the brain (40−50) and ventral nerve cord (VNC) (about 100), which expressed sNPFR. In the brain, the presence of sNPFR was confirmed in several cell types including MNCs, insulin-producing cells (IPCs), and neurons innervated by MB lobes and calyx, including Kenyon cells (Lee et al., 2008; Nässel and Wegener, 2011; Kapan et al., 2012; Carlsson et al., 2013). Additionally, Kahsai et al. (2012) showed that sNPFR is abundant in the central complex in adult *D. melanogaster*, with a prominent set of midline neuropils in the insect brain. Receptor distribution in olfactory sensory neurons in the antennal lobe and maxillary palps has also been proven (Root et al., 2011; Carlsson et al., 2013; Knapek et al., 2013). Interestingly, sNPFRs were also found in clock neurons (Johard et al., 2009) and optic lobes (Dillen et al., 2013). In addition, the previous finding of sNPFR localization in the brain of *D. melanogaster* larvae was supported by the results of a study on fire ant queens by Lu and Pietrantonio (2011).

As well as being present in the insect brain, recent research has confirmed the distribution of sNPFR in neurons of *D. melanogaster* larvae associated with the hypocerebral ganglion (HCG) and ring glands, a structure composed of the prothoracic gland (PG), the corpora allata (CA), and the corpora cardiaca (CC) (Siegmund and Korge, 2001; Carlsson et al., 2013). These results are partially consistent with those of Yamanaka et al. (2008) in larvae of the moth *Bombyx mori*, which confirmed the presence of sNPFR in the CA. Additionally, transcripts of the gene encoding sNPFR were found in the CC and CA of adults of the locust *S. gregaria* and larvae of the fly *Glossina morsitans morsitans* (Dillen et al., 2013; Caers et al., 2016). In *S. invicta* and *S. gregaria*, sNPFR was also abundant in the subesophageal ganglion (Lu and Pietrantonio, 2011; Nässel and Wegener, 2011; Dillen et al., 2013). In the case of the VNC, in *Drosophila* larvae, large cell bodies that expressed sNPFR were located ventro-medially and ventro-laterally in the first abdominal segments. Additionally, smaller cell bodies were observed in all VNC segments (Carlsson et al., 2013). The presence of sNPFR in the VNC was also proven in larvae and adults of the beetle *Dendroctonus armandi* (Liu et al., 2021), *B. mori*, and adults of *S. gregaria* (Dillen et al., 2013).

In addition to the significant abundance of sNPFR in nervous tissues, current research indicates the potential presence of this receptor in most analyzed organs, such as the midgut, hindgut, Malpighian tubules, fat body, ovaries, and testes (Nässel and Wegener, 2011; Kahsai et al., 2012; Dillen et al., 2013; Caers et al., 2016; Ma et al., 2017). Interestingly, some differences in sNPFR distribution exist in peripheral tissues in different species. For example, in adult mealworm beetles, the transcript for the gene encoding sNPFR was detected in the ejaculatory duct but not in the testes or accessory glands (Marciniak et al., 2020). In *D. armandi* adults and larvae, sNPFR was expressed in different tissues but not the pheromone gland or Malpighian tubules (Liu et al., 2021). The existence of these differences is probably closely related to the physiological state of the tested individuals.

Fig. 3 Phylogenetic tree of short neuropeptide F (sNPF) receptors of selected insect species. The data were retrieved from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov). The phylogenetic tree was made with NCBI Tree Viewer (https://www.ncbi.nlm.nih.gov/tools/treeviewer). The phylogenetic distance was calculated based on the Grishin method (fast minimum evolution, max sequence difference=0.85). Distinct colors indicate different insect groups. The IDs of NCBI sequences were listed in brackets.

A good example was reported by Lu and Pietrantonio (2011), in which the presence of sNPFR in ovaries was detected only in mated *S. invicta* queens.

4 sNPF downstream signaling

Although the signaling pathway of sNPF has been studied mainly in *Drosophila*, data are available for several other insect species. In *D. melanogaster*, one of the most important factors in sNPF signaling is the minibrain (*mnb*) gene, which encodes the Mnb/dual-specificity tyrosine phosphorylation-regulated kinase 1a (Dyrk1a) kinase. This enzyme activates the transcription factor Forkhead box class O (FOXO) through the action of silent information regulator factor 2 (Sir2)/Sirtuin 1 (Sirt1) deacetylase (Hong et al., 2012). FOXO then activates *sNpf* transcription, providing positive feedback. Mnb/Dyrk1a kinase is located in neurons expressing sNPFR1. Studies using RNA interference (RNAi) and an sNPFR1 overexpression line have shown that activation of *mnb* transcription is mediated by $G_{\alpha s}$ (a subunit of the heterotrimeric Gs protein that stimulates the cAMP-dependent pathway through activation of adenylyl cyclase), protein kinase A (PKA), and cAMP-response element-binding protein (CREB, a cellular transcription factor). CREB can dimerize with CREB-regulated transcription coactivator (CrtC, a coactivator of cAMP-regulated transcription) to stimulate sNPF expression, resulting in suppression of the immune response and enhanced starvation resistance (Shen et al., 2016).

Notably, sNPF signaling is also connected with other critical factors, such as *Drosophila* ILPs (DILPs, counterparts of insulin peptides in mammals) or IPCs. Neurons that produce sNPF are involved, along with IPCs, in positive feedback regulation to manage insulin signaling, sNPF levels, and food intake during brief periods of starvation. The sNPFs activate IPCs, which in turn maintain *sNPF* gene expression and stimulate the desire to eat.

More specifically, the interaction of sNPF with insect ILPs begins with the extracellular activation of receptor kinases (extracellular signal-related kinases (ERKs)) in IPCs, which in turn modulate insulin expression (Kapan et al., 2012). In addition, the insulin signaling pathway is involved in a negative feedback loop controlling sNPF expression and inhibiting food intake (Ko et al., 2015). In starved flies with low DILP levels, sNPFR1 expression is increased, promoting foraging behavior (Root et al., 2011). Additionally, another study indicated a strict connection between sNPF and DILPs. Suh et al. (2015) showed that the microRNA-9a (miR-9a), as a regulator of DILP signaling, binds to sNPFR1 messenger RNA (mRNA). These results suggest that miR-9a can regulate insect life processes, such as growth, by controlling sNPFR1/NPYR-mediated modulation of insulin signaling.

5 Physiological properties of sNPF

5.1 Regulation of feeding

Feeding is one of several key life processes in insects that is strongly influenced by sNPF (Cui and Zhao, 2020). These peptides were first designated "hunger hormones" (Lee et al., 2004), but recent data obtained in different insect species have shown contradictory results (Table 1). The expression levels of genes that determine the expression of sNPF proteins and their characteristic cellular receptors have a significant impact on issues related to insect food intake (Fig. 4). In various species of insects, different levels of expression of sNPF can trigger inhibition or stimulation of food intake. There are many examples of the stimulation of food intake behavior that leads to increased foraging. In *D. melanogaster*, enhanced *sNpf* expression increases food intake in larvae and adults, often resulting in obesity or larger body size (Lee et al., 2004). Another example in which hunger prompts food intake and causes an increase in sNPFR1 expression level is observed in adult *A. mellifera* (Ament et al., 2011). sNPFs were also detected during foraging of adult *L. decemlineata* (Huybrechts et al., 2004). Interestingly, when adult queen fire ants are starved for 5 or 10 d and the urge for food is enhanced, sNPFR transcripts are downregulated (Chen and Pietrantonio, 2006). Similar to starved queen fire ants, *B. mori* larvae experiencing starvation can also show a decrease in *sNPF* and *sNPFR* expression levels, which prompts the larval stages of the nocturnal butterfly to search for food (Nagata et al., 2012). On the other hand, sNPF can also inhibit foraging. This has been observed in adult *A. aegypti* (Nässel et al., 2008), adult *S. gregaria* (Dillen et al., 2013), and adult *Culex quinquefasciatus* (Christ et al., 2018).

Table 1 Summary of the physiological functions of short neuropeptide F (sNPF) in various insect species

Physiological process	Development	Order	Species	Stage	Mode of action	References
Feeding	Holometabolous	Diptera	*Aedes aegypti*	Adult	**Inhibition** of host seeking	Nässel et al., 2008
				Larva	**Inhibition** of serotonin-induced peristaltic contractions and ion transport in the anterior stomach	Onken et al., 2004
			Drosophila melanogaster	Adult and larva	**Increased** food intake	Lee et al., 2004
			Culex quinquefasciatus	Adult	**Inhibition** of host seeking	Christ et al., 2018
		Hymenoptera	*Apis mellifera*	Adult	**Increased** foraging	Ament et al., 2011
			Solenopsis invicta	Adult queen	**Increased** urge for food	Chen and Pietrantonio, 2006
		Lepidoptera	*Bombyx mori*	Larva	**Stimulation** of search for food	Nagata et al., 2012
		Coleoptera	*Dendroctonus armandi*	Adult	**Decreased** food intake	Nagata et al., 2012
			Leptinotarsa decemlineata	Adult	**Increased** foraging	Huybrechts et al., 2004
	Hemimetabolous	Hemiptera	*Acyrthosiphon pisum*	Adult	**Delayed** food intake	Huybrechts et al., 2004
		Blattodea	*Periplaneta americana*	Adult	**Increased** food intake by decreasing digestive enzymes	Mikani et al., 2012
		Orthoptera	*Schistocerca gregaria*	Adult	**Decreased** foraging	Mikani et al., 2012
Gustation	Holometabolous	Hymenoptera	*A. mellifera*	Adult	**Stimulation** of responsiveness to sucrose	Bestea et al., 2022a
		Diptera	*D. melanogaster*	Food-deprived, homozygous $sNPF^{c00448}$ and $sNPF^{f07577}$ mutant adult	**Stimulation** of bitter sensitivity when sNPFR overexpressed panneuronally	Inagaki et al., 2014
Olfaction	Holometabolous	Diptera	*A. aegypti*	Adult female	**Reduced** odor-mediated seeking behavior	Christ et al., 2017
			Bactrocera dorsalis	Adult	**Stimulation** of responsiveness to ethyl acetate (attractive odorant)	Jiang et al., 2017
				Null sNPFR mutant adult	**Strongly lowered** antennal responses to attractive odorants	Li et al., 2022
		Hymenoptera	*A. mellifera*	Adult female	**Stimulation** of responsiveness to attractive odorants	Bestea et al., 2022a
		Diptera	*D. melanogaster*	Adult	**Improved** sugar-rewarded olfactory memory	Nässel et al., 2008; Carlsson et al., 2010; Root et al., 2011
		Diptera	*D. melanogaster*	Adult	**Stimulation** of necessary for proper appetitive memory	Inagaki et al., 2014
Reproduction	Holometabolous	Diptera	*Anopheles gambiae*	Adult female	**Stimulation (indirect)** of food intake	Garczynski et al., 2006, 2007
			D. melanogaster	Adult female	**Inhibition** of reproductive arrest	Nagy et al., 2019
					Stimulation (indirect) of egg development by DILP1	Liu et al., 2016
				Adult male	**Stimulation** of SMD behavior	Kim et al., 2016

To be continued

Table 1 (continued)

Physiological process	Development	Order	Species	Stage	Mode of action	References
Reproduction	Holometabolous	Coleoptera	*Tenebrio molitor*	Adult female	**Inhibition** of oviduct muscle contraction	Marciniak et al., 2013
					Decreased laid egg number	Marciniak et al., 2020
				Adult male	**Stimulation** of functioning of reproductive system by Lepde-sNPF-1	Marciniak et al., 2017
					Inhibition of functioning of reproductive system by Trica-sNPF	Marciniak et al., 2020
			Zophobas atratus	Adult female	**Inhibition** of oviduct muscle contraction by Lepde-sNPF-1	Marciniak et al., 2013
	Hemimetabolous	Hemiptera	*A. pisum*	Nymph	**Increased** born nymph number	Amir et al., 2022
			Rhopalosiphum padi	Adult female	**Increased** fecundity	Peng et al., 2021
		Orthoptera	*Locusta migratoria*	Adult female	**Stimulation** of development of penultimate follicles	Cerstiaens et al., 1999; Schoofs et al., 2001
					Acceleration of egg development	Schoofs et al., 2001
					Stimulation of release of OMP	de Loof et al., 2001
Development	Holometabolous	Lepidoptera	*B. mori*	Larva	**Inhibition** of biosynthesis of JH	Kaneko and Hiruma, 2014
		Coleoptera	*T. molitor*	Larva	**Delayed** molting into next instar	Marciniak et al., 2013
				Pupa	**Acceleration** of pupal eclosion	Marciniak et al., 2013
Locomotor activity	Holometabolous	Diptera	*D. melanogaster*	Adult	**Increased** walked distance	Kahsai et al., 2010
				Adult female	**Increased** speed of walking	Kahsai et al., 2010
	Hemimetabolous	Blattodea	*P. americana*	Adult male	**Stimulation** of locomotor activity	Mikani et al., 2015
					Stimulation of food searching behavior	Mikani et al., 2015
Circadian rhythm	Holometabolous	Diptera	*D. melanogaster*	Adult	**Increased** daytime and nighttime sleep	Chen et al., 2013; Shang et al., 2013; Juneau et al., 2019
					Increased quiescence under light-dark and dark-dark conditions	Shang et al., 2013
					Interplay with PDF and modulate IPCs	Nagy et al., 2019
					Decreased level of free Ca^{2+} in PTTH neurons	Selcho et al., 2017
					Cotransmitter in sLN_vs and LN_ds	Johard et al., 2009
				Larva	**Suppressed** Ca^{2+} necessary for rhythmicity in DN1	Rosato and Kyriacou, 2017
Water and ion balance	Holometabolous	Diptera	*D. melanogaster*	Adult male	**Inhibition** of increasing secretion of resting fluid	Chintapalli et al., 2012
				Adult	**Suppressed** water lost at desiccation	Kahsai et al., 2010
Visual and color learning memory	Hemimetabolous	Orthoptera	*S. gregaria*	Male and female 5th larval state	**Deficiency** of visual memory	Dillen et al., 2015
	Holometabolous	Hymenoptera	*A. mellifera*	Partially fed forager	**Stimulation**	Bestea et al., 2022b

DILP1: *Drosophila* insulin-like peptide 1; SMD: shorter mating duration; OMP: ovary maturating parsin; JH: juvenile hormone; PDF: pigment-dispersing factor; IPCs: insulin-producing cells; PTTH: prothoracicotropic hormone; sLN_v: small ventral lateral neuron; LN_d: dorsal lateral neuron; DN1: dorsal neuron 1.

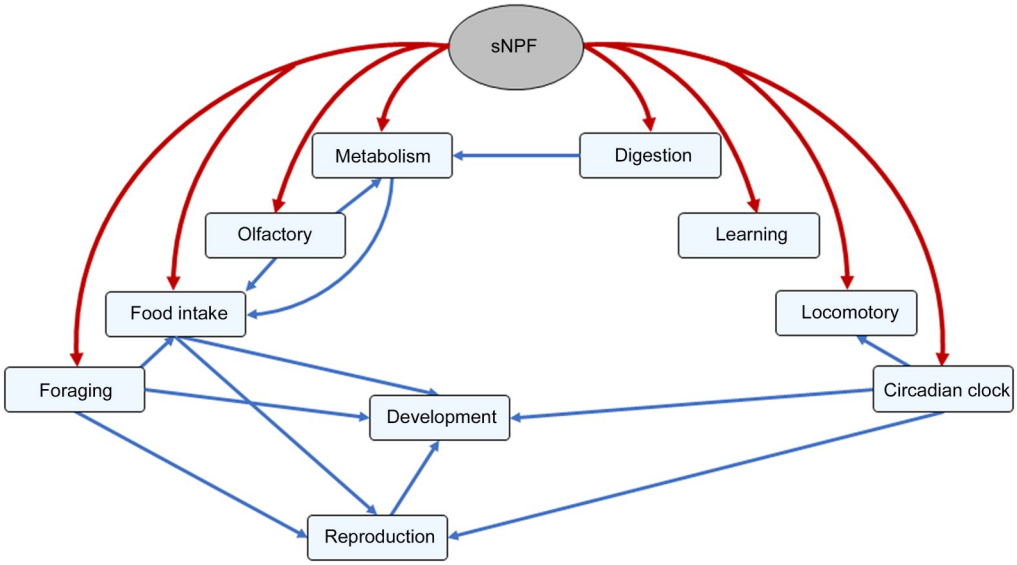

Fig. 4 Possible involvement of short neuropeptide F (sNPF) in the regulation of various physiological processes in insects. sNPF may directly (red arrows) or indirectly (blue arrows) influence different functions.

Lower levels of sNPF can inhibit food intake in different insects (Dillen et al., 2014). For example, in adult *D. melanogaster*, decreased expression of sNPFs results in decreased food consumption (Toprak, 2020). Some insects undergo a period in their life cycle called diapause, in which the suspension of some life activities occurs. The purpose of diapause is to overcome unfavorable environmental conditions. Growth and development are stopped during this time. sNPFs were not found during the diapause state of adult *L. decemlineata*, which is a period of inhibited food intake (Huybrechts et al., 2004). An example of the inhibition of food consumption in insects induced by sNPF levels occurs in adult *S. gregaria*, in which feeding is reduced following sNPF injection (Dillen et al., 2014).

Increasing sNPF levels also inhibit the release of digestive enzymes in the midgut of adult *P. americana* (Mikani et al., 2012). Both starving these insects for four weeks and co-incubating their isolated midgut with sNPF, as well as injection of sNPF into the hemocoel of normally fed insects, cause a significant decrease in the activity of three digestive enzymes: α-amylase, protease, and lipase. In the case of starvation, the activity level of the α-amylase enzyme dropped by 69%, protease by 62%, and lipase by 47%. Administration of sNPF to the hemocoel in an amount equal to or greater than 1×10^{-8} mol reduced the activity of α-amylase by more than 40%. Administration of sNPF in an amount equal to or greater than 1×10^{-9} mol resulted in a 45% decrease in protease activity and a 44% decrease in lipase activity. Moreover, it has also been shown that sNPFs inhibit serotonin-induced peristaltic contractions and ion transport in the anterior stomach of *A. aegypti* larvae (Onken et al., 2004).

Beyond the inhibition and stimulation of food intake, there is also an interesting example of the effect of the type of food intake on sNPF levels in honeybees. The levels of the peptide in the body change depending on whether they forage on nectar or pollen (Brockmann et al., 2009).

Recent research applying RNAi has shown that sNPF actions on the regulation of feeding can also be inhibitory. In the pea aphid *Acyrthosiphon pisum* the function of transcripts encoding AcypiNPF or AcypiNPFR was investigated via RNAi. The use of RNAi assays caused decreases in probing time and phloem sucking duration on broad bean plants (Amir et al., 2022). Additionally, injection of the double-stranded RNA (dsRNA) into the adult insects caused a delay in the activity of their stylet (the protruding mouthpiece that pierces the plant's sieve tube to allow sap to be extracted) to reach the phloem. Early entry of the stylet into the phloem was delayed from 2.2 h (control group) to 2.9–3.5 h in the treatment groups (Amir et al., 2022).

Other interesting studies based on RNAi assays concern the adult *D. armandi*, which is a devastating

pest of coniferous forests in China. The knockdown of *sNPF* and *sNPFR* in these insects reduced their food intake and body weight through shifts in biosynthesis and metabolism pathways (Liu et al., 2021). Injection of dsRNA into beetles also decreased glycogen and free fatty acid levels and increased trehalose level. The above examples based on numerous studies show that sNPF in insects can act as a stimulant or an inhibitor of feeding-related processes.

sNPFs also strongly regulate the olfactory system (Table 1), which is obviously involved in the regulation of feeding, particularly under starvation conditions (Cui and Zhao, 2020). Olfaction in insects is predominantly mediated by antennae that are covered with numerous sensory cells—sensillae. For example, in the adult fly *Bactrocera dorsalis*, sNPFs modulate olfactory sensitivity upon starvation. In flies subjected to starvation, sNPF and NPFR transcripts were upregulated in the head (Jiang et al., 2017). Moreover, electroantennogram assays showed significantly elevated electrophysiological responses of antennae to increasing concentrations of ethyl acetate in starved insects. When the sNPF precursor transcript was knocked down with RNAi in flies subjected to starvation, a significant decrease in the electrophysiological responses of antennae in response to ethyl acetate was observed (Jiang et al., 2017).

Similar results were obtained in adult *B. dorsalis* null *sNPFR* mutants using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system. The mutant flies showed strongly reduced antennal responses to all tested odorants. Furthermore, in mutant flies, significant depletion of transcripts representing eight olfactory receptor (OR)-like genes was reported (Li et al., 2022). The localization of odorant receptor co-receptor (Orco, an OR co-receptor) and sNPFR in *B. dorsalis* antennae allowed the identification of many odorant receptor neurons (ORNs) but only a few $sNPFR^+$ cells in the sensillum of wild-type antennae. The $sNPFR^+$ cells were closely located, but not identical, to the $Orco^+$ cells, which suggests that sNPFR may interact with multiple odorant receptors in a subset of ORNs (Li et al., 2022).

Moreover, studies of adult *D. melanogaster* confirmed that sNPF is commonly expressed in ORNs (Nässel et al., 2008; Carlsson et al., 2010; Root et al., 2011). In this species, a reduction in olfactory behavior was noted when knockdown of *sNPFR* was applied to starved flies (Ko et al., 2015). Root et al. (2011) suggested that sNPF signaling mediates presynaptic facilitation in ORNs. Exogenous application of sNPF resulted in increased intercellular Ca^{2+} levels in ORNs but not in all types. The DM1, DM2, and DM4 glomeruli exhibited enhanced activity in response to the neuropeptide, whereas the VM2 or VA3 glomeruli did not. It seems that sNPF signaling in DM1 is necessary and sufficient for starvation-dependent olfactory-based food-searching behavior (Root et al., 2011).

sNPF also impacts olfactory memory. Downregulation of *sNpf* expression in MBs disturbs the olfactory memory response of adult males and females of *Drosophila* to sugar stimulation (Knapek et al., 2013). Modulation of odor-mediated host-seeking behavior by sNPF also occurs in adult *A. aegypti* females. In the antennal lobes, the level of endogenous Aedae-sNPF-2 was significantly lower in blood- and sugar-fed females than in control and starved females. The application of exogenous sNPF also reduced odor-mediated host-seeking behavior (Christ et al., 2017).

The activity of gustatory cells is also modulated by sNPF. Inagaki et al. (2014) demonstrated that food-deprived $snpf^{+/-}$-mutant flies of *D. melanogaster* were more bitter-sensitive than starved genetic controls, but they showed normal changes in sugar sensitivity. Importantly, under fed conditions, $snpf^{+/-}$-mutant flies did not show any change in bitter sensitivity, indicating that the mutation affected starvation-dependent changes rather than baseline responsiveness. Panneuronal overexpression of sNPFR enhances the starvation-dependent decrease in bitter sensitivity, whereas panneuronal knockdown of *sNpfr* attenuates it. It seems that γ-aminobutyric acid (GABA)-ergic neurons are involved in the modulation of bitter sensitivity by sNPF. In *D. melanogaster*, starvation drives the overexpression of sNPF, which activates GABA-ergic neurons that in turn inhibit bitter-taste gustatory neurons (Inagaki et al., 2014). Topical application of sNPF to fed adult honeybees (*A. mellifera*) increased their proboscis extension response to increasing concentrations of sucrose solution and to attractive odorants, thus showing that sNPF modulates gustatory and olfactory processes prior to ingestion (Bestea et al., 2022a). This suggests that sNPF may modulate the sensitivity of sweet-sensing gustatory neurons, thereby changing the threshold for responding to sucrose and/or central

processing of tastes in the brain of adult *A. mellifera* (Bestea et al., 2022a).

5.2 Reproduction and development

sNPFs control growth, molting (van Wielendaele et al., 2013), stress responses, lifespan (Liu et al., 2016), nociception, the circadian clock, and learning (Fadda et al., 2019). Since they play a significant role in food intake and metabolism (Liu et al., 2016; Fadda et al., 2019), the question about whether they have an impact on reproduction, which is strictly related to the nutrition state, seems justified (Table 1, Fig. 4).

As described above, Mertens et al. (2002) revealed the presence of *Drosophila* sNPFR (*Drome-sNPFR*) gene transcripts in the ovaries of adult *D. melanogaster* flies. Chen and Pietrantonio (2006) came to similar conclusions and reported the presence of sNPFR transcripts in the ovaries of mated fire ant (*S. invicta*) queens, which was confirmed a few years later by Lu and Pietrantonio (2011). Additionally, they noted the occurrence of sNPFRs in the ovaries of mated but not virgin queens. The signal was detected at the posterior end of oocytes (strictly the oocyte membrane) in the early- and mid-oogenesis stages, which suggests that the sNPFR is related to processes at the pole of the oocyte. Western blot analysis revealed three bands in samples isolated from ovaries (46.2, 55.3, and 51.1 kDa), which suggests that receptor proteins undergo posttranslational modifications (Lu and Pietrantonio, 2011). The presence of sNPFR in the ovaries suggests the possibility of a direct influence of sNPF on the activity of the female reproductive system. Cerstiaens et al. (1999) showed that injections of pure Lepde-sNPF-1 (ARGPQLRLRF-NH_2) at a concentration of 0.5 mg daily for 10 d caused changes in the ovaries of adult *Locusta migratoria*, which were better developed than the ovaries of control females. The penultimate follicles were 3.2-mm long and in the vitellogenic phase unlike in the control in which they were in the previtellogenous stage (Cerstiaens et al., 1999). Additionally, Schge-sNPF (YSQVARPRF-NH_2) has gonadostimulating activity in adult females of *L. migratoria.* Application of Lepde-sNPF1 at a dose of 0.05 mg per animal to 6-d-old virgin females (5 times, every 12 h) caused acceleration of egg development (Schoofs et al., 2001). Schoofs et al. (2001) also observed acceleration of vitellogenic processes in penultimate oocytes, as shown by Cerstiaens et al. (1999). Acceleration of the development of the penultimate oocytes is not common. Usually, the first terminal oocytes undergo choriogenesis and are deposited into the lateral oviducts, which stimulates the development of the penultimate oocytes and their shift to the terminal part of the ovary and further development (de Loof et al., 2001). It can therefore be said that Lepde-sNPF-1 acts as an oocyte growth accelerator (de Loof et al., 2001). The authors suggested that Lepde-sNPF-1 might act as a humoral and/or neural releasing factor that acts on the pars intercerebralis, which projects to the CC to release gonadotrophic ovary maturating parsin (OMP). This was supported by the results of immunocytochemistry confirming the presence of FMRFamide in the CC. On the other hand, Kaneko and Hiruma (2014) showed that in *B. mori* larvae, sNPF might be an important factor that regulates the biosynthesis of juvenile hormone (JH). Research showed upregulation of sNPFR expression on Day 3 of the 4th larval stadium isoform bombyx neuropeptide G protein-coupled receptor (BNGR)-A10 and the 0−5th larval stadium isoform BNGR-A11 in the CA. These changes suppressed the expression of mevalonate kinase and 3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA) reductase, and biosynthetic enzymes involved in the mevalonate pathway of JH synthesis, resulting in the initiation of pupal metamorphosis (Kaneko and Hiruma, 2014).

The results of Garczynski et al. (2006), who confirmed the presence of sNPFs (Drome-sNPF-1 and Drome-sNPF-3 as well as Drome-$sNPF2_{9–19}$ and Drome-$sNPF2_{11–19}$) in *D. melanogaster* adult hemolymph by high-performance liquid chromatography (HPLC), also support the argument that sNPF functions as a humoral factor.

sNPF may indirectly influence processes related to reproduction not only by acting as a humoral factor but also by regulating food intake. In adult *A. gambiae*, females require blood meals to start and complete their reproductive processes (Garczynski et al., 2006). In turn, food intake might be regulated by sNPF, so it might be considered a factor that indirectly regulates reproduction success in mosquitos (Garczynski et al., 2007) (for more information, see Section 5.1). Liu et al. (2016) also indicated that sNPF may function as a regulator of DILP1 expression in brain IPCs of adult *D. melanogaster.* Mutation of *dilp1* in female flies caused a reduction in oviposition, which suggests that

sNPF is indirectly related to egg development (Liu et al., 2016).

The most important determinant of proper sexual development of females is their ability to produce offspring. Peng et al. (2021) showed that knockdown of the *sNPF* gene changed the fecundity of adult *Rhopalosiphum padi.* Fifteen days after injection of dsRNA against sNPF (dssNPF), fecundity was decreased. A similar effect was observed after knockdown of the *sNPFR* gene (Peng et al., 2021). As both knockdowns also caused a decrease in food intake, the effect might be indirect (Peng et al., 2021). Similar effects were observed in the aphid *A. pisum* (Amir et al., 2022). Silencing of both sNPF and sNPFR caused a significant reduction in the number of born nymphs. However, feeding was also reduced, so as in *R. padi*, the results might be due to nutritional deficiency rather than a direct effect (Amir et al., 2022). On the other hand, the results presented by Nagy et al. (2019) showed that sNPF caused inhibition of reproductive arrest in adult female *D. melanogaster* flies. They found that in vitro bath application of neuropeptide on fly brains changed the level of Ca^{2+} in IPCs. This may have maintained the IPCs in an "active state," leading to inhibition of gonadal arrest. Similar effects were observed after repressing sNPFR, suggesting the function of sNPF in maintaining diapause in flies (Nagy et al., 2019).

sNPF might also act as a myotropic factor indirectly affecting fecundity. Marciniak et al. (2013) found that sNPF has dose-dependent myoinhibitory activity. After applying Lepde-sNPF-1 to isolated oviducts of *T. molitor* and *Z. atratus* adults, they observed inhibitory effects, where the frequency of oviduct muscle contractions was decreased. Myotropic effects were also observed in isolated ejaculatory ducts of *T. molitor*, but the effects were opposite to those in females—Trica-sNPF and Lepde-sNPF-1 caused an increase in muscle contractility (Marciniak et al., 2017, 2020).

sNPF also affects male reproductive processes. Marciniak et al. (2017) presented the results of injection of Lepde-sNPF-1 into adult males of *T. molitor*. They found that the neuropeptide increases the concentration of the soluble fraction of proteins and changes the protein concentration in the testes at the 3rd and 7th days after adult appearance. In 4-d-old beetles, they also observed an increase in the weight of the testes. At a concentration of 1×10^{-5} mol, it also caused an increase in the number of spermatocytes. In further studies, using Trica-sNPF derived from *T. castaneum*, a beetle closely related to *T. molitor*, they observed slightly different results (Marciniak et al., 2020). They showed that 24 h after injection of neuropeptide into 4-d-old males, the concentration of the soluble protein fraction in testes had decreased. A decrease in the dry mass of the testes was also observed in the case of 8-d-old males. Analysis of the accessory glands dry mass of 4-d-old males showed that the mass was reduced as well as the concentration of soluble protein. Injection of Trica-sNPF at a concentration of 1×10^{-7} mol significantly decreased the total sperm cell number. In turn, injection of 4-d-old males followed by introduction to noninjected females caused slight changes in the numbers of laid eggs and hatched larvae. Injection of Trica-sNPF decreased the number of laid eggs by about 22% but the changes were not significant (Marciniak et al., 2020), and whether this effect is direct or indirect remains to be confirmed. sNPF is also involved in the regulation of behaviors related to reproduction. For example, sNPF is a crucial component of shorter mating duration (SMD) behavior in *D. melanogaster* adult males (a shortened mating duration when males are sexually satiated) (Kim et al., 2016). SMD was abolished by the expression of sNPF-RNAi in all neuronal populations but not in glial cells. Interestingly, the knockdown of *sNpf* or *sNpfr* in cells coexpressing sNPF and its receptors strongly abolished SMD, indicating that sNPF expression in a subset of cells that also express its receptor is important for inducing SMD. Moreover, knockdown of *sNPF* in PDF-expressing cells does not affect SMD behavior, whereas knockdown in cryptochrome-positive and gustatory Gr5a-positive neuron cells arrests SMD behavior (Kim et al., 2016).

Finally, sNPF is also considered a modulator of development. Research showed that injection of the Ledpe-sNPF-1 neuropeptide into *T. molitor* larvae resulted in a slight delay in molting into the next instar, whereas injection into pupae accelerated pupal eclosion (Marciniak et al., 2013).

5.3 Influence on locomotor activity and circadian rhythm

sNPF is one of the peptides expressed in the insect brain neuropil—the tissue considered the locomotor control center for walking and flying (Nässel et al.,

2008). Therefore, this neuropeptide is considered a potential controller or modulator of motor activity (Kahsai et al., 2010) (Table 1, Fig. 4). Studies of spontaneous walking were conducted on *D. melanogaster* adult flies, in which the neurons of the central complex showed decreased levels of sNPF-induced walking following sNPF RNAi. At the same time, the results suggested that these effects might be sex-specific. In female flies, the speed of walking was also increased (Kahsai et al., 2010). Opposite effects were observed after injecting adult male cockroaches (*P. americana*) with sNPF (Mikani et al., 2015). They observed that application of sNPF at concentrations of 1×10^{-8} and 1×10^{-7} mol/L in a volume of 5 μL per insect stimulated the locomotor activity of insects. This level was comparable to the locomotor activity of starved insects, which suggests that sNPF plays a role in modulating food searching behavior, as stated earlier (Fadda et al., 2019).

sNPF is expressed in a large population of neurons in MBs, the most prominent insect bilateral CNS structures that regulate memory and sleep. Thus, it seems that sNPF must play an important role in the regulation of these processes (Chen et al., 2013; Shang et al., 2013; Juneau et al., 2019). Adult *D. melanogaster* $sNPF^{c00448}$ mutants (which showed an about 50% decline in the levels of the sNPF peptide precursor in the MB) showed a significant increase in daytime and nighttime sleep, but daytime sleep was more strongly affected (mean increase of 177% for both sexes) than nighttime sleep (mean increase of 32% for both sexes). Moreover, the sleep bout duration and sleep numbers of female and male flies were also higher than those of the control flies (Chen et al., 2013). Cell-specific silencing of *sNpf* expression showed that *sNpf* suppression only in ventral lateral neuron (LN_v) and dorsal lateral neuron (LN_d) clock neurons did not affect sleep. However, when *sNpf* was suppressed in a subpopulation of MBs, the flies slept significantly more than the control flies because of longer sleep bout durations, while the sleep number of males was lower than that of the controls. The overexpression of *sNpf* in the pars intercerebralis and MBs reduced the total sleep amount. Moreover, studies have shown that both sNPF1 and sNPF2 regulate sleep. The role of sNPF in sleep control was also confirmed in experiments with silencing and overexpression of sNPFR1. Furthermore, when measuring the sNPF transcript in flies both with and without sleep deprivation, it was found that sleep deprivation increased the transcription level of *sNpf*. The regulation of sleep modulation by sNPF is suggested to occur through the cAMP-PKA-CREB signaling pathway (Chen et al., 2013). Similar results were obtained in adult *Drosophila* flies by Shang et al. (2013). Activation of sNPF-ergic neurons (by warmth-activated *Drosophila* transient receptor potential ankyrin 1 (dTRPA1) cation channels) increased quiescence under both light-dark (LD) and dark-dark (DD) conditions. Upon inactivation, the effects were abolished, and the duration of quiescent episodes shortened. Unusually, the reversal of dTRPA1 activation caused flies to sleep even less after inactivation of sNPF-ergic neurons than before activation. Knockdown of the $GABA_A$ receptor in sNPF neurons led to significant increases in both daytime and total sleep time as well as longer sleep bouts. The use of sNPFGAL4 to strongly drive expression in many specific brain regions showed that sNPF promotes nighttime sleep through the small LN_v (sLN_v)-to-large LN_v circuit (Shang et al., 2013). Studies in which optogenetic activation of sNPF neurons was used showed that activation for as little as 3 s at a time of day when most flies were awake caused a rapid transition to sleep that persisted for another 2 h following stimulation. However, when the stimulation was moved to times of day when flies were already asleep and had activated sNPF neurons, they stayed asleep through the light pulse and then showed further increases in sleep, while the control flies woke up (Juneau et al., 2019).

sNPF expression, however, extends beyond the CC to include some "clock neurons" (Nässel et al., 2008). Among insects, there are many groups whose vital functions, such as diapause, are related to the functioning of the so-called "circadian clock." The activity of the circadian clock is related to the expression (transcription-translation loop) of many genes, such as *period* (*per*), *timeless* (*tim*), *mammalian-type cryptochrome* (*cry-m* or *cry2*), *cycle* (*cyc*), and *Clock* (*Clk*) (Tomioka and Matsumoto, 2015; Patke et al., 2020). Studies have shown that between CRY-positive cells, we can distinguish a group of sNPF-expressing neurons called E1 (Johard et al., 2009). After generating mutants and mosaic insects, it was possible to analyze whether sNPF is important in the cycling of PER (Rosato and Kyriacou, 2017). Vecsey et al. (2014) reported that in *D. melanogaster* larvae, sNPF acts

through the sNPFR via G protein α subunit o ($G\alpha_o$), causing hyperpolarization of the neurons. This hyperpolarization blocks spontaneous waves of propagation in the VNC (Vecsey et al., 2014). Its action was also correlated with a small but significant decrease in cAMP levels in neurons (Vecsey et al., 2014). It was also found that sNPF acts as a suppressor of Ca^{2+} and is necessary for its rhythmicity in dorsal neuron 1 (DN1) but is not required for PER cycling (Rosato and Kyriacou, 2017). Based on that knowledge, des Marteaux et al. (2022) conducted studies on the adult bean bug *Riptortus pedestris* and proposed a hypothesis about the functioning of sNPF as a regulator of photoperiodic responses. However, knockdown of the *sNPF* gene did not result in any changes in the development of ovaries under short-day conditions. Those results undermine the hypothesis and eliminate sNPF as an output of the circadian clock (des Marteaux et al., 2022).

In *Drosophila*, clock neurons produce cryptochrome (Yoshii et al., 2008) and might also coexpress PDF, which is also engaged in the regulation of the circadian cycle (Johard et al., 2009). The interplay of PDF and sNPF in controlling circadian clock events was shown by Nagy et al. (2019). These authors indicated that in adult *D. melanogaster*, PDF and sNPF inhibit reproductive arrest (see above), likely through modulation of the IPCs. Genetic manipulations of the PDF-expressing neurons, which include the sNPF-producing sLN_vs, modulate the levels of reproductive dormancy, suggesting the involvement of both neuropeptides. Moreover, a synergistic effect of the two neurohormones was suggested. Separate applications of each neuropeptide increased cAMP levels in the IPCs, but the effect was even stronger when they were applied together. These studies suggest that PDF and sNPF inhibit reproductive dormancy by maintaining IPCs in an active state. In addition, inhibition of sNFR1 in IPCs in late larval development stages of *D. melanogaster* caused a significantly higher proportion of flies to show gonadal arrest than in control flies (Nagy et al., 2019).

Another hypothesis about the functions of sNPF in the circadian clock activity of *Drosophila* adults was proposed by Selcho et al. (2017). They proposed that sNPF could function as a messenger between the central clock, especially the neuron subclass sLN_vs, and nonclock neurons that produce prothoracicotropic hormone (PTTH). This in turn regulates the production of ecdysone by the PG, which is considered a peripheral clock (Myers et al., 2003). Selcho et al. (2017) found that sNPF rapidly decreases the level of free Ca^{2+} in PTTH neurons and suggested that via sNPF, sLN_vs inhibit the action of PTTH-producing neurons. Moreover, those effects were confirmed by experiments with RNAi and sNPFRs localized in PTTH neurons. RNAi-mediated knockdown of receptors caused the distribution of circadian rhythmicity of emergence, designated eclosion events (Selcho et al., 2017). Additionally, the results obtained by Johard et al. (2009) from experiments conducted on adult *D. melanogaster* suggested that sNPF might play a role as a modulator of, or cotransmitter between, sLN_v and LN_d sets of clock neurons, as it is coexpressed with choline acetyltransferase, which is a main neurotransmitter, in two LN_d and with PDF in the four sLN_vs.

5.4 Involvement of sNPF in the regulation of other physiological processes

sNPFs are considered mainly as regulators of feeding and metabolism as well as reproduction and locomotion. Nevertheless, they are hypothesized to be involved in the regulation of other physiological processes, such as water and ion balance (Fig. 4). In *D. melanogaster*, sNPF inhibits resting fluid secretion but only of adult male Malpighian tubules (Chintapalli et al., 2012). The role of sNPF in water homeostasis also seems to confirm the results obtained by Kahsai et al. (2010). They specifically knocked down the expression of sNPF in IPC-1 and IPC-2a cells, which resulted in decreased resistance of flies to desiccation. However, the effects might be direct or indirect. *D. melanogaster* IPC-1 and IPC-2 cells also expressed two other neuropeptides involved in water balance regulation: ITP and TRP.

Like other animals, insects can learn via training and can store information that may help them in future situations. Thus, the control of learning and memory processes is very important. The key structures involved in these processes are Kenyon cells, a large portion of the intrinsic neurons of MBs (Schoofs et al., 2017). Johard et al. (2008) confirmed the expression of sNPF in numerous *D. melanogaster* Kenyon cells, suggesting its role in learning and memory processes. When the knockdown of *sNPF* was applied to Kenyon

cells, it impaired sugar-rewarded olfactory memory, whereas knockdown of *sNPFR* outside the MBs caused deficits in appetitive memory (Knapek et al., 2013). Moreover, data obtained by Dillen et al. (2015) suggested that sNPFs are involved in the efficiency of learning and visual memory in *S. gregaria* 5th instar nymphs of both sexes. Similar results were shown for the honeybee, *A. mellifera*. In foragers with exogenously increased levels of sNPF, the efficiency of color learning and memory was significantly higher when they were partially fed (i.e., with reduced motivation to learn colors) than when starved (Bestea et al., 2022a).

6 Are there any similarities between sNPF and mammalian neuropeptides?

The neuropeptides that have been identified in invertebrates are often orthologs of peptides identified in vertebrates (Hewes and Taghert, 2001; Vanden Broeck, 2001). This is the case, for example, with insulin and ILPs, TRPs, sulfakinin and cholecystokinin, and capability peptide (CAPA) and neuromedin U (Urbanski and Rosinski, 2018). Research conducted on NPF signaling showed that these peptides in insects are equivalent to mammalian NPY based on their structural similarities and, more importantly, physiology. This similarity was long considered also to apply to sNPFs. This was before the discovery that NPF and sNPF are distinct peptide families. Detailed phylogenetic analysis performed by Jékely (2013) revealed that sNPFR clusters together with mammalian PrPR. The alignment of both receptor sequences showed similarity of 40% (Marciniak et al., 2020). Based on these assumptions, sNPF is now considered an ortholog of PrPRs rather than NPY, even if sNPFR is considered an ortholog of the vertebrate neuropeptide Y type 2 (Y2) receptor (Jékely, 2013). Similarities between sNPF and PrPR occur not only at the structural but also at the functional level. Both peptides are pleiotropic, but their main role is predominantly associated with the regulation of food intake and energy homeostasis. PrPRs decrease food intake (Pražienková et al., 2019) as do sNPFs, at least in several insect species. This is another argument in favor of considering both peptides as orthologs. The functions of NPF and sNPF overlap in terms of regulating food consumption and foraging, which is why they are considered to be ancestrally related. However, as summarized by Fadda et al. (2019), over the course of evolution, sNPF signaling was retained only in invertebrates, whereas the PrPR signaling system evolved in vertebrates.

7 Summary and future perspectives

The main physiological roles of sNPF, as an ortholog of mammalian PrPR, seem to be in food intake and energy metabolism. The mammalian PrPR may have other specific roles, such as the regulation of cardiac function, stress response, and reproduction or modulation of hormonal axes (mainly the hypothalamic–pituitary–adrenal (HPA) axis) (Pražienková et al., 2019). In insects, involvement in the regulation of feeding and metabolism has been confirmed. However, the observed effects are contradictory and seem to be species- or order-specific. Why sNPF acts as a satiety or hunger hormone in different species remains to be resolved.

Undoubtedly, energy metabolism affects the proper functioning of the organism and maintains its homeostasis, and deregulation can adversely impact vital processes such as development and reproduction. Many studies have shown that sNPF is involved in tuning reproductive processes. This is in agreement with findings for mammalian PrPR, which has been shown to be involved in gonadal axis regulation. The major question is whether sNPF effects are direct or indirect. Thus far, it seems that sNPFs may directly influence some events, such as reproductive muscle contractions, but there is no proof that they directly regulate gametogenesis and reproductive organs (Fig. 4). The developmental processes, however, are probably indirectly regulated by sNPF. This is probably due to interplay with various other neurohormones. Interplay is especially important between sNPFs and ILPs in energy metabolism and sNPF and SIFamides together with PDFs in the regulation of locomotor activity and circadian rhythm. The exact mode of this interplay is still unknown.

As shown in this review, sNPFs are important pleiotropic neuromolecules responsible for vital biological processes. The complete mode of their action has not been discovered but the importance of sNPFs in the regulation of insect physiology indicates that

they are a potential source for the design and production of ecofriendly biopesticides.

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Author contributions

Paweł MARCINIAK designed the conception and coordinated the preparation of the manuscript. Marcin CHOLEWIŃSKI, Szymon CHOWAŃSKI, Jan LUBAWY, Arkadiusz URBAŃSKI, and Karolina WALKOWIAK-NOWICKA wrote and edited the manuscript. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Marcin CHOLEWIŃSKI, Szymon CHOWAŃSKI, Jan LUBAWY, Arkadiusz URBAŃSKI, Karolina WALKOWIAK-NOWICKA, and Paweł MARCINIAK declare that they have no conflict of interest.

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