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FEEDING STATE MODULATES NOCICEPION IN *CAENORHABDITIS ELEGANS*

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ABSTRACT

An important function of the nervous system is to respond to changes in the environment. The nematode *C. elegans* chemotaxes towards attractants and escapes noxious stimuli. Chemotaxis to salts requires the two ASE neurons ASEL and ASER, and escape responses require the nociceptive ASH neurons. To study the mechanisms underlying these behaviors, we adopted a combination of genetics and *in vivo* calcium imaging, which allows monitoring of neuronal activity in living animals.

Calcium imaging revealed that ASEL and ASER are functionally asymmetric. ASEL is an ON-cell activated by increases in NaCl, and ASER is an OFF-cell activated by decreases in NaCl. Activation of ASEL results in forward runs and activation of ASER results in turns. Signal transduction in the ASE neurons involve cGMP signaling, and activation of both neurons require the TAX-2/TAX-4 nucleotide gated channel and the EGL-4 cGMP-dependent kinase. Together ASEL and ASER function to regulate chemotaxis up a concentration gradient (Paper I).

To study of neurons in isolation from input to other sensory neurons, we developed a method called Functional Rescue in Single Sensory Cilia (FRISSC). In FRISSC, a null mutation in the RFX transcription factor DAF-19C, which is required for ciliogenesis, is rescued cell-specifically. This allows restoration of cilia and sensory function in individual neurons. We tested if the restored cilia are fully functional by performing calcium imaging in the ASE neurons. Our results show that FRISSC generates fully functional cilia, and that the rescue is cell-specific and cell-autonomous. Thus, FRISSC is a useful method to study sensory neurons in isolation (Paper II).

We used FRISSC to study how nociception is modulated by feeding state. We found that the ASH neurons are enhanced by food through dopamine signaling. In a food-rich environment, escape responses to soluble repellents are increased, and the ASH neurons are sensitized. This effect requires sensory input to the dopaminergic neurons and the dopamine receptor DOP-4. Our results indicate that dopamine functions as a food signal to sensitize the ASH neurons and increase escape responses (Paper III).

In contrast to dopamine, neuropeptide signaling inhibits nociception in the absence of food. Overexpression analysis revealed that in the absence of food, the FMRFamide-related peptide FLP-8 inhibits responses to soluble repellents. This effect requires the neuropeptide receptor NPR-1, which acts on the ASH neurons to increase adaptation to repellents. These results demonstrate that feeding state modulates nociception through a complex network of bioamine and neuropeptide signaling (Paper IV).

LIST OF PUBLICATIONS

I. Suzuki, H., Thiele, T.R., Faumont, S., **Ezcurra, M.**, Lockery, S.R., and Schafer, W.R. (2008). Functional asymmetry in *Caenorhabditis elegans* taste neurons and its computational role in chemotaxis. Nature *454*, 114-117.

II. Senti, G., **Ezcurra, M.**, Löbner, J., Schafer, W.R., and Swoboda, P. (2009). Worms with a single functional sensory cilium generate proper neuron-specific behavioral output. Genetics *183*, 595-605.

III. **Ezcurra, M.**, Tanizawa, Y., Swoboda, P., and Schafer, W.R. (2011). Food sensitizes *C. elegans* avoidance behaviours through acute dopamine signalling. EMBO J *30*, 1110-1122.

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CONTENTS

LIST OF ABBREVIATIONS

ASIC	Acid-sensing ion channel
ADE	Anterior deirid neuron
ASE	Amphid single-ciliated neuron class E
ASEL	Amphid single-ciliated neuron class E, left
ASER	Amphid single-ciliated neuron class, right
ASH	Amphid single-ciliated neuron class H
ATP	Adenosine triphosphate
AVA	Ventral cord interneuron class A
AVB	Ventral cord interneuron class B
AVD	Ventral cord interneuron class D
cAMP	Cyclic adenosine monophosphate
CAT	Abnormal catecolamine distribution
CEP	Cephalic
CFP	Cyan fluorescent protein
DA	Ventral cord dorsal neurons class A
DAF	Abnormal dauer formation
DB	Ventral cord dorsal neuron class B
DEG	Degenerin
DOP	Dopamine receptor
EGL	Egg laying defective
ENAC	Epithelial sodium channel
FARP	FMRF-amide related peptide
FLP	FMRFamide-like peptide
FRET	Fluorescence resonance energy transfer
FRISSC	Functional rescue in single sensory cilia
GABA	Gamma-aminobutyric acid
GCY	Guanylyl cyclase
GFP	Green fluorescent protein
GPA	G protein, alpha subunit

GPC	G protein, gamma subunit
NPFF	Neuropeptide FF
NPR	Neuropeptide receptor family
NPY	Neuropeptide Y
OCR	Osm-9 and capsaicin receptor related
ODR	Odorant response abnormal
OSM	Osmotic avoidance abnormal
P2X	Purinergic receptor 2X
PDE	Posterior deirid neuron
QUI	Quinine non-avoider
rGC	Receptor guanylate cyclase

1 INTRODUCTION

An important function of the nervous system is to respond in a dynamic way to changes in the environment. This kind of plasticity allows animals to adjust their behaviors to the present context, so that the behavior is optimal for the current conditions. Nociception is an essential sensory modality, which results in pain sensation and protects us during tissue damage. Normal pain responses lead to rest and withdrawal, which helps an injured animal to recover and heal. Pain sensation is not a static response, and several modulatory pathways acting to increase and decrease pain have evolved. Understanding modulation of nociception, and specifically modulation of peripheral sensory neurons, would provide useful insights into the mechanisms of pain pathways.

The main objective of this thesis is to study the molecular and cellular basis of behavior. My major focus has been to study modulation of nociceptive responses and nociceptive neurons. For this task, I have chosen to work with the nervous system of the nematode *C. elegans*. *C. elegans* is ideal for studying the nervous system at the molecular and cellular level, due to its simple nervous system and genetics, and short generation time. In addition, *C. elegans* exhibits a number of different behaviors, which can easily be quantified. These traits allow us to perform an array of different genetic manipulations in order to study the role of specific cells and genes in modulating nociception.

The major finding of my thesis is that avoidance behaviors in *C. elegans* are modulated by the feeding state of the animal. This modulation is mediated by dopamine and neuropeptides acting directly on primary nociceptive neurons, resulting in increased nociception in food-rich environments, and in decreased nociception in food-poor environments. The work described in this thesis reveals that the nervous system acts in a dynamic way to fine tune nociceptors and avoidance in response to changes in the environment. Another important part of my thesis involves the development of a method, Functional Rescue in Single Sensory Cilia (FRISSC). FRISSC was designed to correlate input to sensory neurons with behavioral output, and we later used FRISSC to make important findings regarding modulation of nociception. The thesis also includes work outside my major focus, regarding another important behavior, chemotaxis. Even though this work is not related to nociception, it is highly relevant to the thesis, since the findings of this work played an important role in the development of FRISSC.

The introduction of this thesis is divided into three parts. Part 1 is a general introduction to nociception. Part 2 describes the nervous system of *C. elegans*, and explains many of the *C. elegans* specific terms used the thesis. It also points out important similarities and differences between *C. elegans* and mammalian systems. Part 3 describes the specific behaviors and methods discussed in the thesis.

1.1 NOXIOUS STIMULI ARE SENSED BY POLYMODAL NOCICEPTIVE NEURONS

The ability to sense harmful stimuli is crucial for survival and warns an animal of danger and tissue damage. Most, if not all, animals respond to noxious stimuli and have polymodal nociceptive neurons, which are activated by high intensity mechanical, chemical and thermal stimuli (Smith and Lewin, 2009). In mammals, nociceptive neurons have their sensory endings in the skin, and terminate in the dorsal horn of the spinal cord. In the dorsal horn they connect to projection neurons that relay information to the brain, leading to pain perception. The main brain areas that receive input from the projection neurons are the thalamus and the reticular formation (Kandel et al., 1991).

1.1.1 Signal transduction in nociceptive neurons

Nociception is remarkable in the sense that the nociceptive neurons are capable of sensing a wide range of stimuli, including mechanical, thermal and chemical stimulation. Polymodal nociceptors are equipped with a wide range of signal transduction molecules, including members of the transient receptor potential (TRP) and DEG/ENaC families.

Thermal stimuli activate thermosensitive TRP channels, in particular TRPV1. Noxious chemical stimuli like protons and capsaicin also activate TRPV1 channels. In addition, TRP channels can be activated by G-protein signaling or depolarization of the membrane (Caterina et al., 1997; Tominaga et al., 1998; Vennekens, 2011)

DEG/ENaC channels are gated by diverse stimuli such as protons and mechanical forces (Chalfie and Sulston, 1981; Lingueglia et al., 1997; Price et al., 2000; Sutherland et al., 2001). Activation of TRP and DEG/ENaC channels results in membrane depolarization and initiation of action potentials, a process that requires nociceptor-specific voltage-gated sodium channels.

1.1.2 Endogenous pathways modulate nociception

Environmental and emotional factors are known to modulate nociception, making nociception a highly complex sensory modality. Pathways to increase and decrease pain sensation are present in the nervous system and nociception can be modulated at all levels of the circuit. Some of the molecular players in endogenous analgesia, or pain relief, have been identified, for example opioid peptides, but the mechanisms underlying their release remain unknown.

Increased pain sensitivity, or hyperalgesia, can occur when peripheral tissues are damaged. The damaged tissue releases signaling molecules, which act on receptors and ion channels on the nociceptor nerve endings. Signaling

molecules include bradykinin, which acts on metabotropic receptors to initiate second messenger signaling, and protons and ATP, which act on ASIC3 channels and P2X channels respectively (Burgess et al., 1989; Lingueglia et al., 1997; Sutherland et al., 2001; Tsuda et al., 2010). These signaling pathways act to increase excitability of the nociceptors, resulting in increased pain sensitivity. In turn, nociceptors can release peptides and neurotransmitters from their peripheral terminals, which promote release of inflammatory molecules from neighboring tissue.

The most studied antinociceptive molecules are the opioid peptides and their receptors. Opioids modulate pain perception by acting on receptors both in the brain and directly on peripheral nociceptors. Opioid receptor agonists, such as morphine, have a long history of clinical use due to their analgesic effects, but their use is problematic due to their effects on the central nervous system. Opioid receptors are involved in mechanisms like reward and fear behaviors, and use of morphine and other opiates can result in cognitive effects and addiction (Butler and Finn, 2009; Kandel et al., 1991; Stein and Zollner, 2009). Other peptides with antinociceptive effects include cannabinoids and NPY (Guindon and Beaulieu, 2009; Smith et al., 2007).

1.2 *C. ELEGANS* AS A MODEL FOR STUDYING NOCICEPTION AND OTHER SENSORY SYSTEMS

The nematode *C. elegans* offers a useful system to study sensory behaviors both at the behavioral, cellular and molecular level. *C. elegans* has amenable genetics and consists of self-fertilizing hermaphrodites with a short generation time of about 3 days. In addition, *C. elegans* produces males in low frequency. The combination of the hermaphrodite and male sexes, and the short generation time, makes *C. elegans* ideal for generating and crossbreeding mutant and transgenic strains.

The structure of the nervous system is simple and its connectivity completely characterized (Figure 1). The nervous system shares signaling molecules with mammalian systems; for example the classical neurotransmitters serotonin and dopamine, neuropeptides and signal transduction molecules. In addition, *C. elegans* offers sophisticated tools for studying the mechanisms underlying sensory behaviors. Neuronal activity can be measured *in vivo* using the genetically encoded calcium sensor cameleon (Ferkey et al., 2007; Hilliard et al., 2005; Kerr et al., 2000; Shyn et al., 2003), and particular neurons can be activated using channelrhodopsin (Guo et al., 2009; Nagel et al., 2005; Nagel et al., 2003).

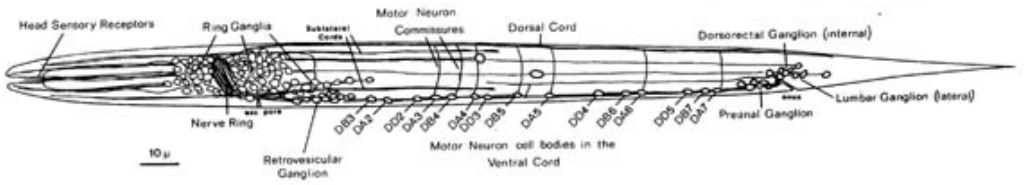

Figure 1. *The structure of the C. elegans nervous system*
The position and connectivity of each neuron has been characterized using electron micrographs. The head and tail ganglia mainly consist of sensory neurons and interneurons. The motor neurons form the ventral and dorsal cords, and innervate the body wall and vulval muscles (from www.wormatlas.org).

C. elegans can sense noxious stimuli and displays strong avoidance behaviors in response to noxious chemicals, mechanical stimulation and thermal stimulation. The *C. elegans* sensory system includes several classes of nociceptive neurons, which allow detection and avoidance of harmful stimuli. The nociceptors are strongly activated by noxious stimuli, and require transduction molecules like TRP channels and DEG/ENaC channels (Chatzigeorgiou et al., 2010a; Chatzigeorgiou et al., 2010b; Colbert et al., 1997; Hilliard et al., 2005; Kindt et al., 2007; Suzuki et al., 2003; Tobin et al., 2002), making *C. elegans* an amenable system to study circuits and molecules involved in nociception.

1.2.1 The anatomy of the *C. elegans* nervous system

The anatomy of the *C. elegans* nervous system has been reconstructed using serial section electron microscopy, which has generated detailed information on the anatomy, position and connectivity of each individual neuron (Figure 1). *C. elegans* has a simple nervous system consisting of 302 neurons, of which 32 are chemosensory and 28 are mechanosensory (Table 1). 113 neurons are motor neurons, which innervate the body wall muscles, the vulva muscles and the pharyngeal muscles. The remaining neurons are interneurons (Ward et al., 1975; White, 1986). However, sensory neurons also receive significant input from other neurons, and most likely function as interneurons in addition to their sensory role. A majority of the neurons are clustered in the head, forming the nerve ring, and in the tail, forming the tail ganglia.

Every *C. elegans* neuron class name consists of either two or three uppercase letters indicating class, for example ASH and ASE. Most sensory neuron classes consist of a symmetrical pair, with one cell on the left side and one on the right side of the head. In these cases, the pair is indicated by the class name, for example ASE, and each individual cell is indicated by the class name followed by L (left) or R (right), for example ASEL and ASER.

Chemosensors	Odorsensors	Oxygen sensors	Thermosensors
Amphids (head)	*Amphids (head)*	*Head*	*Amphids (head)*
ADF	ASH	AQR	AFD
ASE	AWA	URX	
ASG	AWB	BAG	
ASH	AWC		
ASI		Tail	
ASK		PQR	
ADL			

Mechanosensors		Polymodal nociceptors	Dopaminergic
Body	*Deirids*	*Amphids (head)*	*Head*
ALM	ADE	ASH	CEP
AVM	PDE		ADE
PLM		*Head*	
PVM	*Branched*	FLP	*Body*
	FLP		PDE
Cephalic	PVD	*Body*	
CEP		PVD	
	Amphids		
Outer labials	ASH		
OLQ			
OLL			

Table 1. *Summary of function of sensory neurons in C. elegans*
The *C. elegans* nervous system detects a wide range of stimuli. Some sensory neurons respond to one modality only, while others detect several different modalities. The polymodal nociceptive neuron ASH detects mechanosensation, chemical repellents, high osmolarity and odors. The dopaminergic neurons are believed to be mechanosensors.

1.2.2 The amphids are the primary chemosensory organs

Even though the *C. elegans* nervous system is small and simple, it is capable of sensing and responding to a variety of different stimuli. *C. elegans* navigates its environment and finds favorable conditions by sensing chemicals, temperature, oxygen, CO_2 and mechanical stimulation (Bargmann et al., 1993; Bretscher et al., 2008; Cheung et al., 2005; Coates and de Bono, 2002; Colbert et al., 1997; Culotti and Russell, 1978; Gray et al., 2004; Hilliard et al., 2002; Ward, 1973).

The primary chemosensory organs in *C. elegans* are the two amphids, which are located on the left and right side of the head and contain the left-right symmetrical neuron pairs. The amphid neurons have long dendrites, which extend through the amphid pore to the exterior of the animal at the tip of the nose where they terminate with a sensory cilium (Ward et al., 1975; White, 1986). Some of the amphid neurons can be easily identified through their ability to take up fluorescent dyes, since sensory neurons that have unexposed cilia terminating in the subcuticle or body cavity do not take up fluorescent dyes (Hedgecock et al., 1985; Herman, 1984).

1.2.3 Sensory neurons in *C. elegans* are ciliated

Cilia are slender subcellular organelles, which project from the cell. Non-motile, or primary cilia are microtubule-based and used for sensing the

chemical or physical environment of the cell. Most mammalian cell types have primary cilia, but in *C. elegans*, cilia exist only in the sensory neurons. Sensory cilia are rich in sensory transduction molecules, and sensory transduction is believed to occur in these compartments (Ward et al., 1975; White, 1986).

A number of cilia structure mutants have been isolated based on chemosensory phenotypes, inability to take up fluorescent dyes, and cilia morphology. One example is the RFX-type transcription factor DAF-19, which regulates ciliogenesis. DAF-19 recognizes a promoter element termed X-box, and by binding this element DAF-19 drives expression of genes involved in cilium biogenesis in the 60 ciliated neurons. Mutations in *daf-19* lead to the complete lack of cilia in all ciliated neurons, while disruption of individual cilium formation genes leads to shortened or abnormal cilia (Perkins et al., 1986; Swoboda et al., 2000)

1.2.4 Dopamine and serotonin systems in *C. elegans*

The *C. elegans* nervous system uses classical neurotransmitters, including serotonin, dopamine, acetylcholine, GABA and glutamate (Horvitz et al., 1982; Sanyal et al., 2004; Schaeffer and Bergstrom, 1988; Sulston et al., 1975). In contrast to mammals, *C. elegans* and other invertebrates also use octopamine, which is closely related to norepinephrine, and its precursor tyramine, as neurotransmitters (Alkema et al., 2005; Horvitz et al., 1982). Mammalian signaling molecules such as histamine, epinephrine and norepinephrine are not present in *C. elegans*.

Biogenic amines such as dopamine and serotonin are involved in responses to changes in the environment, in particular food availability. Dopamine and serotonin can act both as neurotransmitters, and extrasynaptically as neurohormones, to alter feeding-related behaviors such as pharyngeal pumping and locomotion (Avery and Thomas, 1997; Chase et al., 2004; Sawin et al., 2000). The *C. elegans* nervous system also expresses a large number of neuropeptides, which are involved in modulating many behaviors (Li, 2005; Li et al., 1999).

1.2.4.1 Dopamine

In mammals, dopamine is known to be important in reward and motivation of actions, and creates a state of motivation to seek rewards. Drugs that enhance dopamine transmission often act as reinforcers and have addictive effects (Bromberg-Martin et al., 2010). In addition, dopamine also plays a role in movement by acting on dopamine receptors in the basal ganglia. Dopamine is produced in the substantia nigra and the ventral tegmental area, and can also act as a neurohormone when released from the hypothalamus (Kandel et al., 1991).

Humans have five distinct dopamine receptors, D1 to D5. The dopamine receptors are categorized in two different classes, the D1-like (D1 and D5) and the D2-like receptors (D2, D3 and D4), which have different affinities to dopamine and different anatomical localizations. Both types are linked to G-proteins but have opposite effects; D1-like receptors stimulate adenylate cyclase, while D2-like receptors inhibit it (Kandel et al., 1991).

In *C. elegans*, dopamine is produced by 8 neurons: four CEPs in the nose, 2 ADEs in the head, and 2 PDEs in the body (Sulston et al., 1975). All are ciliated neurons with putative mechanosensory dendrites, and the CEPs in particular have been shown to respond directly to mechanical stimuli (Kang et al., 2010; Kindt et al., 2007). Four G-protein coupled dopamine receptors have been identified in *C. elegans*; DOP-1, DOP-2, DOP-3 and DOP-4. DOP-1 is homologous to the mammalian D1-like dopamine receptors, and DOP-2 and DOP-3 are homologous to the mammalian D2-like dopamine receptors (Chase et al., 2004). DOP-4 is an invertebrate specific D1-like dopamine receptor (Sugiura et al., 2005). Invertebrate specific dopamine receptors have also been identified in *Drosophila melanogaster* (Feng et al., 1996; Han et al., 1996). Invertebrate specific dopamine receptors are pharmacologically similar to D1-like receptors, but sequence analysis has revealed that they are a distinct type of receptors (Suo et al., 2004).

1.2.4.2 Serotonin

In *C. elegans*, serotonin is produced by the neurosecretory NSM neurons, the egg-laying HSN neurons, ADF, the RIH and AIM interneurons and the VC4 and VC5 motor neurons (Duerr et al., 1999; Horvitz et al., 1982). The NSMs have sensory endings in the pharynx, and might respond directly to food being ingested by the animal. The *tph-1* gene encodes tryptophan hydroxylase, and mutant analysis of *tph-1* has shown that serotonin is involved in pharyngeal pumping and egg-laying (Sze et al., 2000).

1.2.5 *C. elegans* has a complex neuropeptide system

Neuropeptides are a diverse group of chemical messengers, whose role mainly is to modulate the action of classical neurotransmitters. Neuropeptides can both act as short-range signals, released close to the site of action, or as hormones, acting on tissues outside the nervous system. Neuropeptides have been implicated in modulating a range of different behaviors. For example, opioid peptides modulate pain sensation, NPY and leptin regulate hunger and satiety, and vasopressin and oxytocin are involved in pair bonding and maternal behaviors (Levine and Morley, 1984; Nair and Young, 2006; Pedersen and Prange, 1979; Stanley and Leibowitz, 1984; Williams et al., 1992; Williams et al., 1994; Winslow et al., 1993; Zhang et al., 1994).

C. elegans has a surprisingly large system of neuropeptides, including over 100 neuropeptide genes. Some of these have mammalian counterparts, like the insulin-like peptides and the FMRF-related peptides. Around 50 putative neuropeptide receptors have been identified, suggesting that multiple peptides have overlapping functions and bind to the same receptor (Li and Kim, 2010; McVeigh et al., 2006).

1.2.5.1 FMRFamide-related peptides

One important neuropeptide family in *C. elegans* is the FMRFamide-related peptide family (FaRP, in *C. elegans* the abbreviation is FLP). The family is named after FMRFamide, a peptide isolated from the clam *Macrocallista nimbosa* (Price and Greenberg, 1977), and members of this family end with the amino acid sequence RF-amide. Interestingly, the amino acid sequence of FMRFamide (Phe-Met-Arg-Phe-amide) is identical to that of a mammalian opioid peptide derived from the Met-enkephalin precursor, suggesting possible functional links between opioids and FaRPs. Since the discovery of FMRFamide, a large number of related peptides have been discovered in all invertebrate groups, and more recently a handful of FaRPs have been found in mammals; *farp-1* (encodes NPFF and NPAF peptides), *farp-2* (PrRP), *farp-3* (RFRP, NPSF and others), *farp-4* (metastin/kisspeptins) and *farp-5* (26RFamide) (Dockray, 2004). These peptides are involved in various processes. For example, NPFF is expressed in the dorsal horn and modulates pain (Dong et al., 2001; Panula et al., 1999). NPFF is also expressed in the hypothalamus, where it inhibits feeding behavior, while 26RF stimulates feeding (Chartrel et al., 2003; Murase et al., 1996).

C. elegans has a more complex FaRP system, including 32 FLP genes, encoding over 60 peptides, and at least 11 receptors. Some receptor-ligand pairs have been identified by using heterologously-expressed receptors, and measuring cellular responses or binding to peptides (McVeigh et al., 2006). Using such approaches, the neuropeptide receptor NPR-1 has been found to be activated by the FLP-18 and FLP-21 peptides (Rogers et al., 2003).

1.2.5.2 The neuropeptide receptor NPR-1 regulates aggregation behaviors

The first neuropeptide receptor in *C. elegans* to be functionally characterized was NPR-1. NPR-1 is homologous to the mammalian NPY receptor, which regulates mood, appetite and pain sensation (Morales-Medina et al., 2010; Smith et al., 2007; Valassi et al., 2008). In *C. elegans*, NPR-1 is strongly implicated in aggregation behavior. Wildtype animals have a high activity variant of NPR-1. On a bacterial food lawn, wildtype animals slow down and feed in a solitary fashion. In contrast, low activity variants or null mutants of *npr-1* display dramatic changes in behavior, and move rapidly on food, accumulate at the border of the lawn and form aggregates (de Bono and Bargmann, 1998).

NPR-1 is widely expressed in the nervous system, and acts in the oxygen sensing neurons AQR, URX and PQR to regulate aggregation (Coates and de Bono, 2002; de Bono and Bargmann, 1998). In *npr-1* mutants, the oxygen sensing neurons promote aggregation in response to oxygen levels (Cheung et al., 2005; Gray et al., 2004). Aggregation is also affected by sensing of ascaroside pheromones by the ASK neurons (Macosko et al., 2009). Aggregation behaviors are coordinated by the RMG interneuron, which is connected to URX, ASK and other sensory neurons by gap junctions (Macosko et al., 2009). In addition, NPR-1 has also been shown to play a role in the nociceptive ASH neurons. NPR-1 is expressed in the ASH and ADL neurons, and aggregating *npr-1* mutants become solitary when ASH and ADL are ablated (de Bono et al., 2002). It is not known if NPR-1 affects nociception or how it acts to regulate ASH function.

NPR-1 has also been implicated in other responses such as ethanol tolerance, heat avoidance, gustation, immunity and CO_2 avoidance, and seems to be an important modulator of the *C. elegans* nervous system (Bretscher et al., 2008; Davies et al., 2004; Glauser et al., 2011; Hallem and Sternberg, 2008; Pocock and Hobert, 2010; Reddy et al., 2009)

1.3 CHEMOTAXIS AND NOCICEPTION IN *C. ELEGANS*

C. elegans explores its environment and moves to favorable surroundings by chemotaxis, thermotaxis, and aerotaxis and escapes from noxious stimuli through avoidance behaviors. Behavioral assays have been developed to quantify these behaviors allowing the comparison of behavioral responses of different genotypes and different conditions. The behaviors discussed in this thesis are chemotaxis and avoidance. *C. elegans* is attracted to various water-soluble molecules and the gustatory ASE neurons have an important role in attraction to these chemicals. Aversive stimuli are mainly sensed by the ASH neurons, which are polymodal nociceptors and detect chemical and mechanical stimuli.

1.3.1 The ASE neurons are required for chemotaxis

Chemotaxis is the movement of an organism along a concentration gradient towards a chemical stimulus. *C. elegans* is attracted to water-soluble molecules such as Na^+, Cl^-, cAMP, biotin and lysine, and displays chemotaxis towards these stimuli (Bargmann and Horvitz, 1991; Ward, 1973). When *C. elegans* travels up a gradient of attractant, it moves in long forward runs with rare changes of direction. When an animal travels down a gradient of attractant, it changes direction frequently, making turns. By changing the rate of turns, the animal makes long movements up the gradient and short movements down

the gradient. Over time, it migrates to the peak of the gradient (Pierce-Shimomura et al., 1999).

The gustatory ASE neurons are required for chemotaxis to NaCl and other salts (Bargmann and Horvitz, 1991). Signal transduction in the ASE neurons is likely to involve cGMP signaling, since chemotaxis also requires the TAX-2/TAX-4 cGMP gated ion channel, which is expressed in the ASE neurons (Coburn and Bargmann, 1996; Komatsu et al., 1996). In contrast to most sensory neurons, where the two members of each symmetrical pair are identical, the two ASE neurons show genetic and functional differences. ASEL is primarily sensitive to Na^+ ions and ASER to Cl^- ions (Pierce-Shimomura et al., 2001). Gene expression studies have shown that receptor guanylate cyclases (rGCs) are asymmetrically expressed in the ASE neurons. For example, *gcy-6* and *gcy-14* are expressed in ASEL and *gcy-1*, *gcy-4*, *gcy-5* and *gcy-22* are expressed in ASER (Yu et al., 1997), and these rGCs are required for sensitivity to different sets of ions (Ortiz et al., 2009). This suggests that rGC are salt receptors, or possible molecules connected to salt receptors, that produce cGMP upon activation, resulting in opening of the TAX-2/TAX-4 cGMP gated channel.

1.3.2 The ASH neurons are polymodal nociceptors

Aversive stimuli are mainly sensed by one pair of nociceptive neurons, the ASH neurons. In contrast to most *C. elegans* neurons, ASH is polymodal, and can detect soluble chemicals, volatile chemicals, high osmolarity and nose touch (Bargmann et al., 1990; Hilliard et al., 2002; Hilliard et al., 2004; Kaplan and Horvitz, 1993; Sambongi et al., 1999). ASH forms glutamatergic synapses to the command interneurons AVA, AVB and AVD. AVA and AVD innervate the VA and DA motor neurons, which coordinate backward movement, while AVB innervates the VB and DB motor neurons that coordinate forward movement (Chalfie et al., 1985; Hart et al., 1995; Maricq et al., 1995; White, 1986). Activation of ASH by noxious stimuli generates immediate escape responses involving reversals followed by forward movement in a different direction (Culotti and Russell, 1978).

Studies using the calcium indicator cameleon have shown that aversive stimuli activate ASH and give rise to large intracellular calcium transients in the cell. Calcium transients require a TRPV channel encoded by OSM-9 and OCR-2, and the L-type voltage-gated calcium channel EGL-19, suggesting that sensory activation depolarizes the cell (Hilliard et al., 2005). Not much is known about signal transduction in ASH and how the different modalities are sensed, but some components have been identified. The G_i protein ODR-3 is required for normal ASH responses to all soluble repellents tested (Hilliard et al., 2005), suggesting that ODR-3 acts downstream of chemosensory receptors in ASH. Responses to the bitter compound quinine involve the G_α protein GPA-3, and QUI-1, a cytoplasmic protein with WD40 domains, but these proteins are not involved in responses to high osmolarity (Hilliard et al., 2004;

Lans and Jansen, 2007). The intracellular protein OSM-10 is required for responses to high osmolarity and nose touch, but not other chemical repellents. Together these results suggest that repellents activate different pathways, which converge at the level of the ODR-3 G-protein and the OSM-9/OCR-2 TRPV channel.

1.3.3 Adaptation to repeated noxious stimulation

Adaptation to continuous and repeated stimulation is an important feature of sensory systems. In general, mammalian polymodal nociceptors do not adapt to continuous stimulation, but in *C. elegans* ASH adapts by decreasing the response over time (Hilliard et al., 2005). *C. elegans* adapts behaviorally to both constant and repeated noxious stimulation, and repeated stimulation of ASH results in smaller calcium transients. The adaptation is reversible; after a rest period of 5 minutes, ASH responses are restored to normal levels. ASH adaptation is not dependent on synaptic input from other neurons, since ASH also undergoes adaptation in *unc-13* mutants (Hilliard et al., 2005), which are defective in synaptic transmission. This indicates that intrinsic changes within ASH lead to adaptation, but which mechanisms might be involved are not known. One important component has been identified, the G-protein γ subunit GPC-1. Mutations in *gpc-1* do not affect initial responses, but lead to decreased adaptation, as shown both in behavioral assays and ASH imaging (Hilliard et al., 2005; Jansen et al., 2002). These results indicate that GPC-1 initiates signaling cascades leading to decreased responsiveness.

1.4 DOPAMINE AND SEROTONIN MODULATE C. ELEGANS BEHAVIORS

C. elegans modulates its behaviors in response to changes in the environment, and this modulation is mediated by bioamines like dopamine and serotonin, and to some extent neuropeptides. One particularly important factor guiding behavior is feeding state. In *C. elegans*, a number of behaviors are affected by the presence or absence of food, for example speed, locomotion, egg-laying and pharyngeal pumping, and also some sensory behaviors (Avery and Thomas, 1997; Chao et al., 2004; Ezak and Ferkey, 2010; Harris et al., 2009; Kindt et al., 2007; Sawin et al., 2000; Trent, 1982).

Dopamine is strongly implicated with food signaling in *C. elegans*, and is involved in modulating locomotion to ensure that animals remain on a food source. One such mechanism is the basal slowing response, which is a decrease in speed when worms encounter food (Sawin et al., 2000). This effect involves the D2-like dopamine receptor DOP-3 acting in the cholinergic motor neurons to inhibit locomotion (Chase et al., 2004). Another mechanism is area restricted

searching. When leaving or exhausting a food source, *C. elegans* searches the immediate environment for new food sources. This search is called area restricted searching and is accomplished by an increase in turn frequency (Hills et al., 2004). *cat-2* encodes tyrosine hydroxylase, an enzyme required for dopamine synthesis, and *cat-2* mutants are defective both in the basal slowing response and in area restricted searching, showing that dopamine is required for these behaviors. After a period of starvation, food-sensing leads to a more prominent slowing to ensure the animal remains on food, called the enhanced slowing response. This mechanism involves serotonin signaling (Sawin et al., 2000).

Neuropeptides have also been implicated in signaling feeding state. For example the *flp-1* neuropeptide gene is required for the stimulation of egg-laying by bacteria (Waggoner et al., 2000) and the neuropeptide FLP-18 acts through the receptors NPR-4 and NPR-5 to regulate foraging and fat accumulation (Cohen et al., 2009).

In addition to locomotion, sensory behaviors have also been shown to be modulated by serotonin and dopamine. Dopamine dampens responses to dilute octanol through the DOP-3 receptor, which acts on ASH (Ezak and Ferkey, 2010), and slows habituation to touch through DOP-1 acting on ALM anterior-body mechanoreceptors (Kindt et al., 2007). Serotonin enhances avoidance of nose touch and dilute octanol by modulating ASH through the serotonin receptor SER-5 and the G-protein GPA-11 (Chao et al., 2004; Harris et al., 2009; Hilliard et al., 2005).

1.5 METHODS FOR STUDYING BEHAVIOR IN *C. ELEGANS*

Within the *C. elegans* field, a large variety of behavioral assays have been developed to study behaviors. The use of behavioral genetics has allowed the identification of numerous genes involved in sensory transduction. Behavior reflects activity of the nervous system, and therefore it is important to determine the activity pattern of neurons and the effect of individual genes on neuronal activity. Electrophysiology and the use of calcium dyes are two common methods used in many systems to record electrical activity and changes in intracellular calcium, but these methods are not always feasible in *C. elegans*. Positioning of recording electrodes and delivery of dyes is difficult due to the small neuron size and the collagenous cuticle, which encloses the body and maintains its shape (Kerr et al., 2000). In recent years, non-invasive optical techniques based on genetically encoded fluorescent sensors have been used successfully to measure neuronal activity in intact living worms (Hilliard et al., 2005; Kerr et al., 2000; Shyn et al., 2003; Suzuki et al., 2003).

1.5.1 Identification of genes involved in nociception

Escape responses to water soluble repellents can be measured using the drop assay. In the drop assay, a small drop of the repellent is delivered to an animal while it is moving forward. If the animal senses the substance as a noxious stimulus, it will display an escape response consisting of a reversal followed by a change of direction of forward movement. Testing a population of animals gives the avoidance index, which is the number of animals responding divided by total number of animals tested (Hilliard et al., 2002; Hilliard et al., 2004). Comparison of the avoidance index of different genotypes allows identification of genes involved in nociception.

1.5.2 Monitoring neuronal activity in *C. elegans*

Optical techniques using fluorescent calcium sensors are particularly useful in *C. elegans*. *C. elegans* is transparent, allowing recordings in intact, living animals without dissection. Genetically encoded calcium sensors like cameleon can be expressed in neurons and muscle using a wide variety of cell-specific promoters. In addition, *C. elegans* lacks voltage-gated sodium channels, and depolarization of neurons is believed to occur through calcium entry.

The genetically encoded calcium sensor cameleon (Miyawaki et al., 1997) is based on fluorescence resonance energy transfer (FRET) changes upon calcium binding. Cameleon consists of two fluorescent molecules, cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP), connected by a calmodulin domain. A calcium increase in the cell results in binding of Ca^{2+} ions to the calmodulin domain and a conformational change of the cameleon. This change brings the CFP and YFP domains closer, allowing fluorescence resonance energy transfer (FRET) to occur. By measuring the intensities of YFP and CFP, and calculating the ratio, Ca^{2+} levels and neuronal activity can be monitored.

2 AIMS

The objective of this thesis is to study the molecular and cellular basis of behavior in *C. elegans*, with a major focus on modulation of nociceptive neurons.

The specific aims of this work are:

1. To discriminate the individual roles of the ASER and ASEL taste neurons in chemotaxis.

2. To develop a method enabling the study of different components of neural circuits by studying individual sensory neurons in isolation from other sensory input.

3. To investigate the effect of feeding state on nociception and nociceptive neurons, and identify cellular and molecular components mediating the effect of feeding state on responses to noxious stimuli.

4. To investigate the effect of neuropeptide signaling on nociceptive neurons and identifying neuropeptides and neuropeptide receptors with antinociceptive effects.

3 RESULTS AND DISCUSSION

3.1 PAPER I: FUNCTIONAL ASYMMETRY IN THE ASE TASTE NEURONS

C. elegans navigates its environment in order to find food and beneficial habitats. The principal neurons required for chemotaxis are the ASE neurons. *C. elegans* chemotaxes towards attractants like NaCl by increasing forward runs and suppressing turns when the animals moves up the gradient, and increasing the number of turns when the animal moves down the ion gradient. To ask how the ASE neurons can transform changes in concentrations into forward runs and turns, we used the calcium indicator cameleon to measure the calcium transients in the ASE neurons in animals when NaCl concentrations were increased and decreased. The two ASE neurons, ASEL and ASER, are anatomically homologous, but are genetically and functionally distinct. Using cameleon, we found that increases in NaCl give rise to calcium transients in ASEL but not ASER, and that decreases in NaCl give rise to calcium transients in ASER but not in ASEL. Genetic analysis indicates that sensory transduction in ASE involves the cGMP-gated channel subunits TAX-2 and TAX-4. We imaged *tax-2* and *tax-4* mutants and found that responses to upsteps and downsteps in ASEL and ASER are completely abolished. Together these data indicate that the ASE neurons act as ON- and OFF-cells and that ASE responses are mediated by cGMP signaling, which most likely acts downstream of chemoreceptors.

We tested the roles of the two ASE neurons in generating forward runs and turns by expressing the mammalian TRPV1 channel, which opens in response to the exogenous ligand capsaicin, in either ASEL or ASER. Behavioral assays showed that capsaicin activation of ASEL increased forward probability, and that activation of ASER decreased the forward probability. These results show the two ASE neurons have asymmetric neuronal responses to increases and decreases in NaCl concentration, leading to ASEL positively regulating forward runs, and ASER negatively regulating runs, causing turns.

3.2 PAPER II: FUNCTIONAL RESCUE IN SINGLE SENSORY CILIA

A number of useful tools have been developed to study the genetic and cellular basis of behavior in *C. elegans*. For example, the calcium sensor cameleon allows *in vivo* recordings of neuronal activity in response to sensory stimulation, and the sensory neurons required for specific sensory responses have been identified by laser ablation. However, these methods do not allow the study of a single sensory neuron in isolation from other sensory input, nor the direct correlation between a sensory cell and behavioral output. To tackle

these problems, we developed a method called Functional rescue in single sensory cilia (FRISSC), in which a null mutation in the RFX transcription factor gene *daf-19,* which is required for the production of sensory cilia, is rescued cell-specifically. Mutations in *daf-19* result in absence of sensory cilia and sensory input, and rescue of *daf-19* using cell-specific promoters restores ciliogenesis and sensory function in individual neurons. We expressed DAF-19 in a *daf-19* mutant background using several different promoters, including the ASER-specific promoter *gcy-5*, and using fluorescent markers and behavioral assays we confirmed rescue of cilia formation in the generated transgenic lines. To test if neuronal activity is normal in neurons with restored cilia, we used the ASE calcium imaging protocol described in Paper I. We found that in *daf-19* animals, the calcium transients in both ASE neurons were completely abolished, as expected from the lack of cilia and sensory function. When we tested rescue lines expressing DAF-19 in ASER using the *gcy-5* promoter, we found that the calcium transients were fully rescued in ASER, but not in ASEL. These results confirm that FRISSC restores the ASER cilium, and that the rescue is cell-specific and cell autonomous. Thus, FRISSC can restore cilia that are fully functional and capable of transmitting physiological responses.

3.3 PAPER III: FOOD ENHANCES NOICEPTIVE RESPONSES

Sensory systems and behavioral responses are not static, but can switch between different behavioral states. This allows animals to adjust their behaviors in response to changes in the internal or external environment, such as feeding state, nutritional state and stress. We investigated the cellular and molecular basis of how feeding state alters nociception in *C. elegans*. Using behavioral assays we found that in the presence of food, in the form of the bacterial *E. coli* strain OP50, behavioral avoidance to chemical repellents like copper, high osmolarity and bitter compounds is increased. Using calcium imaging, we found that neuronal responses to chemical repellents in the ASH neurons are also increased in the presence of food. These results indicate that escape responses to soluble repellents are enhanced by food and that this enhancement results at least in part from changes in the response properties of the primary chemosensory neurons.

Both dopamine and serotonin are involved in food signaling in *C. elegans*. Using several different approaches, we showed that food modulation involves dopamine signaling through the activation of the dopaminergic neurons, but does not involve serotonin. Pharmacological and genetic studies showed that exogenous dopamine mimics the effect of food and that mutation of the gene *cat-2*, which is required for dopamine synthesis, abolishes the effect of food on escape responses. We then used an optogenetic approach and found that direct activation of the dopaminergic neurons using blue light enhances escape responses immediately, and that this effect lasts up to 2 minutes after the

activation. This demonstrates that the activation of dopaminergic neurons modulate avoidance responses transiently.

The dopaminergic neurons are mechanosensory neurons that directly sense the presence of bacterial food. These neurons also receive input from other neurons via synapses and gap junctions and could be responding indirectly to internal signals of food availability. To determine if food modulation requires sensory input to the dopaminergic neurons, we used FRISSC to restore sensory cilia to the dopaminergic and ASH neurons only, in a *daf-19* background devoid of sensory cilia and sensory input. These experiments revealed that the cilia of the dopamine neurons are specifically required for food modulation, indicating that dopamine signaling is directly activated by external sensory cues.

In an effort to identify the receptor mediating the effects of dopamine on ASH, we found that a deletion mutant for the dopamine receptor DOP-4 is defective in food modulation of escape responses and neuronal responses in ASH. Rescue studies using several ASH and non-ASH promoters showed that DOP-4 acts in ASH to modulate neuronal responses and avoidance. Further studies using cell-specific RNAi confirmed the role of DOP-4 in ASH. Knock down of DOP-4 in ASH abolishes the effect of food on avoidance but does not affect responses off food.

One important feature of sensory neurons is adaptation to repeated stimulation. To determine if food and dopamine only modulate acute responses or also affect responses to repeated stimulation, we tested how food affects adaptation using behavioral assays and ASH imaging. We found that food and dopamine not only enhance acute responses, but also inhibit adaptation to repeated stimulation with repellents, leading to increased responses over time in a food-rich environment.

Together, our results suggest that dopamine is released by the dopaminergic neurons upon food sensation, functioning as an on-food signal. Dopamine acts directly on ASH through the dopamine receptor DOP-4 to alter response properties in ASH, leading to enhanced acute responses, and acts through an unknown receptor to inhibit adaptation. Interestingly, the dopaminergic neurons are not connected to the ASH through synapses or gap junctions, indicating that dopamine acts extrasynaptically to enhance ASH activity.

3.4 PAPER IV: NEUROPEPTIDE MODULATION OF NOCICEPTIVE NEURONS

Food availability and nutritional status are important cues affecting various behaviors, and stressful conditions such as absence of food and starvation induce behavioral changes to increase the chance of survival. In Paper 3 we

reported that animals adapt slower to chemical repellents in the presence of food compared to in the absence of food. This effect is mediated by dopamine, which acts as an on-food signal. In Paper IV we show that in contrast to dopamine, the neuropeptide receptor NPR-1 inhibits nociception in the absence of food. *npr-1* null mutants adapted normally to repeated stimulation with 10 mM $CuCl_2$, and several other repellents, in the presence of food, but adapted slower than wildtype to copper in the absence of food. Consistent with this behavioral phenotype, *npr-1* animals showed normal $CuCl_2$-evoked calcium responses in ASH in the presence of food, but showed larger responses than wildtype after continuous stimulation, and adapted more slowly, in the absence of food. Both behavioral and calcium imaging phenotypes were rescued by expression of an *npr-1(+)* transgene under the ASH-specific *sra-6* promoter. Together, these results suggest that NPR-1 functions in ASH to inhibit repellent sensation in response to neuropeptide signals indicating the absence of food. NPR-1 and dopamine signaling have opposing effects on adaptation to repellents: NPR-1 increases adaptation in unfed animals, whereas dopamine decreases adaptation in well-fed animals. To determine how these modulatory pathways interact, we analyzed copper adaptation in *cat-2;npr-1* double mutants. We found that *npr-1* is completely epistatic to *cat-2* with respect to ASH adaptation phenotypes. These results indicate that the effect of dopamine on ASH adaptation requires NPR-1 and suggest that dopamine affects adaptation indirectly through changes in neuropeptide signaling.

To identify which neuropeptides might be involved in increasing adaptation, we screened through a number of FLP mutants and FLP overexpression lines. We found that overexpression of the peptide FLP-8 in wildtype animals leads to increased avoidance in off-food conditions, but not on food. Thus, FLP-8 seems to inhibit avoidance off food, but not on food, and like NPR-1 acts to indicate the absence of food. To test if FLP-8 and NPR-1 act in the same pathway, we tested adaptation in *flp-8::flp-8;npr-1* animals. We found that the FLP-8 overexpression phenotype was completely suppressed by the *npr-1* mutation, indicating that FLP-8 functions to inhibit avoidance responses through the NPR-1 pathway, either directly by binding to NPR-1 or indirectly by acting further upstream. Interestingly, *flp-8* mutant animals, which carry a large deletion in the *flp-8* gene, have no defects in adaptation. This indicates that in addition to FLP-8, other neuropeptides might have overlapping functions, and might act to inhibit nociception.

Together, these results suggest that FLP-8 acts as an 'off-food' signal, resulting in decreased responsiveness in the absence of food. This effect requires NPR-1, which acts directly in ASH to increase adaptation. We speculate that FLP-8 is released in the absence of food or under starvation, and has as an antinociceptive function resulting in decreased escape responses under stressful conditions.

4 CONCLUSIONS

Paper I: The two gustatory ASE neurons, which are required for chemotaxis to NaCl, are functionally asymmetric. ASEL is an ON-cell, which is activated by increases in NaCl concentration, and regulates forward movement. ASER is an OFF-cell, which is activated by decreases in NaCl concentration, resulting in turns. Signal transduction in both neurons involves cGMP signaling, and together ASEL and ASER function to regulate chemotaxis up a concentration gradient.

Paper II: Functional Rescue in Single Sensory Cilia (FRISSC) is a genetic method based on cell-specific rescue of the RFX transcription factor DAF-19. Expression of DAF-19 in a cilia-deficient *daf-19* mutant background results in rescue of cilia in sensory neurons. FRISSC allows formation of cilia, which are anatomically and functionally normal, and the rescue is cell-specific and cell-autonomous. Thus, FRISSC is a useful tool to dissect the contribution of individual neurons in neural circuits and behaviors.

Paper III: In the presence of an external food source, the nociceptive ASH neurons are sensitized through dopaminergic signaling, resulting in increased escape responses to chemical repellents. The dopaminergic neurons release dopamine, which acts directly on ASH through the D1-like dopamine receptor DOP-4. This effect is transient and requires food sensing by the dopaminergic neurons. Thus, dopamine functions as a direct signal of the presence of food to sensitize the ASH neurons and increase escape responses.

Paper IV: In the absence of an external food source, neuropeptide signaling acts on the nociceptive ASH neurons to decrease responses to chemical repellents. The FMRFamide-related peptide FLP-8 and the neuropeptide receptor NPR-1 act in the same pathway to increase adaptation to noxious stimuli in the absence of food. In contrast, dopamine decreases adaptation in the presence of food. This effect requires NPR-1, suggesting that dopamine modulates nociception through changes in neuropeptide signaling. Thus, *C. elegans* downregulates nociception during reduced food availability.

5 PERSPECTIVES

C. elegans offers an excellent system to study behavior and neural circuits. The simplicity of the nervous system, the amenable genetics and short generation time allows a variety of genetic manipulations and in-depth studies of genes and cells in the nervous system. Despite the structural simplicity, the *C. elegans* nervous system has turned out to be surprisingly complex at the molecular and physiological level. Sequencing of the *C. elegans* genome has revealed that *C. elegans* has similar quantities of genes involved in neurotransmission, signal transduction and conductance as mammalian systems. This is unexpectedly high for an organism with only 302 neurons, and functional studies have only started to reveal the role of these molecules in individual neuron classes and behaviors. The gustatory ASE neurons exemplify how a nervous system with a simple structure can achieve relatively complex behaviors. Two neurons, ASEL and ASER, contribute to chemotaxis by responding differently to increases and decreases in attractant concentration. Activation of ASEL and ASER generates distinct behavioral responses; forward runs and turns respectively. Together, ASEL and ASER give rise to chemotaxis, a behavior that is essential for navigating the environment and locating food sources. Recent studies have begun to explore the molecular basis for ASE asymmetry, and have shown that differentially expressed rGCs are essential for chemotaxis and activity in the ASE neurons. However, it remains to be established if these GCs function as direct receptors for NaCl and other salts, or if they function downstream. In addition to understanding the signaling pathways within the ASEs, the neural networks controlling chemotaxis need to be identified. The downstream targets of the ASEs include the interneurons AIY, AIA and AIB, but the roles of these neurons in chemotaxis are not understood. Furthermore, a recent publication has shown that the ASH and ADF neurons also contribute to runs and turns, indicating that chemotaxis is regulated by a complex neural network including several classes of sensory neurons and interneurons (Thiele et al., 2009).

Nutritional state and food availability are essential factors for survival and reproduction, and the feeding state of an animal is an important factor guiding many behaviors. Our work shows that feeding state modulates the sensitivity of primary nociceptive responses to soluble repellents. The dopaminergic neurons act to sensitize the nociceptive ASH neurons in response to food cues, demonstrating that nociceptive responses can be modulated by external signals. These results open up questions regarding the mechanisms involved in changing escape responses. Firstly, how is modulation of ASH achieved? The two receptors modulating ASH activity, DOP-4 and NPR-1 are both G-protein coupled receptors. DOP-4 is a D1-like dopamine receptor, indicating that it acts through cAMP signaling. The downstream signaling pathways of NPR-1 in ASH are completely unknown, and identifying G-proteins activated by DOP-4 and NPR-1 are important steps in

understanding the molecular mechanisms involved in modulating ASH responses. Activation of G-proteins and second messenger systems could result in opening or closing of ion channels, changing the excitability of the cell. Second messengers can also result in desensitization of chemoreceptors or other receptors on ASH, resulting in increased adaptation. A second question involves the regulation of neuropeptidergic modulation of ASH. Where is FLP-8 released from and what kind of cues regulate its release? Interestingly, FLP-8 has been shown to be expressed in mechanosensory neurons in animals that enter dauer-stage, an arrested developmental stage that occurs during starvation, but not in well-fed conditions. This indicates that FLP-8 is regulated by feeding state or nutritional status, and that starvation results in changes in expression of FLP-8. Further investigations of the nutritional cues and molecular mechanisms regulating FLP-8 expression and release will give valuable insight into the effect of feeding state on nociception.

An important step in understanding how dopamine modulates nociception was to distinguish between the effects of sensory input directly to the dopaminergic neurons, and input from other sensory neurons acting indirectly. The use of FRISSC was essential to show that sensory input to the dopaminergic neurons is required for modulation of nociception, and demonstrates the value of FRISSC in determining how individual components of a neural circuit contribute to behavior.

The dopaminergic neurons do not have synaptic connections to ASH, meaning that dopamine acts extrasynaptically. Also other studies have shown that both biogenic amines and neuropeptides act extrasynaptically, adding an extra layer of complexity to a structurally simple nervous system. The expression patterns of the dopamine receptors reveal that they are expressed in many neurons that are not postsynaptic to the dopaminergic neurons. This indicates that when dopamine is released in response to food, it acts extrasynaptically on various neurons to modulate their function and modulate behaviors. Similarly, NPR-1 functions as a modulator of many neurons and behaviors. NPR-1 is widely expressed in the nervous system and is involved in a number of behavioral responses. The FLP genes *flp-18* and *flp-21* have been identified as NPR-1 ligands, but it is not known how their release or expression is regulated. *flp-18* and *flp-21* mutants have in many cases not shown strong phenotypes, indicating that NPR-1 might have additional ligands.

Together with other studies, these results indicate that environmental cues signaling the presence of food modulate behaviors, and we speculate that these changes might increase the chances of survival and reproduction in food-rich conditions. Such behaviors include pharyngeal pumping, slowing, egg-laying and increased nociception. In food-poor conditions, animals display a different set of behaviors; pharyngeal pumping and egg-laying cease, and animals move quickly in search for new food sources. In these conditions, nociception is inhibited through neuropeptidergic signaling, possibly as a measure to increase chances of locating food. Our results demonstrate that

FLP-8 acts as an antinociceptive signal and also signals the absence of food. Interestingly, the mammalian FaRP NPFF inhibits nociception and feeding. This indicates that the function of FaRPs are evolutionary conserved, and that FaRPs are involved in regulating nociception and signaling feeding state both in vertebrates and invertebrates. In humans, pain is known to be a highly complex sensation, affected by various factors like stress and strong emotions. The wide range and complexity of antinociceptive pathways in mammals indicate that analgesia can be induced by many different factors, possibly also feeding state.

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